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# 15th International & 14th European Congress of Endocrinology (ICE-ECE 2012)

5–9 May 2012, Florence, Italy

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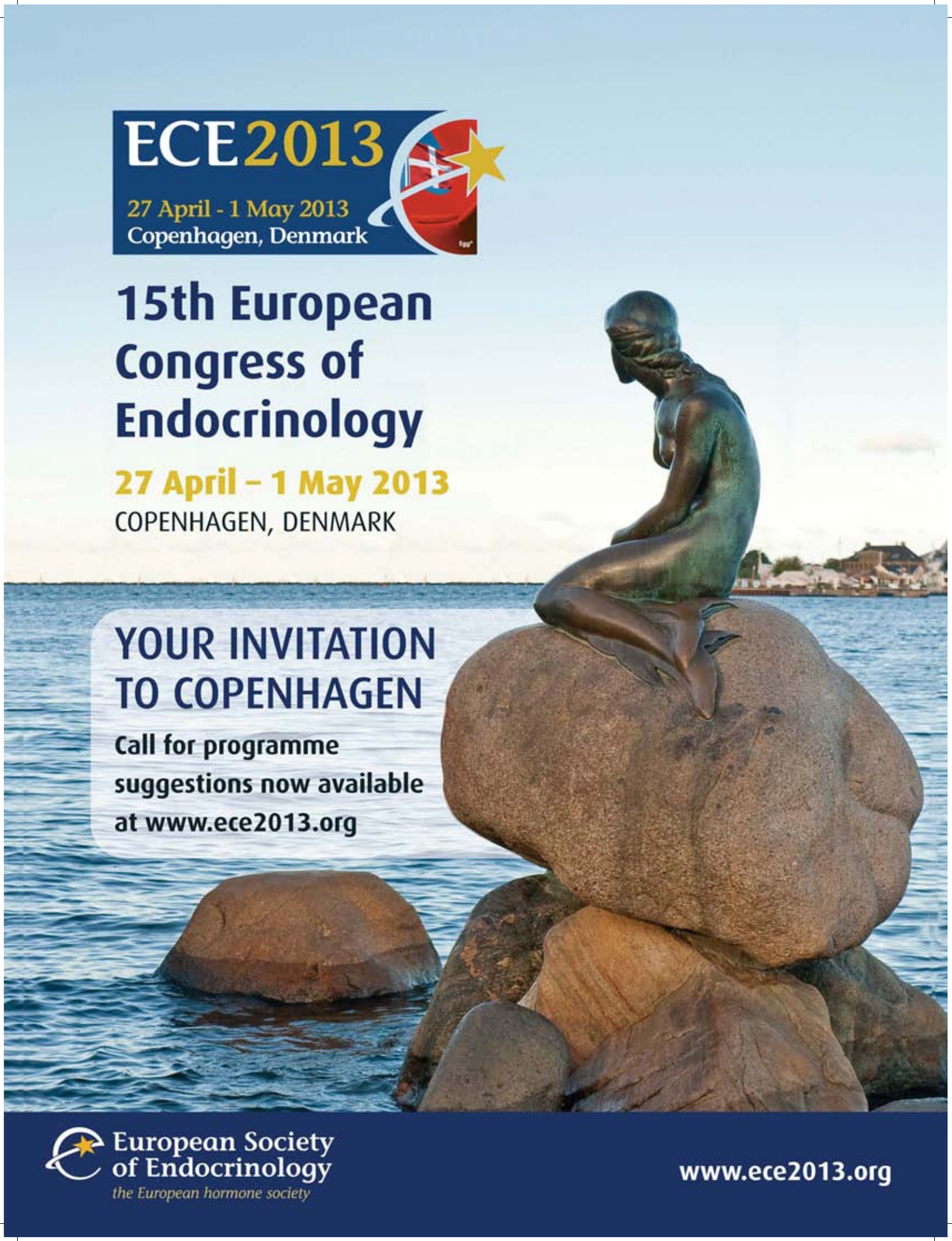
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# European Society of Endocrinology

*the European hormone society*

**The European Society of Endocrinology promotes research, education and clinical practice in endocrinology. It aims to strengthen endocrinology by enabling scientists and doctors across Europe to work together more successfully by providing high-quality education and communication opportunities. The European Society of Endocrinology is a charity and a company limited by guarantee.**

- ESE represents and incorporates the full breadth of endocrinology throughout its activities, which includes not only the core endocrine diseases but also diabetes, metabolism & obesity, bone & calcium, reproduction, and oncology; and through balancing the coverage of common and rare endocrine diseases.
- ESE is the natural home for researchers, clinicians, students, nurses and pharmaceutical companies working in the field of endocrinology and hormonal systems, supporting them through its activities such as education and grants.
- ESE is a community which provides the 'voice of endocrinology', promoting ESE and the understanding of endocrinology to endocrinologists, to European legislative and funding bodies and by providing the public with information on endocrinology, and by supporting endocrine nurses and patient advocacy groups.

### ESE Vision Statement

The European Society of Endocrinology exists to support research, education and patient care in endocrinology and it will continue to achieve this by promoting excellence in endocrine science and medicine and by providing public information on endocrinology.

ESE has a distinctive European flavour, creating an inclusive community that takes advantage of the diversity found within Europe. It will be an innovative society that responds rapidly to the changing needs of clinicians and researchers. It will also provide authoritative and independent information to the public.

## Major activities

Major activities include the annual European Congress of Endocrinology and training courses which provide outstanding updates for basic scientists and clinicians. The Society also runs regular postgraduate educational courses aimed at both basic scientists and clinicians.

## Save the Dates!

**12th ESE Postgraduate Course in Clinical Endocrinology**

Antalya, Turkey
18-21 October 2012

**Summer School on Endocrinology**

Bregenz, Austria
5-9 August 2012

## Membership

### Ordinary Membership

Open to researchers, clinicians and students with an interest in the field of endocrinology and hormonal systems. In particular, the Society welcomes early-career endocrinologists, endocrine nurses and basic scientists.

**Benefits of ordinary membership include:**

- **Free online access to all five official ESE journals:** *European Journal of Endocrinology, Journal of Endocrinology, Journal of Molecular Endocrinology, Endocrine-Related Cancer* and *Endocrine Connections*, an interdisciplinary Open Access journal, launched in 2012
- Reduced rate print subscription to the *European Journal of Endocrinology, Journal of Endocrinology, Journal of Molecular Endocrinology* and *Endocrine-Related Cancer*
- Reduced rate registration to the European Congress of Endocrinology and any other ESE events
- Access to ESE Prizes, Grants and Awards
- Access to a Members' only section with access to a web-based directory of European endocrinologists
- Tri-annual newsletter and regular email alerts advising of news and grants
- Grants forum
- Voting rights and attendance at general meetings

### Affiliated Society Membership

ESE values its links with the 43 national endocrine societies and 10 specialist societies that are currently members.

Membership is open to national endocrine societies in Europe and in countries bordering the Mediterranean sea as well as pan-European and international sub-specialist endocrine societies.

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## ESE Meeting Grant

ESE offer 50 ESE Meeting Grants per year, worth up to €400 each, for ESE members to attend an ESE meeting. The grant is made as a contribution towards the total cost of attending either the annual European Congress of Endocrinology (ECE) or an ESE Training Course in accordance with the eligibility criteria.

## ESE Basic Science Meeting Grant

ESE is making every effort to bring basic scientists to its annual meeting by the introduction of the ESE Basic Science Meeting Grant. ESE offer 100 ESE Basic Science Meeting Grants per year worth up to €450 each for ESE members who are basic scientists to attend the annual European Congress of Endocrinology (ECE) in accordance with the eligibility criteria.


# Journals

The Society has five official journals; between them they cover the full spectrum of endocrinology and help to raise the profile of European endocrinology.

## *European Journal of Endocrinology*

The official clinical journal of the European Society of Endocrinology. The journal publishes original research papers, reviews, short communications and case reports within clinical endocrinology. Invited commentaries also feature regularly. 'Highlights' are concise summaries of new breakthroughs. ESE members receive free online access to the journal.
**IMPACT FACTOR: 3.482** *(as at 2010)*

## *Journal of Endocrinology*

*Journal of Endocrinology* provides a wide coverage of research in all aspects of endocrinology. It is in the top ranks of global endocrine journals and publishes high quality, leading-edge research from all over the world.
**IMPACT FACTOR: 3.099** *(as at 2010)*

## *Journal of Molecular Endocrinology*

*Journal of Molecular Endocrinology* provides a forum for papers devoted to describing molecular mechanisms by which hormones and related compounds regulate physiological and pathological processes. JME publishes high quality, leading-edge research from all over the world and its readership is worldwide.
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## *Endocrine-Related Cancer*

*Endocrine-Related Cancer* offers a global forum for basic, clinical and experimental investigations which concern endocrine-related cancer in human and animal subjects. **IMPACT FACTOR: 4.432** *(as at 2010)*

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## *Endocrine Connections*

A new Open Access journal which offers authors the highest possible visibility for their work and stimulating cross-discipline collaboration.

The European Society of Endocrinology is collaborating with the UK Society for Endocrinology to launch *Endocrine Connections* a new, interdisciplinary, Open Access journal in Spring 2012. The journal focuses on articles that have relevance across the many disciplines that intersect with endocrinology. The broad scope encompasses metabolism, obesity, diabetes, hormones, reproduction and bone disorders.

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# Prize Lectures and Biographical Notes

# The *European Journal of Endocrinology* Prize Winner

Professor Sadaf Farooqi, PhD, FRCP

Sadaf Farooqi qualified with Honours in Medicine from the University of Birmingham, being awarded the gold medal. After hospital posts in Birmingham and Oxford she moved to Cambridge to undertake a PhD. She identified the first single gene defect to cause human obesity in patients with a mutation in the leptin gene (Nature 1997) and described their dramatic response to leptin therapy, establishing leptin's role in the regulation of eating behaviour, puberty and immune function (NEJM 1999; SCIENCE 2007). As a Wellcome Trust Senior Clinical Fellow at the Institute of Metabolic Science in Cambridge, Professor Farooqi co-ordinates a programme of research into the genetic, molecular and physiological basis of human obesity. She has been invited to speak at numerous international meetings, is on several Editorial Boards and has been the recipient of a number of awards in recognition of her work including the RD Lawrence Award (Diabetes UK 2007), Andre Mayer Award (International Association for the Study of Obesity 2006) and the Society for Endocrinology Medal 2012.

The *European Journal of Endocrinology* Prize Lecture

# Tackling obesity: lessons from genetics

S Farooqi, Wellcome Trust Senior Clinical Fellow and Professor of Metabolism and Medicine, University of Cambridge Metabolic Research Laboratories, and NIHR Cambridge Biomedical Research Centre, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK

Whilst the rise in the prevalence of obesity has been driven by environmental factors, there is considerable evidence that body weight and fat mass are highly heritable traits. Differences in susceptibility to obesity between individuals have strong genetic determinants.

Our strategy has been based on studies of patients with severe obesity where we hypothesised that major highly penetrant genetic variants were likely to be playing an important role.

The identification of patients with mutations in the gene encoding the hormone leptin, and their successful treatment with recombinant human leptin, have provided insights into the role of leptin responsive pathways in the regulation of eating behaviour, the onset of puberty and T-cell mediated immunity. Leptin acts by regulating a complex network of brain responses that can be studied using functional imaging, to co-ordinate changes in nutritional state with changes in food intake and the liking of food. A downstream target of leptin action, the melanocortin 4 receptor (MC4R), plays a key role in modulating sympathetic nervous system mediated changes in blood pressure.

Recently, genome wide approaches are proving to be an increasingly important tool in understanding the genetic heterogeneity associated with obesity. The discovery of how genetic variation at an individual and at a population level contributes to weight gain will drive further understanding of the molecular and physiological pathways involved in weight regulation.

# The Geoffrey Harris Prize Winner

Professor Jonathan R Seckl, Moncrieff-Arnott Professor of Molecular Medicine and Executive Dean, College of Medicine and Veterinary Medicine, Endocrinology Unit, Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

Jonathan Seckl is both medically and scientifically qualified (MBBS at UCL, PhD at ICL). A clinical endocrinologist and former Wellcome Trust Senior Clinical Research Fellow, Seckl's research (funded by successive programme grants from the Wellcome Trust, and additional programme awards from MRC, Wellcome and HFSP) focuses on glucocorticoid biology from 'cloning to clinic'. His main themes are the importance of local tissue regeneration of glucocorticoids by 11β-hydroxysteroid dehydrogenases as a cause of and therapeutic target for age-related memory impairments and the metabolic syndrome-diabetes-obesity continuum. His group advanced and supported the glucocorticoid hypothesis of fetal 'programming' and has elucidatedmolecular and epigenetic mechanisms by which this leads to subsequent disorders in adult life. Seckl has authored ~300 peer-reviewed papers (career citations >22,000; h=78), has given ~200 invited lectures at international meetings, has talked to schools, teachers groups, lay audiences and in public fora. Thirty-eight of his students have gained PhDs, several are now Professors around the globe. He has been on committees for the MRC, Wellcome Trust, RSE, RS, UK charities, the EU, the Scottish Government, and the Councils of the Academy of Medical Sciences and the Society for Endocrinology.

The Geoffrey Harris Prize Lecture

# Glucocorticoid metabolism and the brain, from fetal programming to senescence

Jonathan R Seckl, Endocrinology Unit, Centre for Cardiovascular Sciences, University of Edinburgh, Queens Medical Research Institute, Edinburgh EH16 4TJ, UK.

Chronic elevation of glucocorticoids adversely impacts on cognition and the integrity of hippocampal cells. Moreover, inter-individual differences in memory with ageing correlate directly with blood glucocorticoid levels in rodents and humans. Beyond plasma steroid levels, glucocorticoid action on target tissues is determined by the density of nuclear receptors and by intracellular metabolism by 11β-hydroxysteroid dehydrogenases (11β-HSDs) which catalyse interconversion of active cortisol (corticosterone in rodents) and inert cortisone (11-dehydrocorticosterone). In the fetal CNS and placenta 11β-HSD2 predominates. This isozyme catalyses the rapid inactivation of glucocorticoids and acts as a 'barrier' to steroid access to receptors. Inhibition, knockout, bypass or down-regulation (maternal stress, inflammation or malnutrition) of feto-placental 11β-HSD2 allows excess glucocorticoid action on the fetus which 'programmes' many organs, notably the brain. The offspring show affective and cognitive deficits throughout life. Similar effects are seen in the offspring of women who consume liquorice-based 11β-HSD inhibitors in gestation.

In the adult brain 11β-HSD type 1 predominates. This catalyses the reverse reaction, regenerating active steroids and thus amplifying intracellular glucocorticoid action. 11β-HSD1 inhibition protects hippocampal cells from neurotoxic challenge in vitro. 11β-HSD1 is elevated in aged rat hippocampus and correlates with the degree of cognitive decline suggesting an aetiological role. Importantly, 11β-HSD1 null mice resist glucocorticoid-associated cognitive impairments with ageing. Indeed the 11β-HSD inhibitor carbenoxolone improves cognitive performance in elderly humans. Early results suggest selective 11β-HSD1 inhibitors improve memory in aged rodents and even modify pathogenesis in models of Alzheimer's disease. 11β-HSD1 inhibition appears a promising target for therapy of age-related cognitive disorders.

# The IPSEN Foundation Prize Winner

Paolo Sassone-Corsi

Paolo Sassone-Corsi is Donald Bren Professor at the University of California, Irvine, where he is Director of the Center for Epigenetics and Metabolism. He is also External Member of the Max-Planck Institute, Germany. Paolo Sassone-Corsi is an internationally recognized leader in the field of genetics and cell biology. After his PhD in Italy, he has spent time in France and at the Salk Institute (San Diego, CA). He started his research group in Strasbourg, France, where he was Director of Research, before moving back to California in 2006. Early in his career he became fascinated by the world of DNA and gene expression, a field that was beginning to be unraveled at the time, and that he felt had to embody all fundamental facets of cellular and organismal physiology. His research on the regulation of gene expression has been essential to elucidate a remarkable variety of molecular mechanisms, all highly relevant to the fields of endocrinology, neuroscience, metabolism and cancer. All organisms adapt to the environment by readjusting their physiology and metabolism. This plasticity includes chromatin remodeling and epigenetic reprogramming and leads to changes in the activity of genes. Epigenetic processes demonstrate that there is much more to the genome than DNA sequence, permitting plasticity beyond the double helix. The work by Paolo Sassone-Corsi during the past 20 years has been influential and trend-setting, leading to many awards, including the EMBO Gold Medal, the highest recognition for a European molecular biologist. He has been also awarded the Charles-Leopold Meyer Prize of the Academie des Sciences (Paris), the Edwin Astwood Award and the Roy O. Greep Award of the Endocrine Society (USA), the CNRS Medal (France), the Grand Prix Bettencourt for Medical Research and the Endocrine Prize of the Ipsen Foundation.

The IPSEN Foundation Prize Lecture

# Common Threads: Epigenetics, Metabolism and the Clock

Paolo Sassone-Corsi, Center for Epigenetics and Metabolism, University of California, Irvine, California 92697, USA. psc@uci.edu,

Circadian rhythms govern a number of fundamental physiological functions in almost all organisms, from prokaryotes to humans. The circadian clocks are intrinsic time-tracking systems with which organisms can anticipate environmental changes and adapt to the appropriate time of day. Disruption of these rhythms can have a profound influence to human health and has been linked to depression, insomnia, jet lag, coronary heart disease, neurodegenerative disorders and cancer. At the heart of circadian regulatory pathways is the clock machinery, a remarkably coordinated transcription-translation system that utilizes also dynamic changes in chromatin transitions and epigenetic control. Recent findings indicate that regulation goes also the other way, since specific elements of the clock are able to sense changes in the cellular metabolism. Understanding in full detail the intimate links between cellular metabolism and the circadian clock machinery will provide not only critical insights into system physiology and endocrinology, but also novel avenues for pharmacological intervention towards metabolic disorders.

# Plenary Lectures

## Red wine Endocrinology

### PL1

Abstract unavailable.

## Testosterone – more than sex

### PL2

**Testosterone: more than sex**

M. Maggi
Sexual Medicine and Andrology, Florence, Italy.

Testosterone is the main male hormone controlling sexual and reproductive functions. Testosterone deficiency is associated with male hypogonadism, a syndromic condition that encompasses the well-recognised sexual and reproductive failure. Male hypogonadism has been, in fact, recently associated to an increased cardiovascular and metabolic risk (metabolic syndrome), most probably because testosterone deficiency is a stigmata of visceral adiposity. A stepwise reduction in testosterone has been described in human and animal models of increased abdominal adiposity. The biological significance of obesity-induced testosterone deficiency is still a matter of debate, because it can have a detrimental effect, further increasing visceral fat accumulation, or a protective role, by limiting reproductive potential, or it can represent only a marker of a decreased wellness. Nonetheless, meta-analysis of intervention trials on hypogonadism in metabolic syndrome indicate that testosterone supplementation can increase insulin sensitivity and decrease visceral adiposity. Although it is generally assumed that a decreased androgen signalling will restrain prostate overgrowth, having therefore a therapeutic potential for benign prostate hyperplasia (BPH), recent data indicate that both metabolic syndrome and obesity-induced hypogonadism might have a causative role on BPH-associated lower urinary tract symptoms (LUTS). In an animal model of high fat diet-induced metabolic syndrome and hypogonadism, we found that the prostate, as well as the liver, was affected by fibrosis, hypoxia and inflammation. Similar changes, although of a more limited extent, was observed in the bladder. Testosterone supplementation was able to revert hypoxia and fibrosis and to decrease prostate and bladder inflammation. It is therefore conceivable that testosterone supplementation in pathological conditions, such as metabolic syndrome, might have favourable effects not only in increasing insulin sensitivity and decreasing visceral adiposity, but also in ameliorating prostate function and LUTS.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## Mating, sex and the immune system in humans and fish

### PL3

**New insights into the roles of sirtuins in metabolic decline and disease of aging**

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Sirtuins are $NAD^+$-dependent deacetylases that are implicated in mediating health benefits of calorie restriction and possibly exercise. We will report on novel roles of SIRT1-3 in diseases of aging and recent progress in using small molecules to activate SIRT1. Small molecule activators of SIRT1 (STACs), such as resveratrol and SRT1720, improve multiple health parameters in mice including protection from type II diabetes and hepatic steatosis. We developed an system that permits whole-body deletion of SIRT1 in adult mice and have tested whether the effects of resveratrol and SRT1720 on mitochondrial function are SIRT1-dependent. We have also developed novel substrate-agnostic sirtuin assay to discover that SIRT1 activation is facilitated by specific hydrophobic amino acids in native SIRT1 substrates such as PGC-1$\alpha$ and FOXO3$\alpha$, thus explaining substrate specificity. A structured N-terminal domain is critical for the direct activation of SIRT1 by all known STACs and we find that cells with alterations in this domain are insensitive to the effects of STACs. Together, these data point to a common mechanism for allosteric activation of SIRT1 by small molecules.

### PL4

Abstract unavailable.

## Why your bones break - from childhood to old age

### PL5

**Why your bones break: from childhood to old age**

E. Seeman
University of Melbourne, Heidelberg, Victoria, Australia.

Bone's ability to tolerate loading is determined by its material composition and structural design. During growth, growth plate trabeculae condense by appositional growth forming the metaphyseal cortices ('corticalization'). Rapid distal radial longitudinal growth in early puberty outpaces trabecular condensation producing transitory intracortical porosity predisposing to fractures.
In adulthood, remodeling removes and replaces damaged bone. Around midlife in women, the volume of bone formed decreases and the volume resorbed increases producing a negative bone balance which produces structural decay, especially after menopause when remodeling intensity increases. Remodeling is always initiated upon an internal surface of bone. Remodeling upon haversian canal surfaces enlarges the canal focally producing intracortical porosity (in cross section) which fragments ('trabecularizes') the cortex thinning it from 'within'. Pores coalesce increasing the surface area; remodeling becomes self-perpetuating; more bone is lost from a decreasing cortical bone volume so bone loss accelerates. Remodeling upon trabeculae removes them so trabecular remodeling is self-limiting. Resorption upon the endocortical surface adjacent to marrow also thins the cortex. The porous structure loses its ability to resist cracking predisposing to fractures. Most bone loss is cortical, most occurs after 65 years and most fractures are appendicular, not vertebral.
Antiresorptives reduce remodeling intensity slowing structural decay. Structure is partly restored as refilling of resorptive cavities present at the start of treatment proceeds with the concurrent appearance of fewer new remodeling cavities. Osteons formed months earlier mineralize more completely instead of being replaced with younger less fully mineralized bone matrix. More homogenous and fully mineralized bone is more brittle – it cannot absorb energy by deforming. Thus, protracted remodeling suppression reduces structural decay but may compromise bone's material composition. Understanding why and how bone's break requires the study of bone's material composition and structure its 'qualities'. Bone densitometry was a good beginning.

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## Maternal nutrition, metabolism and fetal programming

### PL6

Abstract unavailable.

## Diabetes as a gut disease

### PL7

**Diabetes as a gut disease**

Michael A. Nauck

Gastric inhibitory polypeptide (GIP) is a physiologically important incretin hormone secreted from K cell of the upper small intestine. Glucagon-like peptide 1 (GLP-1) is secreted from L cells in the lower small and large intestine. Both hormones are released after nutrient (especially carbohydrate and fat) ingestion and glucose-dependently augment insulin secretion. An involvement of either incretin hormone in the pathogenesis of diabetes may, in principle, be related to abnormalities of either secretion or action. While some studies suggest reduced GLP-1 secretion after oral glucose or meal ingestion in patients with type 2 diabetes, more recent data do not support any systematic difference to healthy subjects. There is, however, a large inter-individual variation. GIP secretion is also not impaired in type 2 diabetic versus healthy subjects. Nevertheless, type 2 diabetes is associated with a reduced incretin effect. This means that oral glucose has only little more stimulatory effect on insulin secretion than has an isoglycaemic intravenous glucose infusion (i.e. at the same glycaemic profile). In healthy subjects, marked differences are typical, with two thirds of the secretory response being due to factors other than hyperglycaemia, pointing to mechanisms related to the absorption of nutrients from the gut. Insulinotropic GIP effects, in contrast to unchanged secretion, are markedly reduced in patients with type 2 diabetes compared to healthy subjects. Therefore, defective GIP action on β-cells explains impaired insulin secretory responses in patients with type 2 diabetes. This may be pathophysiologically relevant in the case of nurtrient-stimulated insulin secretion, i.e. after meals. A similarly reduced insulinotropic activity of GIP has been described with a variety of other forms of diabetes, whenever there is fasting hyperglycemia. Whether or not such defects are central to the pathophysiology of type 2 diabetes is still unclear. Two hypothesis have been derived: i) there is a specific insensitivity to GIP in type 2 diabetes, so that the type 2-diabetic endocrine pancreas is particularly insensitive to endogenous and exogenous GIP, more than, for example, in response to glucose or other insulinotropic stimuli. The alternative explanation is that ii) there is a generalized functional defect in the endocrine pancreas of patients with type 2 diabetes, which pertains for glucose- as well as GIP-stimulated insulin secretion to a similar degree. Current evidence supports the latter hypothesis. In contrast to markedly reduced insulinotropic GIP effects in type 2 diabetes, GLP-1 actions are much less impaired. If not at physiological concentrations, at pharmacological GLP-1 concentrations, a normalization of plasma glucose can be achieved in most type 2 diabetic patients examined so far. In conclusion, the major abnormality with respect to incretin (GIP and GLP-1) secretion and action in type 2 diabetes is a reduced responsiveness of β cells to GIP. This may partly explain impaired insulin secretion in type 2 diabetes. On the other hand, GLP-1 retains its insulinotropic activity even in advanced type 2 diabetes, why it has become the parent compound for incretin-based medications like GLP-1 receptor agonists and inhibitors of dipeptidyl peptidase-4 (DPP-4).

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## Novel molecular link between the circadian clock and hypertension

### PL8

**Novel molecular link between the circadian clock and hypertension**

H. Okamura
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Rhythmic change of the internal milieu is a fundamental principle in all living organisms. A variety of physiological events show daily rhythms, such as blood pressure, hormonal secretion, cell cycle, and migration of stem cells from the bone marrow to the systemic circulation. This timing is determined by the endogenous oscillatory system called the circadian clock. Within the last ten years, the molecular dissection of circadian clock has progressed dramatically, and it is now known that circadian time is generated by clock genes interlocked in an autoregulatory transcription-(post)translational feedback loop operating in most cells of the body. However, the fundamental timing system evolved in a rhythmic environment is now threatened by recent life-style changes: steady day-night (light-dark) cycles are disrupted by shift-works and long working hours, which increases in the risk of life-style related diseases such as hypertension, metabolic syndrome and cancer, caused by such lifestyles. To clearly demonstrate the importance of the biologic clock for health, we used completely arrhythmic mice with deleted Cry1 and Cry2 clock genes (Cry-null mice). Phenotype survey of these animals revealed Cry-null mice rapidly develop salt-sensitive hypertension. By adrenal transcriptome, we identified a new type of 3-β-hydroxysteroid dehydrogenase/Δ-5-4 isomerase (3-β-HSD) that was dramatically over-expressed in Cry-null mice. This enzyme, Hsd3b6, expressed in the zona glomerulosa (ZG), is regulated by the circadian clock. The lack of a functional circadian clock in Cry-null mice leads to Hsd3b6 over-expression, hyperaldosteronism and, ultimately, salt-sensitive hypertension. We further identified HSD3B1 as the human homologue of mouse HSD3B6, and prevalence of this enzyme in a group of human primary aldosteronism, which represents new possibilities in the treatment of hypertension that are being investigated.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Symposia

## Molecular mechanisms of differentiated thyroid cancer

### S1.1

**Molecular consequences of deregulated RNA genes in papillary thyroid carcinoma**

A. de la Chapelle[1,2]
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Early miR expression analyses in papillary thyroid carcinoma (PTC) disclosed profound differences in the expression of several, microRNAs in thyroid tumor compared with unaffected thyroid tissue. For instance, miR146 and miR221 were 19- and 11-fold upregulated in PTC. These and other miRs target the thyroid hormone receptor THRB gene. Transfection of cell lines with miR146a and other miRs *in vitro* resulted in down-regulation of THRB transcript and protein. Promoter luciferase reporter experiments as well as *in vivo* measurements were concordant. These findings are reflected *in vivo* in thyroid tumor tissue (miRs are up, THRB is down). Thus miRs up-regulated in PTC tumors directly inhibit the expression of THRB, an important tumor suppressor gene.
Examples from other cancers suggest that many more miRs are probably involved in PTC pathogenesis. Indeed the early studies targeted only a fraction ($n=460$) of all miRs known today ($n>1500$). All miRs in the genome can presently be assessed by RNA sequencing. Such studies are in progress and may reveal roles for miR-mediated perturbations in key PTC pathways but definitive data are not presently available.
Recent advances fueled by second-generation deep sequencing implicate long intergenic noncoding RNAs (lincRNAs) in the pathogenesis of cancer. Examples in thyroid pathology include the NAMA gene in 9q22. NAMA is a noncoding RNA associated with the MAP kinase pathway and growth arrest. The PTCSC1 gene in 8q24 is embedded in the introns of two protein-coding genes, thyroglobulin (TG) and Src-like adaptor (SLA). The PTCSC3 gene in 14q13 is a highly thyroid-specific spliced gene that is strongly downregulated in PTC tumors. By functional assays it acts as a tumor suppressor. Some unpublished data on lincRNA genes will be discussed.
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### S1.2

**Correlation between the BRAF V600E mutation, TGFβ pathway and tumour invasiveness in papillary thyroid carcinomas**

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[1]Hospital La Paz Biomedical Research Institute (IDIPAZ), Madrid, Spain; [2]Biomedical Research Institute, CSIC-UAM, Madrid, Spain.

The V600E mutation of BRAF oncogene is one of the most frequent genetic events in thyroid cancer, particularly in papillary thyroid carcinomas (PTC) and in some anaplastic carcinomas that derive from preexisting PTC. It has been associated with advanced clinical stages, extrathyroidal extension, and a high risk of recurrences, particularly those that have lost the ability to accumulate iodide, a property mediated by the sodium iodide symporter (NIS).
Our previous data have shown that BRAFV600E promotes NIS functional repression in thyroid cells and that BRAF-positive tumors exhibit a significantly reduced NIS expression. In addition this oncogene promotes cell migration and invasion. Its mechanism of action is mediated by the MEK-ERK pathway, although MEK inhibition does not fully rescue BRAF-induced NIS repression. We have shown that the mechanism through which BRAF induces NIS repression and promotes epithelial to mesenchimal transition and invasion is based on the operation of an autocrine TGFβ loop.
The role of TGBβ as oncogenic factor is gaining increasing importance. We have analyzed its role in a panel of thyroid cancer cells lines harbouring the main genetic mutations described so far in thyroid cancer. Thyroid cancer cells were classified in two groups depending on whether TGFβ had an oncogenic response or not. In addition preliminary results show that a high TGFβ-Smad pathway activity is present in the most aggressive forms of human thyroid cancer, including well- differentiated carcinomas with radioiodide-refractory metastatic disease, poorly differentiated and anaplastic carcinomas. We suggest that TGFβ is an oncogenic factor in advanced thyroid cancer.
Declaration of interest
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### S1.3

Abstract unavailable.

## 40 years of male hormonal contraception research and no product

### S2.1

Abstract unavailable.

### S2.2

**Clinical trials in male hormonal contraception to date**

M. Meriggiola, S. Cerpolini & A. Costantino
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Over the last 50 years, different studies have been carried out in the field of male hormonal contraception. Two multicenter efficacy studies supported by WHO in which the prototype testosterone-alone regimen was used, have demonstrated the validity of the principle of male hormonal contraception. Contraceptive protection is dependent on the degree of sperm suppression. Azoospermia is certainly the gold standard for this type of contraceptive since no pregnancies were reported among azoospermic men. However, the pregnancy rate among azoospermic and severely oligospermic (sperm count $<1$ million/ml) men was 0.8 per 100 person/year (95% CI 0.1–2.7; 2 pregnancies in 269.5 years of exposure). Therefore, the threshold of 1 million/ml for sperm suppression after hormone administration seems to be an acceptable interim goal for a hormonal male contraceptive. Further clinical studies have shown that testosterone must be combined with a progestin in order to consistently induce profound sperm suppression. Different androgen-progestin regimens have been tested in small pilot trials suggesting that these regimens can effectively suppress sperm counts without inducing major adverse effects. Two efficacy trials testing two different androgen-progestin combinations have recently been performed confirming the high effectiveness of these combinations in terms of sperm suppression and pregnancy rate. Further studies will be needed to confirm the efficacy of these combinations and to select the safest androgen-progestin combination for market entry.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### S2.3

**Acceptability of male hormonal contraception by the pharmaceutical industry and by the consumer**

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[1]Bayer Pharma AG, Berlin, Germany; [2]Gulf Medical University School of Medicine, Ajman, United Arab Emirates.

Introduction
Following extensive research activity to develop an effective hormonal approach to control male fertility, little is known concerning attitudes of men and women

towards male contraception in different countries. Although several pharmaceutical companies have conducted studies in male contraception, a full clinical development programme to obtain approval for one of the hormonal methods has never been initiated.
Methods
Several studies and surveys have been performed in different parts of the world. Major surveys have been funded by Schering (now Bayer Pharma), one in 2002 in 9000 men aged 18–50 years, and a second one in 2006/2007 in 20 000 men in the same age group. An internet survey was conducted in 2000 women in Germany in 2010. In addition, published data are reviewed.
Results
In all surveys, male contraception is seen as an option to share responsibility for contraception in stable relationships. 70% say that the method of contraception is taken in a joint decision. In all surveys, 50% of men and women express a positive attitude towards male contraception. In the two large international surveys, acceptance rates are particularly high in Latin American countries and somewhat lowest in Asia. The majority of men would seek advice on hormonal MFC from a physician.
The two companies with an interest in male contraception have been taken over by two of the largest companies in pharmaceutical industry whose strategic goals are in different areas. Moreover, the premature termination of the WHO study has substantially increased the hurdles for those still willing to consider the development of male contraceptives.
Conclusion
The overall acceptance of male hormonal contraception is broad across both genders and various cultures. However, in the current situation, it is unlikely that pharmaceutical industry will see an opportunity for male hormonal contraception for many years to come.
Declaration of interest
The author declares that there is a conflict of interest.
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## Absolute fracture risk assessment with FRAX

### S3.1

Abstract unavailable.

### S3.2

**Use of FRAX in Asian populations**
L. Koh
Gleneagles Medical Centre, Singapore, Singapore.

Asia occupies 30% of the world's landmass and contains 60% of the world's population encompassing a wide diversity of people and demographics. It has been estimated that by 2050, half of all hip fractures in women >65 years of age will occur in Asia. Hip fracture incidence rates among women vary widely in Asian populations, from as low as 100 up to 500 per 100 000. Within the space of 1 to 3 decades in several countries, hip fracture incidence rates have risen dramatically, constituting an increasing health problem. As in the rest of the world, osteoporosis is under-diagnosed and under-treated in Asia.
FRAX is a computer-based tool which estimates the 10-year probability of major and hip fractures using easily obtained clinical risk factors, with an option to input femoral neck bone mineral density to enhance fracture risk prediction. The recent data that relates high fracture probability with FRAX to densitometric osteoporosis may have important clinical implications in Asia where, except in a few countries, access to bone densitometers is limited.
The FRAX model has been calibrated based on fracture and mortality data for China, Hong Kong, Japan, Philippines, Singapore, South Korea, Sri Lanka, Taiwan, Jordan and Lebanon, and is a work in progress. Unfortunately, there is a lack of epidemiological data from other Asian countries to enable wider calibration. Although using available FRAX models in countries without fracture data may not be suitable, it may be possible to extrapolate fracture risk in neighbouring countries with similar ethnicities and levels of development. Country-specific threshold risk levels for intervention have yet to be established in Asia as health care systems, reimbursement and wealth differ between countries and from the West.
Despite such limitations, with increasing connectivity and easy accessibility, FRAX may prove a useful tool in addressing osteoporosis in Asia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### S3.3

**Beyond FRAXÒ**
John A. Eisman
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The FRAXÒ algorithm represented a major step forward in absolute fracture risk assessment. It took advantage of globally derived information to predict fracture risk that used that as the basis for rational treatment recommendations for a large proportion of the international community. The extrapolation of FRAXÒ to different regional and geographic criteria has been based on two sets of data that can be obtained with relatively high accuracy; these being regional age-adjusted hip fracture and mortality rates. Other fracture risk calculators that have been proposed and validated in other cohorts, including the Garvan FractureRiskCalculator that we developed from the Dubbo Osteoporosis Epidemiology Study data. This differs from FRAXÒ in that it predicts hip fractures and a range of other fragility fractures as outcomes and includes falls risk as an important fracture risk factor. However it does not include other clinical risks that are included in the FRAXÒ algorithm, including diet, smoking and glucocorticoid use amongst others. Since fragility fractures are associated with premature mortality, our work has been focussing on considering the combined risk of initial or recurrent fracture and premature mortality together. As only individuals who are at risk of fracture or other adverse events can benefit from any intervention, identifying individuals at high risk of such 'adverse outcomes' will underpin rational decisions for intervention. Another other major step forward that is yet to take place is randomised controlled interventional studies of novel agents being based on selection of individuals at high absolute risk. This next stage of the validation of treatments based on absolute fracture risk assessment is critical. The future involves more accurate prediction of risk and then selection amongst treatments, validated in specific studies based on such risk selection. This would provide a rational basis for cost-effective osteoporosis risk management.

## The gut nutrient sensing in energy metabolism

### S4.1

**Bile acid metabolism for the control of metabolic disease**
M. Watanabe
School of Medicine, Keio University, Tokyo, Japan.

Over the 5 years, the field of bile acid (BA) research has undergone a considerable evolution. Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, it has recently become clear that BAs are also biological signaling molecules. For instance, BAs were shown to be natural ligands that activate FXR, which controls both the synthesis and enterohepatic recycling of BAs. We have shown that the FXR-mediated SHP induction attenuates the capacity of LXR and LRH-1 to induce the expression of SREBP-1c, explaining the inhibition of hepatic fatty acid and triglyceride biosynthesis and VLDL production by BA administration.
BAs may also signal in peripheral tissues through a newly-identified pathway involving the binding and activation of GPCR, TGR5. The subsequent activation of type 2 iodothyronine deiodinase (D2), the enzyme that converts inactive thyroxine into active 3,5,3′-triiodothyronine and hence determines thyroid hormone receptor saturation in cells, and of PGC-1 then stimulates energy expenditure in brown adipose tissue (BAT) and skeletal muscle. Thus, activation of this pathway explains how administration of BAs to mouse models of 'diabesity' induces weight loss and insulin sensitization. These observations build a strong case that BAs have effects beyond the strict control of BA homeostasis and function as general metabolic integrators.
Bile acid-binding resins (BABRs) are effective drugs for lowering LDL-cholesterol and the primary prevention of coronary heart disease. BABRs absorb BAs in the intestine thereby preventing their uptake in the ileum and interrupting their enterohepatic cycling. We characterize in detail the molecular and functional impact of BABR on metabolic homeostasis in mouse and hamster models and

human subjects with the metabolic syndrome. Interestingly, BABR are not only shown to reduce cholesterol levels but also to decrease body weight and to improve glucose tolerance, qualifying them as ideal agents to treat the metabolic syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S4.2

**Taste receptors in gut regulate endocrine function**
R. Margolskee
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We have found that many of the receptors and downstream signalling elements involved in taste detection and transduction are expressed also in intestinal hormone producing (endocrine) cells where they underlie key chemosensory functions of the gut. In one example of gastrointestinal chemosensation it is known that glucose given orally, but not systemically, induces secretion of the 'incretin' hormone glucagon like peptide-1 (GLP-1), which in turn regulates insulin secretion and glucose homeostasis. We have found that intestinal endocrine cells express sweet taste receptors, the taste G-protein gustducin, and several other taste transduction elements. Knockout mice lacking gustducin or the sweet taste receptor subunit T1R3 have deficiencies in secretion of GLP-1 and in the regulation of plasma levels of insulin and glucose. We have studied intestinal cell lines that express gustducin, taste receptors and other taste signaling elements to identify the roles of these taste proteins in regulating GLP-1 hormone release. In another example of gastrointestinal chemosensation we have found that endocrine cells of the pancreas express multiple taste proteins that are involved in regulating insulin release. Furthermore, taste cells of the oral cavity express GLP-1, other 'gut' hormones and the insulin receptor. Most recently, we have identified intestinal-type glucose transporters and pancreatic-type ATP-gated K+ channels (K-ATP metabolic sensors) as being present in taste cells and potentially functioning in the detection of the sweet taste of sugars. In sum these studies point out similarities in gustation and gut chemosensation and indicate the importance of 'taste cells of the gut' and 'endocrine cells of the tongue' in coordinating the body's hormone responses to regulate glucose homeostasis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S4.3

**Gut microbiome and energy metabolism: the concept of MicrObesity**
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Obesity, type-2 diabetes and low-grade inflammation are becoming worldwide epidemic. We and others have provided evidence that the gut microbiota contribute to the control of energy homeostasis. Therefore, we have defined a novel concept, that we called the 'MicrObesity' (microbes and obesity), devoted to decipher the specific role dysbiosis and its impact on host metabolism and energy storage.
Over the last 15 years, our work has been devoted to examine the way by which the bacteria present in the gut interact with nutrients and host biology to control obesity and associated disorders, including diabetes, inflammation and liver diseases. Recently, we discovered that the gut microbiota contribute to the development of the insulin resistance and the low grade inflammation characterizing obesity. We described the concept of metabolic endotoxemia (increase in plasma LPS levels) as triggering factor in the development of the metabolic alterations associated with obesity.
Following this discovery, we found that the major factor involved in the development of metabolic endotoxemia observed upon obesity is related to the gut barrier function. For instance, we found that both nutritional and genetic obesity are associated with an increased gut permeability leading to the leakage of LPS and possibly other microbiota derived factors. Although the clear mechanisms involved in the bacteria-host interactions are still under investigation, we found that the gut microbiota control enteroendocrine functions such as L-cells (producing GLP-1 and GLP-2) number and differentiation, the endocannabinoid system tone but also leptin sensitivity.
We found that selective changes in the gut microbiota composition by using prebiotics reduce metabolic endotoxemia and gut permeability via different mechanisms (e.g. GLP-2, endocannabinoid system).
Taken together, the compelling data currently published suggest that specific changes in the gut microbiota could be promoted to counteract fat mass development, diabetes and the low levels of inflammation associated with obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Transition from paediatric to adult care - do we have progress?

## S5.1

**GH use in Prader–Willi syndrome (PWS): an evidence-based approach to a clinical practice guideline (CPG), on behalf of the GH Research Society (GRS) and Workshop Participants**
C. Deal
Canada.

GH therapy in children with PWS was approved in the US in 2000, based on short term growth data, and in Europe in 2006, based on growth and body composition data. GH therapy in PWS has been used by the medical community and advocated by parental support groups since this time. The advent of GH therapy for individuals with PWS represents a unique therapeutic challenge with its intersection of GH treatment for individuals with cognitive disability, varied therapeutic goals that are not focused only on increased height, and potential association with life-threatening adverse events. Given the availability of new results regarding efficacy and safety in infants, children, and adults with PWS from randomised controlled trials as well as from a recent Cochrane review, a CPG Workshop sponsored by the GRS systematically reviewed the literature, graded the available evidence and provided concise recommendations for the use of GH in this context. Forty-three experts (pediatric and adult endocrinologists, clinical and basic geneticists, epidemiologists, a nutrition specialist, an orthopaedic surgeon, a psychiatrist, health technology assessment specialists, a bioethicist, health economist, and a patient advocate) participated. The 4-day workshop followed the CPG development recommendations outlined by the AGREE Collaboration (www.agreetrust.org/). Strength of evidence was graded using the EVIDEM Quality Assessment instrument, using questions organized into 8 domains including study population, intervention and comparators, outcomes measures, design, adverse events ascertainment, time horizon, types of analyses and results validity and relevance (www.evidem.org/praderwilli). For clinical evidence in the pediatric population, randomized controlled trials (RCTs), comparative observational studies and long term studies ($>3.5$ years) were included. Adult studies included RCTs of GH treatment for $\geq 6$ months and uncontrolled trials, since data was more limited. Safety data was obtained from pharmaceutical registries (Phase 4) and clinical trials (Phase 3). Data on disease, therapeutic context, and economic, ethical and societal aspects were assessed to reflect the international context. This presentation will review the data discussed in the Workshop and present recommendations for GH treatment of individuals with PWS. We will also address a process for obtaining informed consent/assent of patients with PWS and/or their families.

## S5.2

**Fertility options for adults with panhypopituitarism**
Margaret E. Wierman
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Hypopituitarism and impaired fertility in adulthood may result from congenital genetic defects detected during childhood or acquired pituitary dysfunction after apoplexy or resection of a pituitary tumor or following radiation therapy. This lecture will review the fertility options for hypopituitary patients, success rates and predictors of outcome for conception and live births. In those with normal pituitary function planning surgery and/or radiation to the hypothalamic-pituitary axis, banking of sperm, oocytes or embryos are newer options to preserve future fertility options. In those with partial or complete pituitary hormonal deficiencies, optimization of thyroid, adrenal and perhaps growth hormone function must be obtained before fertility options are discussed. In men, induction of spermatogenesis is usually achieved with intermittent human chorionic gonadotropin (hCG) alone, or in combination with recombinant follicle stimulating hormone (FSH), or

human menopausal gonadotropins. Size of the testes at baseline predicts success, as therapy must restore or recapitulate the pubertal process to achieve normal spermatogenesis. In women with partial gonadotropin deficiency, induction of ovulation with clomiphene citrate may be an option. For most hypopit women with hypogonadotropic hypogonadism, therapeutic options include recombinant FSH with hCG alone or in combination with intrauterine insemination, or *in vitro* fertilization. Despite the expansion of treatment options, decreased response to ovulation induction, decreased live births, increased small for gestational age infants and peripartum complications have been reported in women with hypopituitarism. Further research is needed to plan future options for fertility in those pituitary patients undergoing treatment that will impair reproductive function, and to optimize the treatment options and success rates for patients with hypopituitarism desiring fertility.

## S5.3

**Sex steroid replacement in young males and females**

G. Conway
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Once induction of puberty has been completed the options for sex steroid hormone replacement change to take in to account long term health outcomes and the issues of transition from pediatric to adult care. With regard to the late stages of development, some thought has to be given to the fact that bone mass continues to increase to reach a peak at about the age of 25. It may not be until the age of 18 that meaningful bone density results can be obtained. For those who are slow to reach peak bone mass there might be a benefit to running generous dosing levels for some years.

For many young adults, compliance with treatment can be a problem with some passing through a time of rejection of medical care. The health care team should be alert to the problems of transition and the high rate of drop out from clinic for young adults. With regard to sex steroid replacement, regimes can be adjusted to find one that is most acceptable for the individual. Involving the patient in the process of choice with education may require extending clinic time and a good relationship with nursing staff.

As young adults hypogonadal patients will take stock of their success or otherwise of pubertal development and many express concern about some aspect of physical development or self-confidence in relationships. If these points are not volunteered then skills in eliciting a frank exploration of these psychological issues will often be required. Males in particular may feel that their hypogonadism makes the common sexual insecurities of this age group much more profound.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Subclinical adrenal diseases

## S6.1

**Diagnosis and treatment of subclinical Cushing's syndrome (SCS)**

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SCS is defined as an adrenal tumor (usually adenoma) with autonomous cortisol secretion and no overt symptoms of Cushing's syndrome. SCS has attracted much attention because it often is masked by lifestyle-related diseases such as diabetes mellitus (DM), metabolic syndrome (MS) and hypertension. Because of the broad variation in autonomous cortisol secretion among patients, diagnostic criteria for SCS have not been fully established. Furthermore, the cut-off values of the dexamethasone suppression test (DST) differ among current criteria. Diagnostic problems also arise because of differences in sensitivity among assay kits for the measurement of low serum cortisol levels. We have tried to establish diagnostic criteria for SCS based on the 1-mg DST cut-off value of serum cortisol of $>1.8$ μg/dl by analyzing 118 patients with adrenal incidentaloma. For the diagnosis of SCS, we finally selected two additional values (basal adrenocorticotropic hormone (ACTH) $<10$ pg/ml and serum cortisol $\geq 5$ μg/dl at 23:00 h) from several parameters, because these values showed stronger associations with the results of the 1-mg DST than the other parameters. In some exceptional cases with basal ACTH $\geq 10$ pg/ml, the poor response of ACTH to corticotropin-releasing hormone was equally important for accurate diagnosis. Patients who met these criteria had a significantly higher frequency of IGT/DM than did patients who did not meet these criteria. Improvements in metabolic parameters have been reported after removing the adrenal tumor in patients with SCS. However, it is sometimes a challenge to select operation for individuals with SCS, particularly for asymptomatic individuals showing no significant objective abnormalities. To overcome this problem, some reports have investigated the hormonal conditions of patients with SCS who showed improvements in metabolic profiles following the removal of the adrenal tumor.

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## S6.2

Abstract unavailable.

## S6.3

**Subclinical Addison's disease**

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Autoimmune Addison's disease (AAD) occurs in approximately 1:8000 individuals. Its autoimmune pathogenesis is made evident by the presence of circulating 21-hydroxylase autoantibodies. A recent international program aimed at standardizing adrenal autoantibody measure to optimize both the etiological classification of the disease and the identification of at-risk subjects. Approximately 1% of subjects with autoimmune endocrine diseases and 5% of women with primary ovarian insufficiency are positive for 21OHAb, not necessarily in association with clinical symptoms of adrenal insufficiency. Detection of adrenal autoantibodies in subjects with no clinical signs of adrenal insufficiency defines the so-called subclinical AAD. The overall risk for future development of AAD in 21OHAb-positive individuals is as high as 40% at 10 years. The risk can be stratified on the basis of hormonal parameters, such as basal aldosterone, plasmatic renin activity, basal and ACTH-stimulated serum cortisol and basal plasma ACTH. A subnormal response to the Synacthen test and an increased plasma ACTH mark the irreversible phase of the disease, that evolves towards clinical AAD in over 90% of cases. On the other hand, only a minorityy of cases with normal ACTH levels and normal cortisol increase after the ACTH test progresses towards clinical AAD. Additional risk factors are male gender and high autoantibody titer, while available genetic markers are poor predictors of progression towards the clinical form of the disease. During the last years, studies have addressed the problem of the optimization of the Synacthen test, as the classical stimulation with 250 μg co-syntropin is largely supraphysiological. The low-dose test, performed with 1 μg of synthetic ACTH, is able to accurately discriminate subjects with subnormal cortisol response. An ongoing European study is testing the predictive value of a very low-dose test, performed using 0.5 μg of synthetic ACTH.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Hormones, metabolism and cancer: more than coincidences?

## S7.1

**An epigenetic switch linking inflammation to cancer and the use of metformin as an anti-cancer drug**

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Transient activation of Src oncoprotein can mediate an epigenetic switch from immortalized breast cells to a stably transformed line that forms self-renewing mammospheres that contain cancer stem cells (CSCs). Src activation triggers an

inflammatory response mediated by NF-kB that directly activates Lin28 transcription and rapidly reduces the level of let-7 microRNAs. Let-7 inhibits IL6 expression, and IL6 activates STAT3. Importantly, IL6 activates NF-kB, thereby completing a positive feedback loop. Furthermore, STAT3 activates transcription of the miR-21 and miR-181b microRNAs, and each of these is sufficient to activate the epigenetic switch. This positive feedback loop is enhanced in CSCs as compared to non-stem cancer cells in the same population. Thus, inflammation activates a positive feedback loop that involves NF-kB, Lin28, Let-7, IL6, STAT3, miR-21, and miR-181b that maintains the epigenetic transformed state for many generations in the absence of the inducing signal.
The cancer stem cell (CSC) hypothesis suggests that, unlike most cells within a tumor, CSCs resist chemotherapeutic drugs and can regenerate the various cell types in the tumor, thereby causing relapse of the disease. Relatively low doses of metformin, a standard diabetes drug, inhibits transformation and selectively kills breast CSCs. The combination of metformin and doxorubicin kills both CSCs and non-stem cancer cells in culture, and it prevents relapse much more effectively than either drug alone in mouse xenografts. Metformin is equally effective combined with other chemotherapeutic agents, and with a 4-fold reduced dose of doxorubicin. The combination of metformin and doxorubicin prevents relapse in xenografts involving cancer cell lines from several, but not all, developmental lineages. Metformin appears to inhibit the inflammatory pathway. These results support the CSC hypothesis, and they provide a rationale and experimental basis for using the combination of metformin and chemotherapeutic drugs to improve treatment of patients with breast (and possibly other) cancers.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S7.2

### The insulin-like growth factor system and cancer: what are the implications?

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Insulin and the IGFs, the IGFBPs and the insulin and IGF-1 receptors play an important role in cancer progression. Higher levels of circulating total IGF-1 is often associated with an increased risk of developing cancer. Furthermore, the IGF-1 receptor (IGF-1R) is often overexpressed in tumor tissues. This overexpression has been shown to be due to mutations in tumor suppressor genes such as p53, WT1 and BRCA genes. Inhibition of the IGF-1R in culture and preclinical studies led to the development of humanized antibodies and tyrosine kinase inhibitors for human trials as adjunct therapy for chemotherapy. Thus far human trials have been less impressive than anticipated and suggest compensation by other tyrosine kinase receptors including the IR.
Obesity, metabolic syndrome and early type 2 diabetics have hyperinsulinemia that is associated with increased cancer risk and cancer-related mortality and studies both in mouse models and humans have suggested that hyperinsulinemia via the IR-A receptor on breast cancer is mitogenic, and suggests other therapeutic options.
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## S7.3

### Obesity and cancer

A. Renehan
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By 2008, we[1] and others had established that excess body mass index (BMI), as an approximation of general body adiposity and excess calorie intake, is associated with increased risk of several cancer types. These associations exist for both common (colon, endometrial, post-menopausal breast cancers, oesophageal adenocarcinoma) and less common malignancies (thyroid cancer, non-Hodgkin lymphoma). Given the plausibility of the biological explanations (including insulin/IGF-I; sex steroids; and adipokines/inflammatory cytokines), the consistency of associations, the sufficiently long latency times between BMI measurement and cancer occurrence, and the recent demonstrations of risk reversibility in morbidly obese cohorts undergoing bariatric surgery, many of these associations are probably causal. Approximately 124 000 new cancer cases may be attributable to excess BMI in Europe (2008). Since 2008, it has become clear that associations between BMI and cancer risk may be modified in the presence of other risk factors. For example, in users of hormonal replacement therapy, the associations between BMI and endometrial and post-menopausal breast cancers are attenuated. In turn, these observations point to a strong influence of oestrogen as an intermediary between obesity and cancer development in these cancer groups. Two areas of recent research will be highlighted: (i) it is hypothesised that approximations of central adiposity, such as waist circumference (WC), may better describe associations between adiposity and increased cancer risk, but updated standardised analyses do not fully support this, suggesting a refinement to the proposed mechanistic role of insulin resistance in carcinogenesis; and (ii) studies of the impact of BMI on cancer-specific mortality in patients with a diagnosis of an obesity-related cancer do not necessarily mirror relationships seen for incident risk. These observations suggest that obesity may act through pathways, which in turn, are prognostic pathways for certain cancers. Better understanding of these associations will facilitate refinements of approaches to prevent obesity-related cancers.
1. Renehan *et al. Lancet* 2008 **371** (9612) 569–78.
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# Global phenotypes of endocrine disease

## S8.1

Abstract unavailable.

## S8.2

### Ethnic specific PCOS

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PCOS is the commonest endocrine disorder of young women. It has appreciable impact on wellbeing and quality of life, fertility and reproduction, and long term metabolic and cancer risks. Acknowledged a 'condition of our times', there was no data from Asia until recently. South Asians have early manifestation with more severe symptoms than white Europeans. Young affected Asians have significantly greater insulin resistance than older white Europeans; with approximately a third having the metabolic syndrome. Central obesity, hypertension and abnormal lipids bear greater significance than BMI; a new dimension to the South Asian phenotype. This highlights their propensity to central fat accumulation. Advancing age impacts significantly on metabolic problems, while gestational diabetes is closely interwoven. Studies in China and Thailand also demonstrate ethnic variation with less overt hirsutism.
Country specific epidemiological data reveals substantial intra-regional variation in the Asian phenotype, particularly the BMI and degree of hyperandrogenism. There is also a notable 'culture of silence' among young pre-marital rural Asian women with PCOS; with diminution of quality of life linked to hirsutism rather than obesity that differs from western women. This highlights socio-cultural determinants in differing ethnic groups. The metabolic problems also reveal the need for a life cycle approach. Screening for diabetes pre-conception, weight maintenance through health promotion are simple low cost interventions that require adoption in low income countries.
To study ethnic variations requires evaluation of epidemiological data based on the geographic location of affected women. This warrants a systematic review of population based data or of large clinic databases to ascertain racial and cultural determinants of manifestations of PCOS. In view of its heterogeneous phenotype, an appraisal of possible link(s) between Androgenic, Reproductive, Metabolic phenotypes among different ethnic groups is also required. The 3rd ESHRE/ARSM consensus statement on PCOS (2011) addresses ethnic variation of PCOS.
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## S8.3

**Obesity and diabetes in Asian Indians**

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According to the recent Diabetes Atlas published by the international diabetes federation, globally there are 366 million people with diabetes. The number projected for India was 61.3 million people with diabetes. The recent Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) study which is one the first of its kind to provide accurate and comprehensive state and national level data on diabetes prevalence in India, confirmed that there are 62.4 million people with diabetes in India. Moreover, the study showed that there are 77.2 million people with pre-diabetes, an earlier stage of diabetes, a large percentage of whom will convert to diabetes in the near future.

Obesity is a global health problem and India is facing a rising epidemic of obesity. The prevalence of generalized obesity (as defined by BMI $\geq$25 kg/m$^2$) and abdominal obesity (as defined by waist circumference $\geq$90 cm in men and $\geq$80 cm in women) in the INDIAB study were 21% and 24.8% respectively. Combined obesity (generalized plus abdominal) was present in 28.6% of the population, which translates to 199 million people with obesity in India. The combined diabetes and obesity epidemic is referred to as 'diabesity'. Diabesity in Indians are increased insulin resistance, stronger genetic factors and environmental factors particularly associated with epidemiological transition due to rapid urbanization, industrialization and demographic transition leading to increasing income levels and all of which resulted in altered lifestyle today. Appropriate nutrition measures will help in reducing the risk of not only diabetes and obesity, but also hypertension, dyslipidaemia and hyperinsulinaemia. Thus, effective preventive programmes need to be urgently implemented to tackle the diabesity epidemic threatening the country.

# The risk and benefits of tight glycaemic control

## S9.1

**What do type 2 diabetes outcome trials tell us about the CV risks and benefits of intensive glycaemic control**

Hertzel C. Gerstein

Diabetes is a strong risk factor for cardiovascular (CV) events, and large epidemiologic studies have repeatedly shown that higher fasting or post-load glucose levels predict a higher incidence of CV events regardless of whether or not diabetes is present. In the UKPDS, targeting near-normal fasting glucose levels in newly diagnosed diabetes with a strategy starting with basal insulin or sulfonylurea reduced the 20-year risk of myocardial infarction and mortality by 15% and 13%, respectively. However, this strategy did not maintain stable glucose levels, possibly because it was reactive in nature.

In more recent large clinical trials of different degrees of glucose-lowering in people with advanced type 2 diabetes, stable glucose levels were achieved using a proactive strategy but with more mixed effects on CV outcomes. Indeed, a recent meta-analysis of the major CV outcomes trials in 27 049 people with type 2 diabetes including 5-year data from the UKPDS, the ACCORD trial, VA Diabetes Trial and the ADVANCE trial reported that a strategy of intensive control (which was largely based on insulin therapy) vs conventional glycaemic control reduced the composite CV outcome of myocardial infarction, stroke or cardiovascular death by 9% (95% CI 1–16%), with most of the effect attributable to a 15% reduction in myocardial infarction and no effect on CV death or stroke[1]. However, there was evidence of a mixed effect on mortality with higher deaths in the ACCORD trial.

In addition to these trials of glucose-lowering degrees (i.e. more vs less) several other trials of different glucose-lowering strategies have also been reported or are underway[2]. These include the recently published BARI 2D trial showed that in patients receiving medical therapy for documented coronary artery disease, a strategy of glucose lowering based on insulin-providing therapies had CV effects that were similar to those observed with a strategy based on insulin-sensitizing therapies[3]. To date no clearly effective strategy has been identified however other trials are underway[4].

1. Turnbull FM *et al. Diabetologia* 2009 **52** 2288–98.
2. Gerstein HC. N*at Rev Endocrinol* 2009 **5** 270–275.
3. BARI 2D Study Group. *N Engl J Med* 2009 **360** 2503–15.
4. Origin Trial Investigators. *Am Heart J* 2008 **155** 26–32.

## S9.2

Abstract unavailable.

## S9.3

**Time course of the glucose legacy effect**

R. Holman
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During the 10-year UK Prospective Diabetes Study (UKPDS) post-trial monitoring period, sustained reductions were seen in the risks of diabetic complications, despite the previously randomised conventional and intensive treatment groups becoming identical with respect to median HbA1c values and glucose-lowering therapies. This continuing benefit of an earlier intervention in people with type 2 diabetes has been termed the 'legacy effect'. A similar phenomenon seen in people with type 1 diabetes has been termed the 'metabolic memory'. At the present time, neither the precise mechanism which produces the legacy effect nor the time course over which it operates have been established.

Analyses have been conducted to examine the degree to which the UKPDS glucose legacy effect can be attributed to prolonged benefits of HbA1c levels achieved in each of the previous years since diagnosis of diabetes. The impact of each annual HbA1c value on the future risk of death or myocardial infarction has been estimated, assuming the risk at every moment depended on the contributions from all previous moments.

Major effects of improved glycaemic control are seen 5–10 years later, explaining in part the sustained reductions in risk of death and myocardial infarction seen in the UKPDS post-trial monitoring period.

Declaration of interest

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# Thyroid: From fetal life to adulthood

## S10.1

**Placental transport of thyroid hormone**

J. Franklyn, S. Chan, E. Vasilopolou & M. Kilby
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The transplacental passage of thyroid hormones (TH) from the maternal to fetal circulations is important for normal fetal development, particularly the fetal central nervous system. This is particularly so before the onset of endogenous fetal TH production from the second trimester of pregnancy. The human hemochorial placenta regulates the quantity of TH passing through and the complement of the different forms of TH to ensure requisite levels are present in the fetus for each stage of development. Transplacental TH supply to the fetus is modulated by several factors including plasma membrane TH transporters which regulate the passage of TH in and out of cells, the metabolism of TH by iodothyronine deiodinases, as well as TH binding to several different proteins within placental cells themselves. In pathological situations of either maternal or fetal TH deficiency during pregnancy, the placenta appears to lack the full compensatory mechanisms required to optimize maternal-fetal transfer of TH to achieve normal fetal TH levels, and this may contribute to the development of neurodevelopmental delay associated with such conditions. Thus, maintaining normal maternal TH status is likely to be the primary factor in ensuring adequate transplacental TH passage and iodide supply to the fetus.

Declaration of interest

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## S10.2

Abstract unavailable.

## S10.3

**Thyroid hormones and fetal neurological development**

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The importance of thyroid hormones in neurological development has been well documented in both animals and humans. Thyroid hormones deficit during fetal development can have irreversible adverse effects on an individual's neurological function; including mental retardation, deafness, and spasticity. The only source of thyroid hormones for the developing brain in early pregnancy is from the mother. There is a growing body of epidemiological and experimental evidence to suggest that even mild maternal hypothyroxinemia may lead to abnormalities in fetal neurological development. The fetal thyroid gland reaches maturity by about week 10–12 gestation and begins to produce thyroid hormones at around the 16th week of gestation. Fetal production of thyroid hormones becomes increasingly important in the second half of pregnancy. The molecular and cellular mechanisms by which thyroid hormones affect fetal neurological development are still not well understood. Evidence of the interaction between thyroid hormones and brain development so far has primarily come from animal experiments. Thyroid hormones play an important role in regulating gene expression of myelination and cell differentiation in rats. Severe iodine deficiency (median urinary iodine concentration $<20$ μg/l), which causes both maternal and fetal hypothyroidism, is the most common cause of mental retardation. Moderate iodine deficiency (median urinary iodine concentration 20–49 μg/l) may also be associated with increased risk of impaired fetal neurological development. Future studies are needed to improve the understanding of human iodine nutrition during pregnancy, maternal and fetal thyroid function and mechanisms of fetal neurological development.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Novel genetic and endocrine insights in the Klinefelter's syndrome

## S11.1

**Klinefelter mouse (41XXY): model for human Klinefelter syndrome**

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[1]LABiomed at Harbor-UCLA Medical Center, Torrance, California, USA; [2]Woolcock Research Institute University of Sydney, Sydney, New South Wales, Australia; [3]Hospital for Children and Adolescents University of Helskini, Helskini, Finland.

Klinefelter syndrome (47XXY male) (KS) is the most common sex chromosome disorder in men. The phenotype is diverse including: infertility, hypogonadism, impaired cognition, behavior disorders, increased autoimmunity and osteopenia. We have studied osteopenia, learning dysfunction, germ cell loss, and testosterone deficiency in 41 XXY mice bred by two separate methods. We have characterized the germ cell loss by apoptosis and germ cell migration as the mice progress from day 1 to 10 days of age and subsequently into adulthood. These studies confirm the phenotypic similarities of XXY mice to human KS. Mice have low testicular venous and peripheral blood levels of testosterone (with elevated LH and FSH levels) yet have normal to elevated intratesticular testosterone levels. This suggests a testosterone sequestration effect within the testes. The ability of the testes to synthesize testosterone is present and augmented by response to LH in Leydig cells and in whole testis in culture. Germ cell transplants from XY mice to XXY mice shows seeding of the GFP labeled XY donor germ cells into 41XXY immature and adult recipients with progression to spermatocyte or spermatid germ cells. While overexpression of non-X inactivated genes is believed to be a likely molecular basis for the disorder, the reasons for phenotypic heterogeneity are only partially understood.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S11.2

**Endocrine changes in KS and the effects on growth, bone and body composition**

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Klinefelter syndrome (KS; 47,XXY) is the most common sex chromosome disorder in man, affecting approximately 1:660 men, and is a common cause of infertility. Patients with KS are characterized by tall stature and progressive testicular failure. This results in azoospermia and androgen deficiency with an accompanying risk of developing several phenotypic characteristics in adulthood, e.g., osteopenia or even frank osteoporosis, and metabolic syndrome.

Recent studies on Klinefelter infants during the so-called minipuberty have indicated that serum testosterone (T) concentrations are low or low- normal. Many adults with Klinefelter syndrome have subnormal serum concentrations of total T and the majority of patients have T in the lower half of the normal range, as well as elevated LH. A bivariate LH-T comparison reveals an abnormal LH-T setpoint suggestive of abnormal Leydig cell function.

Sertoli cell function is not impaired in KS patients until puberty, as reflected by normal AMH and inhibin B concentrations in KS boys during infancy and childhood. However, in mid- to late puberty, Sertoli cell function deteriorates progressively, resulting in extremely low or undetectable AMH and inhibin B concentrations, very high FSH levels and small testicular volumes.

Accordingly, patients with Klinefelter syndrome are at increased risk of developing osteopenia or osteoporosis already in young adulthood, whereas normal bone mineralization during childhood and adolescence has been reported. Recent studies showed an increased incidence of an unfavourable change in body composition; with accumulation of body fat and decreased muscle mass already in prepubertal boys with KS. In addition, adult patients have an increased risk of diabetes with decreased insulin sensitivity. It is not known to which degree these symptoms are the result of additional copies of non-inactivated genes on the supernumerary X chromosomes, lifestyle factors, or whether they are partly (or completely) the result of an impaired pituitary-gonadal function.

Special efforts to detect this under-diagnosed chromosome disorder are necessary in order to initiate early androgen therapy, and to prevent the development of a deleterious body composition with the risk of metabolic syndrome.

Declaration of interest
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## S11.3

**The Muenster EXAKT project: epigenetic regulation of the supernumerary X chromosome and its escapee genes**

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Klinefelter syndrome (47,XXY; KS) is a very common chromosomal disorder, affecting 1:500 men and leading to hypergonadotropic hypogonadism as well as an increased incidence of metabolic syndrome. However, our knowledge on the functional role of the supernumerary X chromosome itself and to which extent its origin contributes to the observed pathophysiology is still very limited. Recently we started the EXAKT (Epigenetics, X-Chromosomal features and clinical Applications in Klinefelter syndrome Trial) project which is a Muenster-based prospective study involving Klinefelter patients ($n=130$), and their parents assessing a wide area of biochemical, physiological and genetic parameters in comparison to age-matched healthy male and female controls ($2\times n=50$).

The aim of the genetic and epigenetic part of the EXAKT project is to obtain information on the paternal or maternal origin and the meiotic disjunction events leading to the presence of a supernumerary X chromosome, the inactivation of the second X-chromosome by the non-coding RNA XIST and the expression of X-linked genes which escape X inactivation.

Determination of the origin of the X chromosome by microsatellite analysis in KS and their parents revealed a nearly equal distribution between the paternal (56%)

and maternal (44%) origin of the second X chromosome. Methylation analysis of the XIST promoter displayed a significant higher methylation pattern in KS patients (75%) when compared to women (65%). Moreover analysis of escape genes such as KDM6a in blood RNA samples of KS revealed expression levels comparable to levels detected in women.
The first genetic and epigenetic analyses of KS within the EXAKT project revealed a significantly altered inactivation status and an expression of escapee genes reminiscent of women, indicating a pathophysiological role of the supernumerary X chromosome.
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## Determinants of peak bone mass

### S12.1

**Epidemiology of peak bone mass, structure and strength in males as assessed by high resolution peripheral quantitative CT (HR-pQCT). A cross-sectional study**

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In males, peak bone mass as evaluated by DXA is reached at the age of 20–22 years and may be important for the risk of fracture later in life. DXA, however, only provides two-dimensional images and does not allow assessment of bone structure. We used HR-pQCT to assess trabecular and cortical volumetric BMD (vBMD) and bone microarchitecture at the distal radius and tibia in a population based study including healthy male volunteers. Moreover, bone strength was estimated using finite element analysis. Subjects treated with drugs or diagnosed with conditions affecting bone metabolism were excluded. A total of 247 men aged 20–80 years were included. DXA at the lumbar spine and total hip (Hologic Discovery, US) and HR-pQCT at the non-dominant distal radius and tibia (Scanco Medical, Switzerland) were performed.
Assessed by DXA, areal BMD at the lumbar spine was highest at the age of 35 and had peaked by the age of 20 years at the total hip. Trabecular vBMD in radius and tibia peaked around the age of 30 and 25 years, respectively. Trabecular number in radius had peaked by the age of 20 and peaked around the age of 30 years in tibia. Trabecular thickness in both radius and tibia had peaked by the age of 20 years. Cortical thickness in radius and tibia peaked before the age of 20 and around the age of 40 years, respectively. From the time of peak (or age 20) to the age of 80, pronounced changes in both radius and tibia were seen in trabecular thickness ($-1.4$ and $-1.6$ S.D., respectively) and cortical thickness ($-0.6$ and $-0.9$ S.D., respectively). Data on estimated bone strength will be presented.
In conclusion, bone mass, structure and strength peak at different ages. With age detrimental effects on microarchitecture were most pronounced for trabecular thickness and cortical thickness.
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### S12.2

**The contribution of genetic factors to bone mass**

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An large number of clinical entities are associated with abnormal bone mineral density. On one end of the spectrum, the sclerosing bone dysplasias are characterized by an excess of bone tissue. These conditions are, in most cases, monogenic diseases with involvement of one gene resulting in a clear mode of inheritance. Molecular genetic studies over the last decennia revealed the underlying genetic causes for many of them. The pathogenic mechanism can either be a decreased bone resorption, as in different forms of osteopetrosis, or an increased bone formation. The latter cases were for example very instrumental in illustrating the essential role of canonical Wnt signaling in the process of bone formation.
On the other end of the spectrum osteoporosis is the best known example of a condition with decreased bone mass resulting in an increased risk for low trauma fractures in the elderly. The risk for osteoporosis is influenced by both environmental and genetic factors and is determined both by the peak bone mass reached at young age and by the subsequent bone loss later on in life. Especially genome wide association studies in the last years have been very useful for dissecting the genetic basis for the regulation of bone mineral density. However, despite extremely large scale studies by very extended consortia, the obtained results so far can explain only a small percentage of the assumed genetic variability and further studies are needed. But these genome wide association studies definitely contributed to our current understanding of bone homeostasis by the identification of novel genes of relevance, as was the case by the study of monogenic sclerosing bone dysplasias.
Declaration of interest
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### S12.3

**Estrogens as regulators of bone growth and bone mass**

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Estrogens regulate bone growth and bone mass mainly via estrogen receptor-α (ERα). In addition, the anabolic effect of mechanical loading on cortical bone requires ERα. This lecture summarizes some recent findings characterizing the primary target cells and the crucial ERα domains for the mediation of these estrogenic effects on the skeleton.
Mice with complete inactivation of ERα continue to grow throughout life associated with increased growth plate height, resembling the longitudinal bone growth phenotype of patients with inactivating mutations in ERα or aromatase. It is unknown whether the effects of estrogens on skeletal growth are mediated directly via ERs in growth plate cartilage. Using a mouse model with cartilage-specific inactivation of ERα, we found that during early sexual maturation, ERα in growth plate cartilage is not important for skeletal growth. In contrast, it is essential for high-dose estradiol to reduce the growth plate height in adult mice and for reduction of longitudinal bone growth in elderly mice.
ERα stimulates target gene transcription through two activation functions (AFs), AF-1 in the N-terminal and AF-2 in the ligand binding domain. Using domain-specific ER-inactivated mouse models, we demonstrated that ERα AF-2 is required for the estrogenic effects on all parameters evaluated, whereas the role of ERα AF-1 is tissue-specific, with a crucial role in uterus but not cortical bone, suggesting that selective ER modulators stimulating ERα with minimal activation of ERα AF-1 could retain beneficial actions in cortical bone while minimizing effects on reproductive organs. We recently demonstrated that ERα amplifies the osteogenic response to mechanical loading in a ligand-independent manner involving its AF-1 but not AF-2. Thus AF-2 but not AF-1 is required for the estradiol effect in cortical bone while ERα AF-1 but not AF-2 is required for the osteogenic loading response.
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## Novel therapies for protecting beta cell function at diagnosis of type 1 diabetes

### S13.1

Abstract unavailable.

### S13.2

Abstract unavailable.

## S13.3

Abstract unavailable.

# Wnt/Beta-catenin in pituitary development and disease

## S14.1

### β-catenin deficiency disrupts patterning of the ventral diencephalon, causing adenohypophysis over growth and dysmorphology

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β-catenin is thought to play an important, intrinsic role in early pituitary development by regulating adenohypophysis (anterior pituitary) transcription factor activity. Expression of β-catenin is detected in the adenohypophysis, rostral mesenchyme, and neural ectoderm, including the area that develops into the infundibulum and neurohypophysis, and nearby regions. The importance of β-catenin expression in the neural ectoderm and mesenchyme has not been addressed, and the precise role of β-catenin expression in the anterior pituitary is controversial. We tested the function of β-catenin using a panel of cre alleles to inactivate the gene in specific regions. Wnt1-cre knocks out β-catenin in the midbrain and migratory neural crest during head development, eliminating expression in the neural ectoderm and rostral mesenchyme. This results in dramatic expansion and dysmorphology of the adenohypophysis with no obvious effect on specification or differentiation of hormone producing cells. The organ overgrowth is attributable to induction of an abnormally large area of the oral ectoderm to become Rathke's pouch, marked by ISL1 and LHX3. Elimination of β-catenin in the neural crest shifts the expression of SIX6 and the regions of BMP and FGF signaling that emanate from the infundibulum. Lineage tracing experiments show that P0-cre is active in neural-crest derived mesenchyme and the adenohypophysis. In contrast to Wnt1-cre, P0-cre mediated deletion of β-catenin has no obvious effects on the adenohypophysis. This is surprising given the idea that β-catenin has an intrinsic role in adenohypophysis development. Our results imply that β-catenin is necessary for patterning the ventral diencephalon and underscore the significance of establishing a proper organizing center there for normal pituitary development.
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## S14.2

### Wnt/beta-catenin signalling in craniopharyngioma

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Adamantinomatous craniopharyngioma (ACP) is an epithelial intracranial lesion arising from Rathke's pouch with a median age at diagnosis of 8 years. ACP is the third most common paediatric brain tumour, accounting for approximately 5–13% of brain tumours, and the commonest pituitary tumour among children. Although histologically benign, ACP often behaves aggressively with invasion of the hypothalamus and visual pathways. Consequences associated with both the tumour and its treatment (surgery and/or radiotherapy) include obesity and subsequent Type 2 diabetes mellitus (up to two thirds of patients), learning difficulties (psychological and educational problems), visual impairment, severe multiple pituitary hormone deficiencies and irreversible diabetes insipidus in up to 95% of patients. This high morbidity bears a vast burden to patients and families as well as considerable financial costs to health services. In a significant percentage of patients, complications may result in death.
Activating mutations in the gene encoding β-catenin have been identified in ACP and recently, using a novel mouse model, we have demonstrated a causative role for mutated β-catenin and the subsequent over-activated Wnt/ β-catenin pathway in ACP tumorigenesis. We have utilised this new genetic tool further understand the aetiology and pathogenesis of human ACP, as well as to test the efficacy of small-molecule inhibitors for treatment of these tumours. Our research has revealed that pituitary progenitors/stem cells are likely to play an important role in the oncogenic process by acting as signalling centres promoting cell proliferation and survival. In addition, we have identified several genes/pathways expressed in mouse and human ACP, thus providing novel biomarkers for studying genotype/phenotype correlations. Finally, we show that inhibiting some of these pathways may deliver targeted therapies for the treatment of these devastating childhood tumours.
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## S14.3

### Wnt signalling in oestrogen-induced lactotroph proliferation

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The anterior pituitary gland displays considerable plasticity with a proliferative response to oestrogen in a number of different situations including pregnancy. The nature of pituitary remodelling to this physiological demand is not clear.
Using microarray analysis we found that oestrogen treatment of the rat *in vivo* increased expression of Wnt4 mRNA in adult rat pituitary tissue. Dual immunofluorescence analysis showed that this Wnt4 expression was not confined to lactotrophs but was expressed in all anterior pituitary cell types. Reporter gene assays for canonical Wnt signalling action on transcription showed no effect of Wnt4 or other activators in pituitary lactotrophic GH3 cells, suggesting that an alternative pathway might be in operation. No nuclear localisation of beta-catenin was detected under any conditions in either GH3 cells or normal rat anterior pituitary cells. On the other hand Wnt4 did inhibit calcium oscillations in GH3 cells, indicating that a non-canonical pathway may be activated in the mature lactotrophic cell. Immunocytochemical studies showed that Wnt4 was particularly strongly expressed in the marginal zone, a region of the pituitary containing progenitor cells expressing Sox9. Studies in normal human pituitary tissue showed higher expression of Wnt4 staining in the lateral aspects of the pituitary but no counterpart to this marginal zone expression was clearly identifiable. Wnt4 immunostaining was seen in all types of pituitary adenoma including prolactinomas and somatotroph adenomas, and was weakest in corticotroph adenomas.
In summary Wnt4, a known developmental regulator in the fetal pituitary, is increased during oestrogen-induced pituitary proliferation. It acts through non-canonical signalling pathways in the mature pituitary lactotroph. Wnt4 is expressed in a variety of different human pituitary adenomas, though its role in their pathogenesis remains unclear.
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# Adrenal cancer

## S15.1

### Molecular pathogenesis of adrenocortical cancer

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Adrenocortical carcinoma is a rare heterogeneous neoplasm with a poor prognosis. Most patients present with symptoms related to steroid hormone excess or presence of an abdominal mass, while less frequently they are diagnosed incidentally during clinical exploration for other causes. In children, adrenocortical tumors are most commonly found associated with mutations of the TP53 tumor suppressor gene and have the highest incidence in southern Brazil. Several clinical and pathological features distinguish children from adult adrenocortical tumors.
Much progress has been made recently in our understanding of the pathogenesis of adrenocortical cancer, with an important contribution from genomic studies. A relevant role is played by loss of heterozygosity of the 11p15 chromosomal region, with consequent deregulation of the expression of the IGF2 growth factor and other genes. Clinical studies and mouse models also demonstrated the important role for overexpression of the SF-1 transcription factor and beta-catenin activation in triggering tumorigenesis. mRNA expression profiling of adult adrenocortical tumors allowed their molecular classification and the identification of predictors of malignancy and survival, while reliable markers of clinical outcome are still lacking for childhood tumors. Expression of a distinct set of miRNAs, affecting the function of crucial intracellular signalling pathways, is also deregulated in adrenocortical tumors and may be exploited in the future to

identify circulating markers of malignancy. Furthermore, studies of tumor genomes have shown the presence of recurrent chromosomal alterations and suggested that distinct oncogenic routes may exist in adrenocortical tumors. Finally, preclinical studies have shown the efficacy of drugs targeting the most important signaling pathways deregulated in adrenocortical tumors to slow down tumor cell proliferation *in vitro* and *in vivo*. Those drugs represent promising novel therapeutic tools to be associated to current chemotherapeutic protocols.
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## S15.2

**Imaging of adrenocortical cancer**
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Adrenocortical cancer (ACC) is a rare and challenging malignancy for clinicians. The cornerstone of imaging for the diagnosis of ACC or re-evaluation of known ACC is thin-collimation computed tomography (CT) without contrast and after early and late washout of contrast medium. Although size and shape of adrenal lesions must be taken into consideration to differentiate adenomas from other adrenal lesions, it is pre- and post-contrast adrenal lesion density, measured in Hounsefield units, which is the key parameter not to be omitted; histogram analysis is also useful (sensitivities of 67–98% and specificity of 100% have been reported for both modalities). Chest CT is useful for staging while CT angiography gives important information on tumor extension. Multiplanar magnetic resonance imaging (MRI) is helpful, particularly in delineating tumor vascular extension. MRI refinements such as chemical shift add to its diagnostic potential (with sensitivity of 79–100% and specificity of 94–100%). Approximately one third of adrenal lesions cannot initially be categorized with CT/MRI. Functional (i.e. nuclear medicine) modalities are used to further evaluate patients. 131I-iodonorcholesterol (NP-59) imaging is an adrenal-cortex-specific modality, with sensitivity of 79%, but its availability is limited. Positron-emission tomography (PET) with 11C-metomidate is a recent adrenal-cortex-specific modality giving good results but its availability is also limited due to the very short half-life of 11C (newer relevant clinical studies are being undertaken with radionuclides that have a longer half-life). PET with 18F-fluorodeoxyglucose (FDG) may be non-specific for the adrenal cortex but its wide availability (and the combination of PET/CT) has yielded extensive and useful information on the evaluation of adrenal and extra-adrenal lesions. Bone scintigraphy can detect osseous disease extension. The use of CT plus FDG PET/CT may be time-saving, if not cost-effective, in the evaluation of ACC (with sensitivity of 97% and specificity of 91%).
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## S15.3

**Genetics of micronodular hyperplasias (associated with Cushing syndrome)**
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The overwhelming majority of benign lesions of the adrenal cortex (AC) leading to Cushing syndrome (CS) are linked to one or another abnormality of the cAMP signaling pathway. Benign adrenocortical causes of CS include the common and sporadic cortisol-producing adenoma (CPA) and a spectrum of corticotropin (ACTH)-independent, and almost always bilateral, hyperplasias. Macro-hyperplasias are more common among older patients, whereas micro-hyperplasias are frequent among children and young adults. Massive macronodular adrenocortical disease (MMAD) or ACTH-independent macronodular adrenocortical hyperplasia (AIMAH) describes a heterogeneous group of disorders that are associated with aberrant G-protein-coupled receptor (GPCR) expression (E). Abnormal GPCR-E has been found in CPAs; a small number of both MMADs and CPAs harbor somatic GNAS (Gsa) mutations. AIMAH can also be found in the context of McCune-Albright syndrome. Micro-hyperplasias are either pigmented (the classic form being that of primary pigmented nodular adrenocortical disease or PPNAD) or non-pigmented (NP-MAH) and isolated (i) or in the context of other syndromes (Carney complex – CNC). CNC and iPPNAD are caused by germline PRKAR1A mutations; somatic mutations of this gene that regulates cAMP-dependent protein kinase (PKA) are also found in 10–20% of all CPAs and abnormalities of PKA are present in MMADs. NP-MAH forms of adrenal hyperplasia and some CPAs are associated with phopshodiesterase (PDE)-11A and PDE-8B defects. Mouse models of PRKAR1A deficiency also show that increased cAMP signaling leads to tumors in AC and other tissues. Micro-RNA studies identified the Wnt-signaling pathway as a possible culprit. We also investigated Prkar1a (±) mice when bred within the Rb1 (±) or Trp53 (±) backgrounds. These studies identified Wnt signaling as the main pathway activated by abnormal cAMP signaling. Prkar1a haploinsufficiency is a relatively weak tumorigenic signal that can act synergistically with other defects to induce tumors, mostly through Wnt-signaling activation and cell cycle dysregulation.
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# Understanding growth

## S16.1

**From Galton to GWAS (and beyond): what we have learned about the genetics of human height?**
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Variation in quantitative traits such as human height is caused by a combination of multiple genes and environmental effects. Traditionally, since Galton in the late 1800s, the genetics of such traits has been studied using concepts that refer to the combined effect of all genes (e.g., heritability). Estimation of heritability for adult height are ~0.8, so that 80% of differences between people is due to genetic factors. Genome-wide association studies (GWAS) facilitate the dissection of heritability into individual locus effect. They have been successful in finding many SNPs associated with height and have greatly increased the number of genes involved in height variation. To date, hundreds of loci have been identified that explain in total about 10–15% of heritability. Estimation of heritability explained by all common SNPs together (not just the significant ones) are ~50%, spread over all chromosomes in proportion to their length, implying that there are many more variants with effects sizes too small to be detected with sample sizes employed to date. Many SNPs are in loci that are in meaningful biological pathways (e.g., skeletal growth) and/or in genes for which major (Mendelian) mutations were already known, and there is evidence at a number of genes for multiple segregating variants. The effect sizes of individual detected SNP variants are very small, about 1 to 4 mm per allele, which means that very large sample sizes are needed to detect more variants. The International GIANT (Genetic Investigation of ANthropometric Traits) consortium is analyzing GWAS data on ~250 000 people and is likely to identify hundreds more loci that are associated with adult height. The emerging genetic architecture of human height from DNA evidence is one of many hundreds to thousands of loci scattered throughout the human genome with tiny effect sizes. The identification of hundreds of associated variants facilitates the study of biology and function, in particular when genetic data is combined with data on gene expression.
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## S16.2

**Genes and the investigation of idiopathic short stature (ISS): are we ready for prime time?**
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Strictly speaking, the diagnosis of idiopathic short stature (ISS) can only be made after exclusion of all known causes of growth failure, but for pragmatic and financial reasons there are limits to the investigations that can be carried out. Clinical algorithms can assist decisions on genetic testing. Testing for SHOX is indicated if there are signs of Leri-Weill syndrome, including Madelung deformity, short forearm and lower legs. Short upper arms and legs should lead to testing for FGFR3 (hypochondroplasia). In both disorders sitting height/height ratio is high, and arm span/height ratio low. If serum IGF-I is $< -2$ SDS while

the GH peak in a provocation tests is normal or high, the differential diagnosis includes 3 conditions with normal GH sensitivity (bioinactive GH; neurosecretory dysfunction; GHSR defect) and 4 conditions with low GH sensitivity (GHR, STAT5b, IGFALS and IGF1 defects). The likelihood of such defects can be assessed by measuring GHBP, IGFBP-3, prolactin, and ALS, and performing an IGF-I generation test. While all cases with homozygous IGF1 mutations are born small for gestational age (SGA) and microcephalic, heterozygous carriers can present as ISS, particularly in short families. Children with heterozygous IGF1R defects, usually characterized by a serum IGF-I$>0$ SDS, SGA and low head circumference, but in some cases birth weight and length are $>-2$ SDS (thus 'ISS'). In cases with dysmorphic features, consulting a clinical geneticist is indicated. If a known genetic disorder is suspected, the candidate gene approach may lead to the diagnosis. If not, particularly if there are additional clinical features, a whole genome SNP analysis can be performed to detect copy number variants or uniparental disomy. With nextgen whole exome sequencing novel gene defects can be discovered, particularly if more than one family member is affected, or when various patients with a similar clinical syndrome are studied.

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## S16.3

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# Hormonal control of pregnancy

## S17.1

**Hormone signalling between mother and fetus**

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The developing fetus adapts to an adverse in utero environment, and is 'programmed' to an increased risk of developing disease in later life. Fetal growth is regulated via a complex interplay between mother, placenta and fetus. Maternal nutrients, transported via the placenta, are essential for fetal growth. Conversely, the placenta may also respond to fetal endocrine signals to regulate maternal metabolism. The placenta also regulates fetal growth via production and metabolism of key hormones involved in growth and glucocorticoid metabolism. For example, the enzyme 11 beta hydroxysteroid dehydrogenase type 2 interconverts active cortisol into cortisone, thus protecting the developing fetus from the adverse effects of excess glucocorticoids. Over-exposure to glucocorticoids leads to low birthweight and programmes long term risk of development of diabetes, cardiovascular risk and behavioural problems. While most studies have focused on maternal undernutrition and long-term adverse effects for fetal growth, there is now evidence that overnutrition of the fetus may have similar adverse effects. This is pertinent given the rising prevalence of obesity as currently 1:5 women are obese at the time of pregnancy. We are exploring the factors that influence fetal growth in a cohort of severely obese (BMI$>40$) pregnant women. Ultimately it is hoped that understanding of the mechanisms regulating fetal growth will have long-term benefits for later health.

Declaration of interest
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## S17.2

**What controls labour in humans?**

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In most mammals the onset of labour is regulated by an abrupt fall in circulating progesterone concentrations or a rise in circulating oestrogens. In humans, however, no dramatic change is observed in circulating steroid concentrations prior to labour and the mechanisms regulating the onset of labour have remained obscure. In a series of studies we have shown that placental production of the peptide hormone CRH increases exponentially across gestation peaking at the time of labour. We have also shown that CRH can exert direct actions on the fetal adrenal to stimulate production of DHEA which is 16 hydroxylated in the fetal adrenal and then converted to estriol in the placenta. The exponential increase in CRH drives a rise in estriol production in late gestation leading to a progressive increase in the ratio of estriol to estradiol (which is derived largely from maternal precursors). The increase in ratio of estriol to estradiol leads to activation of estrogen responsive genes and the onset of labour. Recently it has become apparent that the increase in placental CRH production is the result of expression of endogenous retroviruses in the placenta. The retrovirus gene product Syncytin promotes cytotrophoblast fusion and CRH expression. Data also indicates that CRH can directly increase placental estrogen synthesis and inhibit progesterone synthesis. Thus CRH plays a central role in modifying the placental endocrine environment to promote labour. It is not yet clear how this links to the expression of the myometrial proteins that regulate myometrial contraction including the progesterone receptors, connexin 43 and the changes in ion channels that regulate myometrial excitability. It may be that prostaglandins are stimulated by CRH and then regulate progesterone isoforms expression by changing the epigenetic marks on the promoters of the progesterone receptor promoters.

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## S17.3

**Placental hormones as putative markers of gestational diseases**

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Preterm delivery (PTD) and preeclampsia (PE) are 'obstetric syndromes', characterized by complex pathogenesis and representing major causes of perinatal mortality and morbidity. Although therapeutical treatment have been developed, their incidence is not reducing. Prevention aims to preclude development of disease by eliminating/reducing risk in women with known risk factors.

The feto-placental unit produces hormonal/neuronal/immune factors, crucial in maintaining feto-placental homeostasis, that are abnormally secreted in gestational diseases as part of pathogenetic mechanisms and/or feto-maternal adaptive response. Corticotrophin releasing hormone (CRH) and inhibin/activin, expressed by trophoblast, locally modulate hormone secretion, blood vessel tone, myometrial contractility, inflammatory events. Their measurement in biological fluids has been investigated to find putative biochemical markers for early diagnosis/prediction of these conditions.

PE is characterized by poor placentation due to impeded transformation of spiral arteries into low resistance vessels. Uterine artery Doppler at mid gestation allows to screen high-risk population through early detection of abnormally high resistance uteroplacental blood flow. Maternal serum activin A and inhibin A are high at 12 and 24 weeks in patients later developing PE. sFLT-1, soluble VEGF receptor neutralizing angiogenic actions of VEGF and PlGF, is elevated in women developing PE.

Spontaneous PTD is characterized by pathological and premature onset of labor and the ultrasound measurement of cervical length and, more recently, fetal membrane thickness are useful predictors of PTD. High maternal plasma CRH can predict delivery within 48 hours in symptomatic and within 34 weeks in asymptomatic women. High plasma urocortin levels predict delivery within 34 weeks in women with threatened PTD. High salivary estriol levels predict PTD with good accuracy and plasma estriol/estradiol ratio is altered before the onset of preterm labor.

Given the complex pathogenesis of PTD and PE, a multiple approach through the combination of biophysical and biochemical parameters may add significant prognostic information in women at risk.

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## Molecular mechanisms in autoimmune thyroid disease

### S18.1

**Predictors of relapse of Graves' disease after antithyroid drug withdrawal**

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For patients with Graves' disease (GD), the primary goal of antithyroid drug therapy is to temporarily restore the patient to the euthyroid state and wait for a subsequent remission of the disease. This study sought to identify the predictive markers for the relapse of disease.
To do this, we studied 262 GD patients with long enough follow-up after drug withdrawal to determine treatment outcome. The patients were divided into three groups by time of relapse: early relapse group ($n=91$) had an early relapse within 9 months, late relapse group ($n=65$) had a relapse between 10–36 months, and long-term remission group ($n=105$) were either still in remission after at least 3 years or relapsed after 3 years of drug withdrawal. We assessed the treatment outcome of 23 SNPs of costimulatory genes, phenotype and smoking habits. We used permutation to obtain P values for each SNP as an adjustment for multiple testing. Cox proportional hazards models was performed to assess the strength of association between the treatment outcome and clinical and laboratory variables. Four SNPs were significantly associated with disease relapse: rs231775 (OR, 1.96, 95% CI, 1.18–3.26) at CTLA-4 and rs745307 (OR, 7.97, 95% CI, 1.01–62.7), rs11569309 (OR, 8.09, 95% CI, 1.03–63.7), and rs3765457 (OR, 2.60, 95% CI, 1.08–6.28) at CD40. Combining risk alleles at CTLA-4 and CD40 improved the predictability of relapse. Using 3 years as the cutoff point for multivariate analysis, we found several independent predictors of disease relapse: number of risk alleles (HR, 1.30; 95% CI, 1.09–1.56), a large goiter size at the end of the treatment (HR, 1.30; 95% CI, 1.05–1.61), persistent TSH-binding inhibitory Ig (HR, 1.64; 95% CI, 1.15–2.35), and smoking habit (HR, 1.60; 95% CI, 1.05–2.42).
In conclusion, genetic polymorphism of costimulatory genes, smoking status, persistent goiter, and TSH-binding inhibitory Ig predict disease relapse.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### S18.2

**Genotype-phenotype correlations in GD**

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Graves' disease (GD) is a heterogeneous disorder affecting with varying degrees of severity the thyroid and eyes. This variety may suggest that complex interactions between genetic, environmental and endogenous factors influence the clinical course of GD. Although the genetic predisposition to GD is well established, the significance of genotype-phenotype correlations remains controversial. Moreover, the lack of complete phenotypic concordance in twin studies suggested that environmental and/endogenous factors are of great importance. The major environmental factor influencing the clinical course of GD is cigarette smoking.
Most evidence for genotype-phenotype correlations in GD is based now on case-control association studies with candidate genes. The following loci have been proposed to influence susceptibility to orbitopathy, age of onset of GD and/or severity of hyperthyroidism: HLA, PTPN22, CTLA4, CD40, TSHR, TG, TNF, ICAM-1, IFN-γ, IL-23R, NFKB1. Unfortunately the results of these studies have to be judged very carefully. The vast majority of studies are small and underpowered; replication studies are lacking or were unable to confirm the initial finding; the studied groups were often not characterized in detail. At present, while some genetic differences between subgroups of GD patients have been identified, none of the polymorphisms justifies genetic testing to guide therapy or preventive strategies. Genotype-phenotype correlations in GD remain to be elucidated in future, well designed and appropriately powered studies.
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### S18.3

**Regulatory cells, Th17 lymphocytes, and dendritic cells in autoimmune thyroid disease**

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Human autoimmune thyroid diseases (AITD), mainly including Hashimoto's thyroiditis (HT) and Graves' disease (GD), are characterized by reactivity to self-thyroid antigens. Regulatory T cells have an important role in immune tolerance to self-antigens. We have reported that different regulatory T cell subsets are abundant in the peripheral blood and in inflamed thyroid tissue in AITD and are able to synthesize the immunoregulatory cytokines IL-10 and TGF-beta. However, they are apparently unable to downmodulate the autoimmune response and the tissue damage seen in AITD.
Since there is a reciprocal negative regulation between Treg and Th17 lymphocytes, we hypothesized that the defect in T regulatory cells could be related to an expansion of Th17 cells. Accordingly, we found a higher proportion of Th17 cells and raised levels of Th17 cytokines in the peripheral blood and thyroid gland from patients with AITD, mainly those with HT. Thus, our data suggest that Th17 may have a relevant role in the inflammatory phenomenon and tissue destruction seen in HT.
Dendritic cells (DCs) bear the great responsibility of discerning whether a particular antigen must be interpreted as harmful or innocuous; whether specific T cells must develop to self-reactive T cells or to regulatory T cells. In humans, two subtypes of DCs: myeloid (mDCs) and plasmacytoid (PDCs) have been reported, each one with different phenotype and function. We have studied the phenotype and function of both DCs (mDCs and PDCs) including the expression and function of inhibitory receptors. We have found phenotypic and functional abnormalities in DCs from peripheral blood and the thyroid gland in patients with AITD that point to a role in the amplification and/or perpetuation of the immune process. These findings add additional elements to the complex pathogenesis of AITD.
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## Controversies in testosterone replacement

### S19.1

Abstract unavailable.

### S19.2

**Late onset hypogonadism: who should receive testosterone?**

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The search for eternal youth has created a market for treatments that might affect the process of aging, and testosterone (T) is one of the hormones that have been in focus. It is well accepted that T levels decline with increasing age, although the individual variation is large. Male hypogonadism is characterised by a low serum T level in combination with a diversity of symptoms and signs such as reduced libido and vitality, decreased muscle mass, increased fat mass and depression. Similar symptoms in combination with subnormal T levels are seen in some elderly men, and several attempts have been made to identify symptoms and corresponding T levels that would define late-onset hypogonadism as a syndrome. However, symptoms of T deficiency in elderly men are nonspecific and are difficult to discriminate from symptoms of other conditions and diseases common in older men. Despite this uncertainty, the sale of T has increased tremendously over the last few years.
In observational studies low T levels tends to predict the development of metabolic syndrome and type 2 diabetes, and have been associated with all-cause mortality and cardiovascular disease mortality. Results from small intervention studies, however, are diverging but have mainly shown that T treatment in older men increase fat-free mass and decrease overall body fat while the effect on glucose and lipid metabolism have been slight. Weight reduction, on the other

hand, in obese men with low T levels has been shown to normalize T levels as well as significantly improve both lipid and glucose levels. Thus, low T levels may be seen as a marker of general health, and the primary treatment should probably be lifestyle changes. T treatment should not routinely be initiated in elderly men with subnormal T levels until benefits and safety are confirmed in well performed studies.

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## S19.3

**Testosterone for women: why when and how?**

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Testosterone is a vital hormone in women, circulating in nanomolar concentrations. Not only is testosterone a precursor for estradiol biosynthesis in the ovaries and extragonadal tissues, but testosterone acts directly via androgen receptors throughout the body. Levels decline with age in women with the greatest fall in total and free testosterone occurring before the menopause. Large RCTs involving naturally and surgically postmenopausal women presenting with hypoactive sexual desire disorder (HSDD) demonstrate that testosterone therapy, with/without concurrent estrogen therapy, improves the quality of the sexual experience. More recent studies demonstrate the use of testosterone therapy improves sexual wellbeing in premenopausal women with HSDD. The effects appear not to be mediated by aromatization of testosterone to estrogen. The other potential benefits of testosterone in women include favorable effects on bone density, muscle mass, vascular endothelial function and cognitive function.

Data from observational studies mostly show an inverse relationship between testosterone and CVD risk. Published RCTs indicate that non oral testosterone therapy does not adversely affect plasma lipids or other CVD risk markers. The relationship between endogenous testosterone production and breast cancer risk remains contentious, with recent studies indicating no relationship. There does not appear to be an association between testosterone and endometrial cancer, or other malignancies.

Testosterone use has not been approved other than for surgically menopausal women on estrogen therapy in Europe. Despite this, the use of testosterone by women is widespread, with vast numbers of women using testosterone preparations developed and marketed for men, testosterone preparations compounded on individual prescriptions as oral lozenges and creams, and testosterone implants. Hence there is a clear need for a testosterone therapy delivering an appropriate female dose to be approved, so that women have the option of using a product formulated for women.

Declaration of interest

I fully declare a conflict of interest. Details below:

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# Signalling to and from osteoclast

## S20.1

**Efferent signalling by osteoclasts**

K. Matsuo
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The 'coupling' concept of bone resorption and formation was developed at both the whole-body and microscopic levels in the 1960's. At the whole-body level, 'coupling' served to explain a positive correlation observed between whole body rates of bone resorption and formation, as determined by radiocalcium kinetics. At the microscopic level, 'coupling' describes sequential bone remodeling at each basic multicellular unit (BMU), in which bone resorption is followed by an equivalent amount of bone formation. 'Coupling factors' have been defined as osteoclast-derived molecules that facilitate the transition from bone resorption to formation at the BMU level by either recruiting or promoting differentiation and activation of osteoprogenitors and osteoblasts. Factors derived from osteoblasts and osteocytes have generally been excluded from coupling factors to avoid confusion. Coupling factors are either released from the extracellular matrix, such as TGF-beta and IGFs, secreted from osteoclasts, like sphingosine-1-phosphate, or are membrane-bound.

The transmembrane ligand ephrinB2 is one such membrane-bound factor expressed on osteoclasts, while its receptor EphB4 is expressed on osteoprogenitors and osteoblasts. Significantly, ephrinB2/EphB4 signaling is bidirectional. Forward signaling into EphB4-expressing progenitors enhances osteoblast differentiation, while ephrin-expressing osteoclasts receive 'reverse signaling' upon ephrinB2/EphB4 interaction, suppressing osteoclast differentiation and function. It is increasingly clear that osteoclast-derived factors also negatively regulate osteoblasts. Such negative regulators may be GPI-anchored ephrinA2, which is produced by osteoclasts. Our data suggest that its receptor EphA2 expressed on osteoblasts inhibited mineralization. We propose that ephrinA2 and other osteoclast-efferent negative factors should be designated 'coupling inhibitors'.

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## S20.2

**Immune signaling in osoteoclasts**

Hiroshi Takayanagi
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In addition to cellular interactions via cytokines, the immune and skeletal systems share various molecules, including transcription factors, signaling molecules and membrane receptors[1]. RANKL stimulates osteoclastogenesis through NFATc1 in cooperation with immunoglobulin-like receptors, using the immunomodulatory molecules for signalling[2]. Here I will discuss emerging topics in osteoimmunology including the mechanisms underlying bone cell communication: osteocyte RANKL[3], regulation of bone formation by osteoclast-derived Sema4D[4], and osteoprotection by osteoblast-derived Sema3A[5].

References

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(2) *Nat Rev Rheumatol* **5**, 667–76, 2009.
(3) *Nat Med* **17**, 1231–34, 2011.
(4) *Nat Med*. **17**, 1473–80, 2011.
(5) *Nature*, in press.

## S20.3

**Coupling of bone resorption and formation**

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Coupling of bone formation subsequent to bone resorption is a fundamental mechanism for sustaining bone density in adults. We have demonstrated that TGFβ1 released from the bone matrix during osteoclastic bone resorption induces the migration of bone marrow mesenchymal stem cells (MSCs). We also found that IGF1 released from bone matrix responsible osteoblast differentiation of MSCs recruited by TGFβ1 during bone remodeling. TGFβ1 released from the bone matrix during osteoclastic bone resorption induces the migration of MSCs, whereas IGF1, also released from the bone matrix stimulates osteoblast differentiation of MSCs for new bone formation.

# Obesity and reproduction

## S21.1

**Integrating hypothalamic regulation of energy homeostasis and reproduction**

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Nutrition is a crucial regulatory component of the reproductive physiology. Conditions of negative energy balance or low energy store often causes a

disruption of the neuroendocrine reproductive axis and arrest of sexual maturation. On the other hand, excess energy, as observed in obesity, also negatively impacts the reproductive physiology. For example, high adiposity may induce or aggravate polycystic ovarian syndrome, ovulatory dysfunction and hypothalamic amenorrhea. In obese men, fertility is usually decreased due to altered activity of the hypothalamus-pituitary axis and defective steroidogenesis. Thus, changing levels of key metabolic cues constitutes a fundamental signal for the coordinated control of the reproductive physiology. Of particular importance is the adipocyte-derived hormone leptin. In humans and mice, inactivating mutations of the leptin or leptin receptor genes induce hyperphagic obesity, diabetes and infertility. These individuals exhibit low gonadotropins levels, deficient gonadal development and lack of sexual maturation. Leptin replacement to leptin-deficient subjects restores gonadotropins levels and the reproductive function. Leptin may also contribute to the obesity-associated decrease in fertility as the reproductive deficits observed in obese subjects may be in part caused by excess leptin and leptin resistance at the cellular (receptor/signaling) level. In this Symposium, I will discuss the recent advances in the identification of populations of hypothalamic neurons relaying leptin's action in the integration of metabolism and reproduction. Special attention will be given to glutamatergic neurons located in the ventral premammilary nucleus and kisspeptin neurons of the arcuate nucleus. We will also discuss the use of mouse genetics and Cre/loxP technology to assess the role of specific neuronal populations in the metabolic control of the reproductive function.

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## S21.2

**Impact of obesity on male reproduction**

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The incidence of obesity has dramatically increased, not only in industrialized societies but also in developing countries. Since male fertility shows a parallel decrease, obesity should be considered as possible etiology of male subfertility. Studies exploring this possible association report that obesity may affect fertility by different mechanisms that include: abnormal reproductive hormonal milieu, increased release of adipokines and adipose-derived hormones (mainly leptin and resistin), in addition to other problems such as increased scrotal temperature, obstructive sleep apnea and accumulation of environmental toxins (endocrine disruptors) in adipose tissue. All these factors could provoke erectile dysfunction and/or decline in sperm quality.

Obese reproductive hormonal profile is characterized by reduced levels of total and free testosterone concentration, gonadotrophins, SHBG and/or inhibin B (marker of Sertoli cells function) and excess of estrogens (explained by the aromatase overactivity ascribed to adipose tissue increase).

The enhance in leptin levels could be responsible for at least some of the alterations on the hypothalamic-pituitary-testicular axis (reducing hypothalamic Kiss 1 expression) and could also exert direct deleterious effects on Leydig cells physiology, spermatogenesis and sperm function.

Adipokines (proinflammatory agents), higher scrotal temperature and toxins accumulation are responsible for the increase in testes oxidative stress, and sleep apnea suppresses the nocturnal testosterone rise necessary for normal spermatogenesis.

Finally, although controversial, hormonal misbalance and leptin increase might comprise gametes quality. Some reports indicate that obesity may decrease seminal sperm density/motility and increase sperm DNA fragmentation, probably disturbing spermatogenesis and/or epididymal function. Nonetheless, these alterations are mild.

In summary, although obesity may impair male fertility by some/all of the described mechanisms, the fact is that only a small proportion of obese men are infertile; probably those genetically predisposed or morbidly obese. Nevertheless, since the incidence of obesity is increasing, the number of men with reduced fertility will rise as well.

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## S21.3

**Lifestyle modification and fertility**

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With an increasing prevalence of overweight and obesity, there is abundant evidence linking these conditions with sub- and infertility in women. The evidence in men is less well developed but is emerging gradually. Even when fertility is achieved in women, there are many conditions of pregnancy that are increased including diabetes mellitus, hypertension, congenital abnormalities and peripartum problems. Children born also have an increased long-term problem of obesity and associated disorders. In males, there is evidence for a non-genomic transfer of metabolic disease to the offspring. Use of assisted reproduction is more comon and compromised by weight disorders.

Abnormalities of eggs, sperm, embryos and endometrium have been documented in overweight conditions in animals and humans.

Lifestyle modification to improve fertility is well documented but not accompanied by a strong literature of randomised trials or evidence-based interventions. It is alledged that $>5\%$ weight loss is effective at inducing pregnancy in overweight women. The respective roles of exercise, diet and pharmacology is yet to be determined.

The role of diet has not shown any benefit of any particular composition with most of the effect being due to the amount of calories consumed. It would seem logical to use a low calorie diet with a Mediterranean type composition. Exercise can be arranged to suit the ability of the person to participate and associated medical conditions. There is little evidence for benefit of medications, particularly metformin. A full metabolic profile should be doen prior to pregnancy, especially with respect to glucose.

Much needs to be done to determine, the epidemiology of weight disorders and infertility and outcomes of offspring. Randomised trials are essential to determine the most effective interventions. A better understanding of the pathophysiology of weight and metabolic disorders and fertility is urgently required.

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The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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# Pituitary tumorigenesis

## S22.1

**Molecular mechanisms: the genes involved in pituitary tumorigenesis**

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Pituiatry tumors account for about 15% of all intracranial neoplasms. They arise from pituitary cell types due to both cell cycle trophic dysruptions leading to adenomatous growth, as well as a coupling of specific hormone gene over-expression. Thus, both tumor growth characteristics, as well as hormonal excess are the hallmarks of these invariably benign tumors. They may lead to both central compressive features due to mass expansion or invasion, as well as to specific clinical hormone hypersecretory syndromes. Although several familial syndromes have recently been characterized for specific gene mutations, the etiology of spontaneous non-familial tumorigenesis has remained largely elusive. This talk will emphasize findings for spontaneous pituitary tumorigenesis. Transgenic murine and zebrafish models will be described which recapitulate phenotypic human pituitary adenomas, and genetic mechanisms derived from these models will be translated to human clinical observations. Limited information derived directly from human tumor specimens will also be discussed, inasmuch as they relate directly to cause rather than effect of specific pituiatry adenoma subtypes. An overview of known genetic changes which underly pathogenesis of these tumors include dysrupted cell cycle proteins, including CDKs and CDK inhibitors and the RB-related cascade will be provided. Disordered genetic regulation of HMG proteins, PTTG, tumor supressor genes and cAMP-related genes will also be reviewed. Epigenetic and chromatin-associated changes leading to altered specific transcriptional patterns for cell cycle or hormone-related gene expression, may also contribute to the observed genetic changes. Given that these tumors are predominantly benign, and rarely progress to malignancy, genetic mechanisms underlying premature proliferative arrest and oncogene-induced senescence will also be reviewed. Overall, the myriad of genetic information will be integrated into a unifying hypothesis which determines the etiology of these tumors responsible for considerable patient morbidity and mortality. These insights provide direction for future subcellular approaches to therapy, and examples of such available pharmacologic agents will be presented. Thus,understanding genetic alterations in these tumors will lead to enhanced disease prognostic

marker prediciton, as well as development of novel therapies.
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## S22.2

**Tracing back a Gene's influence**
M. Korbonits
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In the late 1700s, a 22 y patient suffering from pituitary gigantism died and his skeleton was deposited in a medical museum. A contemporary etching showed him standing alongside a set of giant twin brothers who were believed to be his cousins. The existence of such a rare disorder within closely related family members indicates to modern science a genetic dimension to their disease. In 1909 Cushing opened the skull and he found a greatly enlarged pituitary fossa. We identified a mutation in the AIP gene (aryl hydrocarbon receptor interacting protein, a gene known to predispose the young-onset pituitary adenomas) from a tooth of the patient indicating the genetic basis of his gigantism. In addition, several families, originating from the vicinity of the giant's birthplace were also identified with familial isolated pituitary adenoma and exactly the same AIP mutation (c.901C>T/p.R304X). Whilst the geographical location of these families suggests a common ancestral origin, the possibility of a mutational hotspot also had to be considered, as this C>T change occurred at a CpG-site and such sites typically have higher mutation rates. The hotspot theory was supported by the fact that the same mutation has been identified in several patients from various countries around the world. Microsatellites demonstrated that the 18th-century giant and the families inherited this allele from a common ancestor, while other patients with the same mutation from elsewhere do not share this common ancestor. The age of this founder mutation and forward simulations allowed us to estimate the number of carriers alive today.
Genetic screening from this geographical area identified further carriers. Clinical screening of carriers provides earlier diagnosis with an anticipated improved clinical outcome. Our case study provides an interesting example of how medical history combined with molecular genetics has resulted in direct benefit to patients and their families.
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## S22.3

Abstract unavailable.

# Advances in regulation of aldosterone synthesis

## S23.1

**Regulation of aldosterone synthase expression in normal adrenal and primary aldosteronism**
W. Rainey
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The human adrenal cortex can be divided into distinct zones that have both morphologic and biochemical differences. The production of aldosterone in the zona glomerulosa can be traced to the zone-specific expression of the enzymes involved in steroid biosynthesis. This is particularly true for its expression of the enzyme aldosterone synthase (CYP11B2). For aldosterone biosynthesis, CYP11B2 expression has often been called the 'late rate-limiting' step. We and others have shown that the expression of CYP11B2 is controlled at the level of its gene transcription, which is normally under tight control of the renin angiotensin system (RAS). Detailed analysis of the mechanisms regulating transcription of CYP11B2 has demonstrated a key role for the NGFI-B and the CREB/ATF families of transcription factors. In addition, we have extended the molecular studies to human disease and have shown that the recently described mutations in KCNJ5 increase adrenal cell expression of both CYP11B2 and NURR1. Thus, APA expression of KCNJ5 somatic mutations leads to renin-independent aldosterone production. A disruption in the normal regulation of NURR1 and CYP11B2 expression appears to be an important part of the disease process leading to primary aldosteronism. The ongoing studies are providing a detailed understanding of the molecular mechanisms regulating aldosterone and cortisol production within the normal adrenal and should provide insight into diseases of adrenal steroid excess.
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## S23.2

**Extra-adrenal aldosterone biosynthesis**
C. Gomez-Sanchez[1,2]
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Aldosterone (aldo) concentrations in the brain and heart are slightly lower, but parallel those in the plasma. Plasma, heart and urine aldosterone continue to be detected in very small amounts in adrenalectomized (ADX) rats, however the concentrations of aldosterone in the brain, though very low, are higher than plasma levels in ADX rats. StAR protein and all of the steroidogenic enzymes necessary for the synthesis of aldosterone are expressed in the central nervous system (CNS) of rats and humans. Brain tissue from ADX rats synthesizes aldo from endogenous substrate and converts 3H-deoxycorticosterone into 3H-aldosterone *in vitro*. Inappropriately high aldo levels are associated with increased sympathetic drive and hypertension of central origin. Aldo synthesized in the brain appears to play a role in the development of hypertension in the Dahl salt-sensitive rat. Aldo concentration in the hypothalamus of Dahl SS rats is higher than in Sprague-Dawley controls and inhibition of enzymes within the aldosterone biosynthetic pathway, including trilostane, a 3-hydroxysteroid dehydrogenase, and aldosterone synthase, decreases the BP in the Dahl SS rat. Rats in which the aldosterone synthase enzyme cDNA is over-expressed in neurons also have hypertension.
Though most of the aldo in the brain is sequestered from circulating aldo synthesized by the adrenal gland, a small proportion is locally synthesized in the normal rat. As the amount is exceedingly low and number of cells that produce aldo in the brain few, aldo produced in the brain, if relevant, would be expected to have autocrine or paracrine functions. Aldo synthesized in the CNS might have a role in those forms of hypertension and autonomic dysfunction in which circulating aldo is not elevated, but which respond nonetheless to mineralocorticoid receptor antagonists.
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## S23.3

**Role of N-type calcium channel blockers in aldosterone biosynthesis**
M. Yoshimura
The Jikei University School of Medicine, Tokyo, Japan.

The inhibition of aldosterone activity is a significant approach for preventing the progression of cardiovascular diseases in hypertensive patients. We hypothesized that N-type calcium channels might regulate aldosterone biosynthesis and firstly examined *in vivo* studies using rats. We investigated the effects of cilnidipine, an L-/N-type calcium channel blocker (CCB), and nifedipine, an L-type CCB, on aldosterone levels and found that only cilinidipine significantly reduced

aldosterone levels. We secondly examined the direct relationship between N-type calcium channels and aldosterone production in human adrenocortical cells. In this study, the analysis of quantitative reverse transcription-PCR, western blotting, and immunocytological staining indicated the possible presence of N-type calcium channels in human H295R cell line. Patch clamp analysis indicated that omega-conotoxin GVIA (CnTX), an N-type calcium channel inhibitor, suppressed voltage-dependent barium currents. CnTX significantly reduced the transient calcium signaling induced by angiotensin II (Ang II) and partially prevented Ang II-induced aldosterone formation. Knockdown of $\alpha$1B calcium channel subunits significantly decreased Ang II-induced aldosterone formation with increments in CYP11B2 mRNA expression. We investigated the inhibitory activities of cilnidipine and showed a dose-dependent inhibition effect on Ang II-induced aldosterone production. These results suggest that N-type calcium channels have a significant role in transducing the Ang II signal for aldosterone biosynthesis in humans.

Declaration of interest

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## ManagemDiabetes in children

### S24.1

Abstract unavailable.

### S24.2

**Hypotheses explaining the increase in type 1 diabetes**

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The incidence of type 1 diabetes (T1D) has increased markedly after World War II among children and adolescents, and in parallel the mean age at diagnosis has decreased. These trends support a critical role of exogenous factors in the development of T1D, since genetic factors alone can hardly explain the rapid increase. The hygiene hypothesis postulating a relationship between allergic diseases and a childhood environment characterized by a decreased pathogen exposure has recently been expanded to explain the rising incidence of autoimmune diseases such as T1D. It is conceivable that a decreased microbial load in early life may have a major impact on the programming of the immune system. Enterovirus infections have been implicated as potential triggers of the disease process leading to T1D. The frequency of enterovirus infections has, however, lately decreased among the general population in developed countries at the same time as the T1D incidence has increased. This seeming contradiction can be explained by the so called polio hypothesis, according to which the decreasing infection rate in the general population results in a situation where infants have an impaired protection against acute enterovirus infections early in life. This may then result in more invasive enterovirus infections, some of which might lead to the induction of the diabetic disease process as a complication. Bovine insulin have been proposed to function as a driving exogenous antigen in T1D. One can speculate that the increasing processing of milk products may have some impact on the immunogenicity of bovine insulin in commercial milk products. Children grow linearly and gain weight faster now than some decades ago. Rapid growth and weight gain induces beta-cell stress and could thereby lead to an earlier presentation of clinical T1D and also an increasing incidence by expanding the proportion of susceptible individuals progressing to overt disease.

Declaration of interest

The author declares that there is a conflict of interest.

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### S24.3

Abstract unavailable.

## Reproductive hormone action

### S25.1

**GnRH receptor signalling**

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The hypothalamic decapeptide, gonadotropin-releasing hormone (GnRH), is the key neuroendocrine regulator of mammalian reproductive development and function. GnRH binds to its target, the GnRH receptor (GnRHR), on pituitary gonadotropes to stimulate the synthesis and intermittent release of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn stimulate gametogenesis and gonadal hormone synthesis. Thus GnRH, via the GnRHR, plays a pivotal role in the coordination of reproductive events, and dysregulation of GnRH signaling underlies many reproductive disorders, such as polycystic ovarian syndrome (PCOS), hypothalamic amenorrhea, disorders of pubertal maturation, and infertility. GnRH is released in a pulsatile manner, with the frequency and amplitude of GnRH pulses varying temporally and developmentally, for example during different phases of the menstrual or estrous cycle. These patterns of pulsatile GnRH release activate distinct signal transduction cascades to contribute to frequency decoding of GnRH pulsatility by gonaodtropes, resulting in differential LH and FSH synthesis and secretion. The identification and studies of mutations in the GnRHR in patients with disorders of reproductive maturation and function have further highlighted the importance of GnRHR signalling in regulation of gonadotropin synthesis and secretion.

### S25.2

**Light on the structural communication in gonadotropin hormone receptors: implications in genetic diseases**

F. Fanelli

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The network paradigm is being increasingly used to describe the topology and dynamics of complex systems. The representation of protein structures as networks of interacting amino acids has in fact been used to investigate and elucidate complex phenomena such as protein folding and unfolding, protein stability, the role of structurally and functionally important residues, protein-protein and protein-DNA interactions and intra- and inter-protein communication and allosterism.

The graph theory was combined with fluctuation dynamics in the framework of Protein Structure Network (PSN) analysis to investigate the structural communication in the inactive and active states of the Gonadotropin Hormone Receptors (GHRs). The analysis of wild type and spontaneously occurring GHR mutants served to identify key amino acids that are part of the regulatory network responsible for propagating communication between the extracellular and intracellular poles of the receptors. Highly conserved amino acids in the rhodopsin family of G Protein Coupled Receptors (GPCRs) participate in the protein structural stability as highly connected nodes in the network (i.e. hubs) in both the inactive and active states. Moreover, they behave as the most frequent nodes in the communication paths between the extracellular and intracellular sides.

Hub distribution reflects the existence of a diffuse intramolecular communication inside and between the two poles of the helix bundle, which makes pathogenic mutations share similar phenotypes irrespective of topological and physico-chemical differences between them. Spontaneously occurring gain-of-function and loss-of-function mutations induce perturbations in the structure network that characterize the wild type form. In this framework, the computational models are useful tools for structure-based identification of ligands able to correct the genetic defect by restoring the essential features of the native structure network.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S25.3

**Extragonadal FSH action- facts and dreams**
N. Ghinea
Inserm, Paris, France.

Early detection is essential for curative cancer therapy and for achieving a decrease in cancer mortality. Markers for incipient stages of every type of cancer constitute therefore all oncologists' dream. We have recently uncovered a new tumor marker, the FSH receptor (FSHR). Compared with the available markers, FSHR is found in a wide array of cancers, including eleven of the most frequent types. We have shown by various methods that FSHR is expressed in $>1300$ human tumors, in all tumor types examined (colon, prostate, breast, lung, ovary, testis, kidney and others), for all tumor grades and stages examined (Radu *et al. N Engl J Med* 2010 **363** 1621–30). FSHR (as protein and mRNA) is present only in the tumor's endothelial cells, most frequently at the periphery of the tumors. No expression has been noticed at the level of blood vessels in healthy tissues from cancer patients, with the exception of the reproductive organs where it is present in much lower concentrations than in tumors. A mouse tumor model showed that the FSHR is present on the luminal surface of the endothelium in tumors and can internalize ligands delivered in the vasculature.
Based on these results, FSHR appears to be very promising for various applications such as cancer diagnosis, imaging, and therapy. It is also possible that the level of the endothelial FSHR expression could have predictive power regarding the progression of the disease and the efficacy of various therapies, especially those aimed at tumor vasculature. The latter possibility is supported by our recent publication (Siraj *et al. J Cell Mol Med* 2011. doi:10.1111/j.1582-4934.2011.01495.x.) in which we reported that the level of FSHR expression in the primary kidney tumors could predict the response to subsequent therapy with sunitinib, a drug that it is thought to act by inhibiting tumor angiogenesis.
Declaration of interest
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# TSH Receptor

## S26.1

**Low molecular weight antagonists of the TSH receptor**
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Over the last several years, we have generated low molecular weight (LMW) antagonists of the TSH receptor (TSHR) that have the potential to be developed as drugs to treat patients with Graves' hyperthyroidism (GD)/Graves' ophthalmopathy (GO) and thyroid cancer. As GD is caused by persistent, unregulated activation of TSHRs by thyroid-stimulating antibodies (TSAbs) on thyrocytes and GO may be caused by activation of TSHRs on retro-orbital fibroblasts, a LMW TSHR antagonist could be used to treat GD and GO. Some antagonists, termed inverse agonists, in addition to inhibiting receptor stimulation by agonists like TSH and TSAbs, inhibit basal receptor signaling. As TSHR exhibits basal signaling, inverse agonists could be used to inhibit basal TSHR signaling on thyroid cancer cells and thereby further suppress activation of residual thyroid cancer. In this presentation, we will describe the data we have developed concerning the effects of these LMW antagonists in model cell systems over-expressing TSHRs, in primary cultures of human thyrocytes, and in primary cultures of fibroblasts from the retro-orbital space of patients with GO.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S26.2

**Learning from how antibodies interact with the TSH receptor**
Terry F. Davies, Syed. Morshed & Rauf. Latif
Thyroid Research Unit, Mount Sinai School of Medicine, James J Peters VA Medical Center, New York, New York, USA.

Successful crystallization of TSHR residues 21–260 has been reported (Sanders *et al. Thyroid* 2007 **17** 395) using a partial ectodomain (ECD) bound to the Fab fragment of a human stimulating TSHR monoclonal antibody (M22) and its conformational epitopes delineated. We have now studied antibody binding to the entire ECD (residues 1–412) using epitope protection. In this approach, we first protected a highly purified ectodomain fragment with a variety of TSHR antibodies prior to enzyme digestion and the protected peptides were then delineated by mass spectrometry. There were 7 protected linear peptides spanning the TSHR-ECD. Five of these regions corresponded to peptides in leucine rich domains (LRD) but in addition, monoclonal thyroid stimulating antibodies contacted a region on the amino terminal of the TSHR and a protected epitope on the hinge region (residues 263–293) which encompasses a highly conserved motif (SHCCAF) previously implicated in activation of the receptor. These data demonstrate that stimulating TSHR-Abs have epitopes not confined to the LRD but also incorporate epitopes not revealed in the available crystal structure and indicate the potential for such antibodies to activate the receptor by direct contact with the non-LRD regions of the TSHR. Only TSHR-Abs which bound to the LRD were able to activate $G\alpha s$ and $G\alpha q$ pathways and induce thyroid cell proliferation and activation. In contrast, neutral TSHR-Abs which bound only to the hinge region of the ectodomain activated only $G\alpha q$ and in the absence of PKA activation induced thyroid cell apoptosis. These results indicate that the binding sites of TSHR-Abs determine the pattern of signal activation and the resulting transduction pathways involved in thyroid cell responses.

## S26.3

**Structure and function of TSH receptor**
H. Biebermann, G. Kleinau, A. Grüters & H. Krude
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The Thyrotropin receptor (TSHR) is known as the important key-player for regulation of thyroid growth and function. As a G-protein coupled receptor (GPCR) TSHR predominantly activates the Gs/adenylyl cyclase and the Gq/11 phospholipase C signaling pathways, that finally regulate thyroid hormone production. Aberrant thyroid hormone production can be caused by activating TSHR mutations in case of toxic thyroid nodules or non-autoimmune hyperthyroidism or via inactivating mutations identified in patients suffering from hyperthyrotropinemia, TSH resistance or congenital hypothyroidism. Careful functional characterization of naturally occurring TSHR mutations and huge efforts from side-directed mutagenesis studies combined with fragmental structural information have widened our knowledge of signaling related processes at the TSHR significantly. In addition, it is recognized that the TSHR functions as a dimer or oligomer, which is of importance to understand specificities of patient phenotypes caused by heterozygous inactivating TSHR mutations. Strikingly, the available information is a prerequisite for understanding the role of TSHR under different physiological and pathophysiological conditions, which is also important for the development of artificial ligands that modulate controlled TSHR functions. However, several details of the TSHR are still unknown like the assembly of the entire receptor protein and the interplay between receptor components during signal transduction. For that reason, even after nearly twenty years of TSHR mutational screening, naturally occurring mutations are still helpful to reveal new insights into such missing information. Recently identified mutations for example prompted to details of the sixth transmenbrane helix during receptor activation and inactivation, or to a potential relationship between activating mutations and a TSHR oligomer constellation. Under these perspectives latest insights into pathogenic malfunctions are still of enormous importance also to complete the understanding of the TSHR regulation mechanisms and related physiological processes.
Declaration of interest
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## Are endogenous testosterone levels predictors of cardiovascular events?

### S27.1

**Androgen action on the cardiovascular system**

K. Channer
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The most important cause of death as men age is cardiovascular disease and as men age so their androgen status declines. Studies have shown that lowering of endogenous androgen by deprivation therapy as treatment for prostate cancer increases the risk of cardiovascular death. In long term studies, low blood testosterone is associated with accelerated atherosclerosis as manifest in coronary, aortic and carotid vascular territories.

Animal models of hypogonadism with cholesterol feeding have also shown accelerated atherosclerosis, which replacement therapy (TRT) ameliorates. The mechanism is not understood but low testosterone in men, is associated with an adverse lipid profile, increased inflammatory activation, weight gain, insulin resistance and impaired glucose tolerance which is improved by TRT as proven in randomised controlled trials.

Testosterone has also been shown to be an arterial vasodilator, an effect mediated by blocking the L-type calcium channels in the vascular smooth muscle membrane. This may be the mechanism whereby it improves exercise capacity in men with low blood testosterone who also have angina or heart failure.

The disadvantage of a low blood testosterone level is not just related to accelerated atherosclerosis. There have been at least 4 epidemiological studies showing that a low serum testosterone is associated with an excess mortality, in populations of normal men without overt vascular disease. We recently reported a follow up study of 930 men with coronary disease confirmed at angiography which showed that men with low levels of bioavailable testosterone had a highly significant increase in mortality (x2) compared with those with a normal bioavailable testosterone over a follow-up period of 7 years.

It is not known whether TRT to normal physiological levels will prolong life or reduce cardiovascular events, but there is no doubt that TRT improves symptoms, functional capacity and well being.

Declaration of interest

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### S27.2

Abstract unavailable.

### S27.3

**Endogenous androgens, diabetes, and cardiovascular disease in women: the Rancho Bernardo study**

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For $>50$ years female cardioprotection was attributed to premenopausal endogenous estrogen levels. We describe here how cardioprotection is greatly reduced in the presence of diabetes and endogenous testosterone levels.

Forty years ago we measured total and bioavailable testosterone (bioT), total and bioavailable estradiol, and SHBG in community-dwelling older men and women from the Rancho Bernardo Study, using organic solvent extraction and celite column chromatography prior to radioimmunoassay (SSC Yen). Participants were followed to the present. SHBG was used to directly measure bioavailable sex hormones in this cohort; it was not associated with the 19-year risk of heart disease or CVD mortality in men or women.

During menopause, total testosterone levels decrease 35%, later increasing to levels near the lower range in intact women. After bilateral oophorectomy and loss of ovarian stromal cells, women had 38% lower testosterone levels; after hysterectomy without oophorectomy women still had somewhat lower testosterone levels.

In women without diabetes, FPG was positively associated with bioT levels, but not with estrone or total estradiol. Women in the highest quartile of bioT had a 2.9-fold increased risk of developing diabetes (by OGTT criteria). Older women with IGT or diabetes had higher bioT levels than normoglycemic women.

Total testosterone was positively associated with HDL and inversely with triglycerides in both sexes. In contrast, bioT was positively associated with body mass index, waist girth, and cholesterol in women but not in men. Women with higher bioT levels had more metabolic syndrome (MetS) components, while men with lower total T had more MetS components. Women with lower total T had a poorer 20-year cumulative CHD-event-free survival. In contrast, both extremes of bio-T levels predicted the poorest survival in women. Endogenous testosterone appears to be a critical component of diabetes-related sex differences in cardiovascular disease.

Declaration of interest

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## Osteoporosis treatment in 2012 and beyond

### S28.1

**Controversial issues with bisphosphonate treatment**

S. Papapoulos
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Bisphosphonates, because of their efficacy, safety and ease of administration, are accepted as first-line therapy for osteoporosis worldwide. They decrease the rate of bone resorption but have also distinct pharmacological properties including preferential uptake in the skeleton, primarily at sites with increased bone remodeling, and long-term retention in bone. There are differences among bisphosphonates both in their affinity for bone as well as in their antiresorptive potencies and the whole molecule is responsible for their inhibitory effect of bone resorption. Available potent bisphosphonates can be given to patients by different dosing schedules that range from daily oral administration to yearly intravenous infusions. While at the bone surface bisphosphonates decrease the rate of bone resorption and turnover, maintain or improve structural and material properties of bone, increase areal mineral density and thereby decrease the risk of fractures. Bisphosphonates are generally safe provided that recommendations and indications for their use are followed. Selection of a bisphosphonate for the treatment of an individual patient should be based on review of efficacy data, risk profile of the bisphosphonate and values and preferences of the patient. However, despite progress in our understanding of the basic and clinical pharmacology of bisphosphonates there are still controversial issues in the use of bisphosphonates in the management of osteoporosis that remain to be addressed. The most important of these issues is the length of treatment and, consequently, its potential role in the development of the rare, but serious clinical events of osteonecrosis of the jaw and atypical fractures of the femur. In addition, potential, clinically relevant differences among individual bisphosphonates, optimal selection of patients for treatment, use in combination with bone forming agents as well as their action in reducing all-cause mortality in treated patients need to be clarified.

Declaration of interest

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### S28.2

**Sclerostin: a key bone regulatory molecule**

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Sclerostin is an osteocyte-expressed, extracellular cystine-knot protein that is lacking in patients with sclerosteosis, a rare condition characterized by excessive bone formation. Sclerosteosis patients exhibit very high bone mass (lumbar spine Z scores up to $+14$) and are anecdotally resistant to bone fracture. Homozygous patients commonly develop symptoms associated with cranial nerve entrapment from excessive bone formation but do not show signs of heterotopic bone formation or metabolic abnormalities. Carriers demonstrate moderately elevated bone mass without associated untoward symptoms of excessive bone formation. Sclerostin knockout mice recapitulate many of the features of the excessive bone

formation seen in sclerosteosis patients. Sclerostin acts by down modulating signaling through two osteogenic pathways (the Wnt and BMP pathways) and is consequently believed to negatively regulate the anabolic output from cells in the osteoblast lineage Treatment of rodents with monoclonal antibodies that block the function of sclerostin results in significant increases in bone formation, bone mass and bone strength. Further, antibodies to sclerostin can reverse the bone loss in rodents associated with ovariectomy, chronic inflammatory conditions and steroids. In primates, sclerostin antibodies cause a dose-dependent increase in circulating markers of bone formation as well as increasing bone mineral density and bone strength in both trabecular and cortical bone.
Sclerostin antibodies also accelerate bone repair in both rodent and primate fracture repair models. Mechanistic studies have shown that primarily acts to stimulate bone formation through a modeling rather than a remodeling pathway. A Phase 1 clinical study demonstrated that a humanized antibody to sclerostin increased markers of bone formation and also inhibited a marker of bone resorption in a dose-dependent manner. Sclerostin inhibition provides an opportunity to evaluate therapies to increase bone mass to reduce fracture and accelerate fracture healing.
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## S28.3

### Other emerging therapies

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Current osteoporosis therapy is predominantly 'anti-resorptive'. Oestrogen antagonises the action of RANK-Ligand, a potent cytokine for osteoclast differentiation. Amino-bisphosphonates inhibit the HMG CoA reductase pathway, reducing osteoclast activity and viability, while denosumab is a human monoclonal antibody that binds to RANKL. Denosumab treatment reduces fractures. In a study of 7868 women with postmenopausal osteoporosis, denosumab reduced new radiographic vertebral fracture risk (HR, 0.32; 95% CI, 0.26 to 0.41; $P<0.001$); hip fracture risk (HR, 0.60; 95% CI, 0.37 to 0.97), and nonvertebral fracture risk (HR, 0.80; 95% CI, 0.67 to 0.95). Importantly, there was no increase in the risk of cancer, infection (apart from cellulitis), cardiovascular disease, delayed fracture healing, or hypocalcemia, and no cases of osteonecrosis of the jaw. However, two subsequent cases have been reported. Denosumab increases BMD after alendronate therapy, and increased BMD more than alendronate in a head-to-head study. In elderly men on androgen deprivation for prostate cancer, denosumab reduced vertebral fractures by 62%, compared with placebo, after 3 years. Cathepsin-K is a collagenolytic enzyme, relatively specific for bone. It is an 'uncoupling' anti-resorptive that reduces osteoclast activity, but not viability. The cathepsin-K inhibitor, odanacatib, increases BMD and reduces bone resorption markers in a dose-dependent manner. Importantly, bone formation is only modestly and transiently reduced. Fracture data are awaited. A further cathepsin-K inhibitor, ONO-5334, is being assessed. A new drug family inhibits endogenous inhibitors of Wnt-dependent anabolic bone pathways e.g. anti-sclerostin and anti-dikkopf (Dkk-1) antibodies. Anti-sclerostin antibodies increased BMD, bone formation and doubled skeletal strength after only 2 months in primates. A phase-1 study in older women and men showed a single injection of anti-sclerostin antibodies resulted in large increases in bone formation and smaller decreases in bone resorption, resulting in increases in BMD after only 85 days, and may represent a paradigm shift in osteoporosis therapy.
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# Are we ready for novel therapies in obesity?

## S29.1

Abstract unavailable.

## S29.2

### Growth hormone replacement and metabolic syndrome in GH deficient adults

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There is an increased prevalence of metabolic syndrome in patients with severe GH deficiency. Prior studies used different criteria to define metabolic syndrome but in spite of this limitation, several studies indicate that GH therapy improves many of these parameters. The variable that shows the greatest improvement is waist circumference. Some studies show improvement in both systolic and diastolic blood pressure and in HDL cholesterol. Definitive evidence of decreased levels of triglycerides has not been forthcoming. GH results in minimal changes in adiponectin, leptin and resistin. The changes that occur in glucose metabolism are clearly dependent on GH dose. A low dose, e.g. 0.1 mg/day, improves glucose utilization but in studies wherein the entire range of GH doses was utilized, fasting glucose increased in initially 23% and then returned toward normal in all but 11% of patients. Only 6% of patients developed evidence of diabetes mellitus and this also tended to improve over time. Although GH administration clearly increases insulin resistances, most patients do not develop impaired fasting glucose or diabetes. In summary, the prevalence of the characteristic features of the metabolic syndrome is increased in GH deficiency. Institution of GH therapy is likely to improve waist circumference and in many patients it increases HDL. The effects on triglycerides and blood pressure are variable. Some lack of consistency may be due to patient selection and GH dosage. The effects on glucose metabolism are complex and involve increasing insulin resistance and decreasing visceral adiposity. The combined effect of these two changes results in maintenance of glucose homeostasis in most patients although a small subset may develop impaired fasting glucose or overt diabetes which requires modification of dosage. Whether changes in these parameters of metabolic syndrome will result in a benefit in terms of altering cardiovascular mortality has not been definitively determined.
Declaration of interest
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## S29.3

### GLP-1 and analogues in obesity

A. Astrup
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A wealth of evidence has substantiated GLP-1's role in regulating appetite and energy intake in humans. Meta-analyses confirm that i.v. infusion of native GLP-1 reduces energy intake in lean and overweight subjects[1]. A high-protein diet is known to increase satiety and this effect appears to be at least partly mediated by GLP-1. Increased post-prandial GLP-1 levels have been measured in healthy subjects following high-protein meals compared with medium- and low-protein meals, which correlated to increased satiety in the high-protein group[2].
Agonists of the GLP-1 receptor, such as exenatide and liraglutide, have been demonstrated to cause weight reduction and decreased food intake in animal models and in patients with type 2 diabetes. Trials are underway to assess efficacy and tolerability in obese patients.
Liraglutide has been shown to cause weight loss in obese patients. Liraglutide 1.2–3.0 mg was compared with placebo or orlistat over 104 weeks in 564 non-diabetic obese individuals[3,4]. After 52 weeks weight loss was greater with liraglutide 3.0 mg (7.8 kg) vs placebo (2.0 kg) or orlistat (3.9 kg; both $P\leq0.0001$). Nausea and vomiting occurred more often with liraglutide, but adverse events were mainly transient and mild. In another study liraglutide 3.0 mg ($n=207$) maintained weight lost through diet and exercise for 52 weeks in 81% of subjects compared to 49% of subjects on placebo ($n=206$; $P<0.0001$)[5]. In summary, GLP-1 is an endogenous satiety hormone involved in appetite regulation and GLP-1 receptor agonists are being investigated for the management of obesity.
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## New developments in pituitary adenomas

### S30.1

**Symposium 30: new developments in pituitary adenoma cytokines and other new genes in pituitary adenomas**

E. Arzt
IBioBA-CONICET-MPSP, Ciudad de Buenos Aires, Argentina.

With the aim of identifying genes involved in the development of pituitary tumors, we used the mRNA differential display technique comparing tumor and normal pituitary cells. Two genes have been identified to be involved in pathogenesis process: in prolactinomas obtained from Dopamine D2R knockout female mice, we have found differential expression of the cytokine BPMP-4 and in clones of the tumoral lactosomatroph cell line GH3 cell line overexpressing the cytokine IL6 signal transducer gp130, which have enhanced tumorigenecity in nude mice, we found the expression of a novel gene RSUME.
BMP-4 has a dual role in lactotrophas and corticotrophs: it is augmented (and its antagonist noggin decreased) during prolactinoma development stimulating this cell proliferation, while, on the contrary, in corticotrophinomas BMP-4 has an inhibitory action. In both cases the action is different of that of TGF β and involves a cross talk of smad-4 with steroid receptors. RSUME expression is induced under hypoxic conditions, increases VEGF and HIF expression, which correlates with increased angiogenic potential of the lactosomatotrophic gp130 clones, and has a potential role during vascularization. Its mechanism of action involves the stabilization of these proteins through sumoylation. RSUME is overexpressed in human pituitary adenomas, particularly in necrotic areas. These proteins provide new interesting targets for inhibiting differente steps involved in the development of pituitary adenomas.
Declaration of interest
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### S30.2

**'Dopastatin': an evolving story**

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Somatostatin receptors (sstr1–5) and dopamine receptor 2 (D2DR) are expressed and co-localized in several endocrine tumors including pituitary adenomas that express both D2DR and various subtypes of somatostatin receptors, depending on the original cell type. Treatment of pituitary adenomas using somatostatin or dopamine analogs targets only one receptor (octreotide/lanreotide mainly sst2, cabergoline D2DR). Association of octreotide and cabergoline showed limited additive effects in GH adenomas both *in vivo* and *in vitro*. Sst5 agonists showed *in vitro* the capacity to suppress hormonal secretion in pituitary adenomas, and pasireotide, targeting both sst5 and sst2, showed efficacy in controlling GH and ACTH hypersecretion.
Both sst2 and sst5 are able to form heterodimers with D2DR, which modifies ligand binding and signal transduction in a cooperative manner. Sst2-D2DR cooperation may be more efficiently triggered through so-called 'dopastatins' i.e. hybrid dopamine and somatostatin agonists. In GH tumoral cells *in vitro*, characterized by a balanced sst2/D2DR expression, dopastatins showed a synergic effect on cell secretion and proliferation by acting through both sst2 and D2DR receptors. *In vivo* phase IIA single dose study of BIM-23A760 dopastatin showed encouraging efficacy and safety profile. However, in a phase IIb repeat dosing clinical trial, a strong dopaminergic activity was observed, but little evidence of somatostatinergic activity with weak inhibition of both GH and IGF1. In other types of pituitary adenomas presenting lower sst2 expression, dopastatin showed *in vitro* an antisecretory and antiproliferative effects similar to D2DR agonists. In prolactinomas resistant to D2DR agonist treatment, sst2 overexpression induced a sst2/D2DR balance similar to that of GH adenomas. However inhibition of PRL secretion induced by dopastatin remained similar to that obtained by the reference D2DR agonist.
Gastro-entero pancreatic neuroendocrine tumors (GEPNET) and other neuroendocrine tumors, like pheochromocytomas and paragangliomas, express both sstr and D2DR, opening the way for potential sst2–D2DR cooperation in these tumors. Further studies are underway to validate the interest of chimeric agonists in pituitary adenomas and other neuroendocrine tumors.
Declaration of interest
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### S30.3

**The use of temozolomide in pituitary tumours**

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The management of aggressive pituitary tumours is challenging. These tumours are typically resistant to standard medical therapy and progressive tumour growth occurs despite multiple operations and radiotherapy. Chemotherapy has been reserved as salvage therapy, although historically results are often disappointing. However temozolomide, an oral alkylating agent, has recently demonstrated significant activity against these tumours. Over the past 6 years, growing international experience with the use of temozolomide in aggressive pituitary tumours is reflected in more than 40 published cases. A review of these cases demonstrates a 60% response rate overall to temozolomide, with prolactinomas and ACTH-secreting tumours more likely to respond compared with non-functioning pituitary tumours[1]. A degree of publication bias towards reporting of successful outcomes may be overstating the effectiveness of temozolomide in the management of aggressive pituitary tumours.
O-6-Methylguanine-DNA methyltransferase (MGMT), a DNA repair protein, directly removes the alkylating lesion induced by temozolomide. MGMT expression, as determined by immunohistochemistry, shows promise as a biomarker of response to temozolomide, although there is concern about its clinical utility[1]. In addition, low MGMT expression has been reported with increased frequency amongst more aggressive pituitary tumours[2]. This suggests that MGMT may play a role in pituitary tumorigenesis. Indeed, we have recently found a difference in the gene expression profiles between pituitary tumours with low and high MGMT expression.
Temozolomide is the first chemotherapeutic agent to show promising efficacy in the treatment of aggressive pituitary tumours, and clinical trials are needed. Pituitary tumour MGMT expression may serve both as a biomarker of response to temozolomide and a prognostic marker.
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## Progress in treatment in primary aldosteronism

### S31.1

Abstract unavailable.

### S31.2

Abstract unavailable.

### S31.3

Abstract unavailable.

## Disorders of sex development (DSD)

### S32.1

**Genetics of disorders of sex development**

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Recent progress in molecular genetics has successfully identified multiple causative genes for DSD. Such advance is primarily based on the detection of pathologic mutations on coding sequences. However, genetic disorders can be caused by other mechanisms. Here, I will review such mechanisms that have been identified recently.
Aromatase excess syndrome (AEXS) as a model for genomic disorder
AEXS is a rare autosomal dominant disorder characterized by gynecomastia in which no causative mutation has been identified on the coding region of CYP19A1. Here, I will explain three genomic rearrangements leading to AEXS because of CYP19A1overexpression: i) a tandem duplication involving seven of 11 non-coding CYP19A1 exons 1 associated with tissue-specific promoters; ii) two types of deletions at the upstream region of CYP19A1 that have produced a chimeric mRNA between CYP19A1 coding exons and a promoter-associated DMXL2 exon 1; and iii) four types of inversions involving the upstream region of CYP19A1 that have yielded chimeric mRNAs between CYP19A1 coding exons and promoter-associated exon 1 of CGN1, MAPK6, TMOD3, and TLN2. This represents novel mechanisms leading to gain-of-function of CYP19A1.
SOX9-related DSD as a model for a regulatory region disorder
SOX9 is a causative gene for DSD and campomelic dysplasia. Recent studies have indicated that heterozygous deletions of the $5'$ region of SOX9 can lead to 46,XY DSD with gonadal dysgenesis (I will introduce a hitherto unreported patient with this condition), and that heterozygous duplications involving similar regions can result in 46,XX DSD with variable degree of testis development. In addition, larger duplications involving the commonly duplicated segment in 46,XX DSD patients permit normal male sex development. These findings imply that the contrastive DSD phenotypes may be caused by loss or duplication of a testis-specific enhancer(s), or by alteration of a chromatin structure affecting SOX9 expression.
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### S32.2

**Psychosocial and psychosexual outcome in DSD**

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The 2006 DSD consensus statement states that all newborns should receive a gender assignment. Gender assignment decisions are typically guided by a combination of diagnosis and long-term, adult follow-up studies that focus on gender identity stability and other psychosexual outcomes. One relevant question in gender assignment is whether, in newborns, gender identity can be predicted, and if so, whether adult gender identity is related to prenatal brain exposure to testosterone. Sexuality is another area that is closely related to quality of life. It may be hampered by psychological factors, such as uncertainties about one's sexual orientation, fertility, or appearance, but also to medical interventions such as early surgery. From various reviews is appears that gender identity is not closely related to prenatal brain exposure to testosterone. It also seems that adolescent girls with DSD reach psychological milestones somewhat later than their peers and that sexual problems are more common in women with DSD than in control women. Outcomes, however, differ largely between conditions, gender of rearing and treatment centers. This reflects the need for large scale multi-clinic collaboration.
Declaration of interest
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### S32.3

Abstract unavailable.

## Pollution Related Acromegaly

### S33.1

**Pollution mediated acromegaly**

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Acromegaly is a disease due, almost in all cases, to a pituitary adenoma (PA) characterized by GH hypersecretion. Its prevalence seems widely variable among different geographic areas, ranging 36–151 cases $\times 106$ of inhabitants (c.p.m.). Apart from the possible underestimation due to misdiagnosis, reliable epidemiological studies showed that the prevalence of acromegaly could be 4–5 times higher in some geographic areas than in other ones, independently by presumable geographic clustering due to a very rare genetic predisposition. In 2010, we showed that prevalence of acromegaly in the province of Messina (654.601 inhabitants) was 97 c.p.m., similar to that reported in other studies performed in other European country, but it was dramatically higher in a high-risk zone for environmental crisis (RR 2.36, 95% CI 1.20–4.64, $P=0.01$). In this zone the prevalence was 210 c.p.m. and increased to 238 c.p.m. when four neighboring small towns, identified on the basis of increased air pollutants distribution, were enclosed into the high-risk zone. In this wider area, laryngeal, bone, and connectival tumors, among men, and all kind of tumors, among women, were more prevalent than in the population residing in surrounding towns in a 15 km radius area. On the basis of studies performed from 2002, daily atmospheric concentrations of non methanic hydrocarbons (NMHC), and volatile organic compounds (VOC) are dramatically increased in the high-risk zone. The risk to develop PAs after exposition to environmental toxics was evaluated also in Seveso populations, after accidental dioxine exposure, demonstrating that the relative risk of developing PAs was increased, despite not significantly. The effect of several endocrine disruptors in the proliferative promotion and in hormone secretion of pituitary cells (CH3, MtT/E-2, HeLa, etc.) is well known, but their role on pituitary tumorigenesis have been poorly investigated until now.
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## S33.2

**Exposure to endocrine disrupting chemicals may interfere with reproductive development in humans and affect timing of puberty**

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Man-made chemicals in our environment may be harmful, in particular for the developing child with potential irreversible effects into adulthood. Considering the rapid increase in use and distribution of such chemicals over the past decades, it is tempting to speculate whether these changes contribute to the increase in some health problems.

The prevalence of hypospadias and cryptorchidism in boys has increased in some countries, parallel to adult testicular cancer and impaired semen quality. Many countries report an earlier onset of puberty, in particular in girls. In addition, there is evidence that some environmental chemicals contribute to postnatal obesity.

Our data from prospective cohort studies show associations of early pre- and perinatal chemical exposure with reproductive outcomes in both sexes, body composition and puberty.

Exposure to persistent chemicals such as polybrominated diphenyl ethers (flame retardants) and polychlorinated pesticides increased the risk of giving birth to a son with cryptorchidism. Exposure to phthalate monoesters (plastic emollients) was negatively correlated with infant serum testosterone levels and anogenital distance (AGD). AGD is a measure of androgen effect in the newborn, which is positively associated with semen quality in adulthood. Childhood exposure to phthalates additionally lowered thyroid hormone and IGF1 levels and affected growth patterns.

Early prenatal exposure to modern pesticides was associated with earlier onset of breast development in girls, smaller genital size in boys and an increase in body fat percentage. The latter effect was enhanced if the mother also smoked during pregnancy and the child was carrier of a specific genetic polymorphism, underlining the importance of interaction between life style, genetic susceptibility and exposure scenarios.

Thus, current evidence suggests that growth and reproductive development in both sexes may be susceptible to endocrine disrupting chemicals.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## S33.3

**Effect of iodine intake on thyroid diseases in China**

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It has been 14 years since universal salt iodization was conducted legislatively throughout China in 1996, and the residents experienced the period of excessive iodine intake (MUI > 300 μg/l) for 6 years and more than adequate iodine intake (MUI 240 μg/l) for 8 years, and now the status of iodine is normally adequate (MUI 180 μg/l).

During this period, we completed several epidemiological studies on the relationship between iodine intake and thyroid diseases. (1) A 5-year follow-up study ($n = 3761$) conducted in three regions with different levels of iodine intake (MUI 84, 243 and 651 μg/l respectively) showed that the incidence of subclinical hypothyroidism increased by 11.3 and 12.6 times in the communities with more than adequate iodine intake and excessive intake, respectively. The incidence of autoimmune thyroiditis increased by 4.4 and 5.5 times respectively. A shift in iodine intake from mildly deficient to more than adequate was a risk factor for the development of subclinical hypothyroidism to overt hypothyroidism. the incidence of papillary thyroid carcinoma significantly increased in the community with excessive intake of iodine (MUI 651 μg/l). (2) A cross-sectional study ($n = 3813$) conducted in two communities with different levels of iodine intake (MUI 261 and 145 μg/l, respectively) showed that the prevalence of subclinical hypothyroidism and autoimmune thyroiditis were significantly higher in subjects from the region with more than adequate iodine intake than with adequate iodine intake. (3) A follow-up study conducted in pregnant women ($n = 610$) showed that the prevalence of post-partum thyroiditis significantly increased in the subjects with more than adequate and excessive iodine intake. (4) A cross-sectional epidemiologic study of ten cities ($n = 15\,008$) showed that the prevalence of overt hypothyroidism, subclinical hypothyroidism, positive TPOAb and TgAb in the region with more than adequate iodine intake (MUI 240 μg/l) were significantly higher than adequate iodine intake (MUI 189 μg/l).The serum TSH level significantly increased with the increase of iodine intake.

In conclusions, supplementation of iodine should be maintained at a safe range with MUI 100–200 μg/l in order to ensure the thyroid health of susceptible populations (more than 10% of the general population).It is necessary to establish the region iodine-specific reference interval of TSH to avoid the influence of iodine status.

Declaration of interest

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# Effects of thyroid hormone derivatives

## S34.1

**Pathways of thyroid hormone (TH) metabolism**

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TH are derived from the iodinated amino acid tyrosine and coupled to their typical diphenylether structure. Metabolic modifications of TH lead to activation, inactivation or novel quality not exerted by the parent molecule L-$T_4$. Phenolic-5′-(outer) and tyrosyl-3′-(inner ring) deiodination are well known metabolic pathways. Activating 5′deiodination of $T_4$ to $T_3$ is catalysed by two distinct DIO1 and DIO2 selenoproteins, with distinct biological characteristics, developmental profiles, tissue distribution and regulation. DIO2 significantly contributes to local $T_3$ production while DIO1, highly expressed in hepatic, renal and thyroid tissue, mainly generates $T_3$ for systemic circulation. DIO3 represents the key enzyme for $T_4$ and $T_3$ inactivation in various tissues, diseases and during development. Inappropriate function of DIO3 during development alters the HPT set point and over-expression in juvenile hemangioma leads to 'consumptive hypothyroidism'. TH metabolism at the alanine side chain generates Tetrac and Triac, both found in the blood. Tetrac antagonizes $T_4$ and $T_3$ activation of the cell membrane $\alpha_v\beta_3$ integrin receptor, possibly involved in tumour proliferation and angiogenesis. Triac, a potent TR ligand, is clinically used in TH resistance without producing concomitant $T_3$-typic cardiac effects. Recently, 3T1AM has been 're-discovered' as a potent 'cooling' thyroid TH metabolite. Aromatic amino acid decarboxylase had been proposed as the enzyme catalysing decarboxylation of the TH alanin side chain to thyronamines, but this hypothesis has recently been refuted. Oxidative diphenylether-ring cleavage inactivates TH generating DIT, found in septic serum and probably generated by activated macrophages and leucocytes. Conjugation of the 4′ phenolic OH group with sulphate or glucuronide inactivates TH and leads to their biliary excretion and enterohepatic recycling.

Several major and minor pathways of TH metabolism have been characterized during the last decades, but still detection of novel, relevant TH metabolites such as 3T1AM brings surprises for thyroid community. Supported by DFG and BMBF grants.

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## S34.2

**Thyroid hormone mimetic compounds**

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Thyroid hormones $T_4$ and $T_3$ regulate many different physiological processes in different tissues in vertebrates. Most of the actions of thyroid hormones are mediated by the thyroid hormone receptor (TR), which is a member of the nuclear receptor superfamily of ligand-activated transcription regulators. There are two different genes that encode two different TRs, TR alpha and TR beta, and these two TRs are often co-expressed at different levels in different tissues. TRα is the major TR in the heart, and is crucial for heart rate and for cardiac contractility and

relaxation, whereas TRβ is the predominant TR isoform in the liver and is important for lipid metabolism. Compounds that selectively modulate thyroid hormone action by functioning as isoform-selective agonists or antagonists of the TRs might be useful for medical therapy. We present here the design, synthesis and biopharmacological profile of a series of halogen free TH analogs, either agonists or antagonists, structurally related to our TRbeta selective lead compound GC-1, also known as QRX-431 and Sobetirome, which recently completed Phase I clinical studies as a cholesterol-lowering agent. Selective thyroid hormone modulators provide useful experimental probes to better define thyroid hormone actions, and most importantly some of them have the potential to become novel therapeutic agents.

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## S34.3

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# Endocrinology and the Olympics: Will hormones help to win?

## S35.1

### Does the high performance athlete need hormone replacement?

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Endogenous hormones (e.g. catecholamines, glucocorticoids, growth hormones, androgens, growth factors) may affect the characteristics of physical capacity and performance in athletes by influencing, throughout long-term and short term effects, morphological and functional qualities of neuromuscular, cardiovascular, metabolic and adaptive systems. Exercise per se is associated to the release of different hormones: acute exercise stimulates an acute hormones secretion (e.g. catecholamines, growth hormone, CRH-ACTH-cortisol, testosterone) while chronic exercise (training) is able to modify hormones secretion at rest and their activation during acute exercise. A physiological quantitative/qualitative hormone release at rest (e.g. for long-term effects) and an adequate hormones activation during exercise (e.g. for short-term effects) are essential to reduce specific health risks and to guarantee optimal performances in athletes. Consequently, in athletes with reduced/altered hormonal secretion a correct and specifically adapted replacement therapy is necessary. In fact, at least in theory, for health-protective concerns and to guarantee optimal/maximal physiological performances the hormone replacement in athletes should assure the physiological amount of the reduced hormone and to reproduce, as much as possible, its physiological profile/rhythm and specific activation during exercise. In this sense, besides symptomatic classical diseases or conditions that may reduce/alter the qualitative/quantitative hormones secretion, serious clinical concerns exist for asymptomatic endocrine hypo-function (e.g. sub-clinical hypogonadism, growth hormone deficit and hypothyroidism), particularly in adult athletes. For example, in master athletes we observed an high prevalence of undiagnosed severe (12%) and mild (18%) hypo-testosteronemia frequently in the absence of clinical symptoms. In our opinion, a testosterone replacement therapy should be considered in all athletes with true hypogonadism (e.g. no related to anabolic androgens abuse) independently from the presence of classic symptoms of hypogonadism and if no contraindications exist. Unfortunately, few studies evaluated the prevalence of reduced hormones secretion in athletes and the concept of adapted hormone replacement in high competitive athletes.

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## S35.2

### Can an endocrine patient participate in high performance sports?

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The question posed by this title encompasses three issues. First, certain endocrine diseases present physiologic challenges to high-intensity or endurance activities, including type 1 diabetes mellitus and adrenal insufficiency. Using carefully adjusted insulin regimens evolved in concert with training sessions, several athletes with type 1 diabetes have succeeded in elite competitions, but the endocrinologist must 'train with the athlete' and craft regimens that continuously deliver both insulin and glucose. Patients with adrenal insufficiency might require additional hydrocortisone for training in extreme circumstances of heat and intensity, even when fluid and electrolyte replacement appears adequate. Second, specific endocrine disorders require treatment with hormones, which are banned performance-enhancing substances. Men with hypogonadism require testosterone replacement, and type 1 diabetics require insulin therapy. For these athletes, a process called Therapeutic Use Exemption (TUE) has been developed, and this process will be reviewed. Third, certain endocrine disorders and genetic variations might confound testing for doping with androgens and lead to false-positive or false-negative test results. For example, women with 21-hydroxylase deficiency excrete higher amounts of androgens and their metabolites than unaffected women, and depending on the testing method, might be flagged for testosterone doping. A common deletion in the UGT2B17 gene impairs the capacity of some testing methods to detect testosterone doping by lowering the excretion of testosterone glucuronide but not epitestosterone glucuronide. Efforts to improve the accuracy of testing for doping with testosterone or other endogenous androgens will be discussed.

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## S35.3

### Hormone abuse in sports: the knowns and unknowns

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Sports drug testing laboratories are facing multifaceted challenges including the misuse of naturally/endogenously occurring substances, non-approved/discontinued drug candidates, urine manipulation, etc. In order to enable the differentiation of licit drug use and/or the natural variation of hormone concentrations (due to e.g. legal interventions) from doping offences, best-possible analytical performance is required. Therefore, modern doping control analytical assays commonly employ mass spectrometry-based and immunological approaches to detect prohibited substances and methods of doping exploiting the respective advantages.

With the constantly increasing analytical requirements concerning the number of target compounds, the complexity of analytes (e.g. peptides and proteins) as well as the desire to accelerate analyses and obtain information allowing also for retrospective data mining, high resolution/high accuracy mass spectrometry has gained much attention recently. This methodology is successfully complemented and seconded by selected immunoassays targeting in particular peptide hormones such as human growth hormone (hGH) and erythropoietin (EPO). In the course of the presentation, advances and limitations of current methods in sports drug testing will be outlined by means of selected examples demonstrating the complexity of measuring mostly known and partly unknown substances from a limited volume of serum or urine.

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## Bone and metabolism

### S36.1

**Leptin and bone signaling**

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Osteoporosis is caused by a failure of bone homeostasis. The precise molecular mechanism controlling bone homeostasis is largely unknown. Increasing evidences that neurons and neurotransmitters are intimately involved in bone remodeling shed light on a novel regulatory mechanism for bone homeostasis. Namely, like all other homeostatic functions, bone remodeling is under the control of hypothalamus. We have uncovered that leptin, an adipocyte-derived anorexigenic hormone, regulates bone mass through its receptor located in the central nervous system. Subsequent analysis revealed that leptin uses sympathetic nervous system to inhibit bone formation and stimulate bone resorption. Furthermore, serotonin has emerged as an indispensable molecule linking leptin, bone and energy metabolism. We also found that NMU, another anorexigenic neuropeptide, is a novel central mediator of leptin-dependent regulation of bone mass. In addition, many epidemiological studies confirmed the effect of beta blockers on bone mass and fracture. We are currently analyzing the bone abnormality in mice lacking other neuropeptides. I will summarize the advance of this brain-bone-adipo axis research field.

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### S36.2

**Insulin-bone axis**

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Energy homeostasis in mammals is controlled by the actions of circulating hormones, which coordinate fuel production and utilization between metabolically active tissues. Mounting evidence implicates the osteoblast as an important player in the coordination of global energy utilization through its hormonal interactions with other tissues. Leptin produced by adipocytes controls postnatal bone acquisition by activating sympathetic nerves whose efferent outputs target $\beta_2$-adrenergic receptor on osteoblasts to regulate their proliferation and differentiation. Insulin stimulates osteocalcin production by the osteoblast, which in turn stimulates insulin secretion by the pancreas. These observations suggest that skeletal cells such as the osteoblast, whose cellular ancestry is common to fat and muscle, evolved pathways to participate in global energy homeostasis. New basic questions arising from these findings will probe the precise nature of the fuel sensing and processing mechanisms in the osteoblast and their relative contribution to overall glucose disposal and utilization. The answers to these questions should improve our ability to diagnose and manage patients with metabolic diseases.

### S36.3

**Functions and mode of action of osteocalcin**

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We have proposed twelve years ago that bone mass accrual, energy metabolism and reproduction must be coordinately regulated by hormones that would appear during evolution with bone. This hypothesis has been tested and verified in several ways one of them being to show that osteocalcin, the most osteoblast-specific secreted protein, is a hormone that regulates insulin secretion, glucose homeostasis, energy expenditure and male fertility. The metabolic function of osteocalcin has been confirmed, through correlative studies, in humans. Work in our laboratory has begun to unravel the signaling pathway used by osteocalcin to fulfill some of its actions. We will present at the meeting the current state of knowledge signaling the functions and mode of action of this hormone.

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## Diabetes and cancer

### S37.1

Abstract unavailable.

### S37.2

Abstract unavailable.

### S37.3

**Goiter throughout the pediatric ages**

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Goiter may be present before birth in utero, at birth or detected at any age thereafter. The goiter may be caused by increased thyrotropin (TSH) secretion resulting from hypothyroidism; antibodies that activate TSH receptors (Graves' disease) with increased thyroid hormone secretion; or TSH-independent processes, such as inflammation associated with autoimmune thyroiditis, benign and malignant tumors, and infiltrative disease.

The causes of goiter in fetuses, infants, children, and adults are similar, but their relative frequency varies substantially. In the United States and in Europe, for example, most children with a goiter have chronic autoimmune thyroiditis; whereas, among adults, nontoxic nodular goiters predominate. Goiter in neonates and infants will be more often due to dyshormogenesis whereas in older children and fetuses the major causes will be autoimmune disorders.

We will not cover all the etiologies but rather will focus on less known forms of goiter such as in fetuses where the therapeutic options may appear controversial. In pregnant women with past or current Graves' disease, ultrasonography of the foetal thyroid gland by an experienced ultrasonographer is an excellent diagnostic tool that will inform of potential fetal thyroid dysfunction if a goiter is noticed; close teamwork among internists, endocrinologists and obstetricians, echographists and paediatrician, can ensure normal foetal and neonatal thyroid function. Some rare studies have confirmed the feasibility and safety of intrauterine L-thyroxine treatment for nonimmune fetal goitrous hypothyroidism when detected. Whether it allows goiter size reduction in the majority of cases, the effectiveness to reach euthyroidism at birth remains inadequate; monitoring of fetal thyroid function by amniocentesis was not adequate. In the latter situation, the risks to the foetus and the psychological burden on the parents should be factored into the risk to benefit evaluation.

The possibility of delayed detection of goiter due to congenital dyshormonogenesis rather than autoimmune goiter will also be pinpointed.

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## Craniopharyngioma: Hypothalamic complications

### S38.1

**Childhood Craniopharyngioma**

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Craniopharyngiomas are embryogenic tumorous malformations of the sellar region, presumably derived from Rathke cleft epithelium. With an overall

incidence of 0.5–2 new cases per million population per year, approximately 30–50% of all cases represent childhood craniopharyngioma. Typical manifestations at primary diagnosis are headache, visual impairment, polyuria/polydypsia, growth retardation, and weight gain.
Therapy of choice in patients with favorable tumor localization is total resection with the intention to maintain optical nerve and hypothalamic-pituitary functions. In patients with unfavorable tumor localization (hypothalamic tumor involvement), a limited resection followed by local irradiation is recommended.
Survival rates are ranging from 91 to 98%. Recurrences after complete resection and progressions of residual tumor after incomplete resection are anticipated subsequent events after primary surgery. Accordingly, the appropriate time point of irradiation after incomplete resection is currently under investigation in a randomized trial (KRANIOPHARYNGEOM 2007).
Quality of survival is frequently impaired due to the proximity to optical, pituitary and hypothalamic structures. Long-term sequelae substantially reduce the quality of life of ~50% of long-term survivors, notably extreme obesity owing to hypothalamic involvement and/or surgical- or radiation-induced lesions especially of posterior hypothalamic structures.
Initial hypothalamic tumour involvement, especially when both, anterior and posterior areas are involved, has an apriori effect on the clinical course. Therefore, the recommendations are based on recognizing childhood craniopharyngioma as a chronic disease requiring commensurate monitoring and medical resources for treatment and follow-up in order to provide the best lifetime quality of life for the patient.
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## S38.2

**Hypothalamic disorders in Clinical practice; relevance to clinical practice**

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Craniopharyngioma (CP) is associated with considerably higher mortality and morbidity than pituitary adenomas. The tumours are rare, but present a considerable management challenge. In addition to the endocrine and mass effects which are common to all tumours arising from the region of the pituitary fossa, the site, size, and sometimes the treatment of CP, dictate that the endocrinologist must also manage hypothalamic complications. Endocrinologists are aware of the high frequency of diabetes insipidus in CP, which, in contrast to pituitary adenomas, may be present prior to surgical intervention. However diabetes insipidus may be complicated in craniopharyngioma by thirst disturbances. These include adipsia/hypodipsia, which predisposes to hypernatraemia, particularly during intercurrent illness, and polydipsia, which may lead to hyponatraemia when the patient is treated with desmopressin for DI. Obesity is very common in CP patients and is most likely multifactorial; some patients have polyphagia and are sometimes referred to as having hypothalamic obesity, whereas others have somnolence and low exercise levels. The contribution of hormonal disturbances to obesity remains to be fully explained. A number of groups have shown disturbances of sleep, including obstructive sleep apnoea, in CP, and daytime somnolence in the absence of sleep disorders has also been reported. The heterogenous nature of somnolence in CP indicates that patients should be fully evaluated as a number of therapeutic interventions are available which can improve quality life. Many authorities have hypothesised that hypothalamic complications of CP may be related to the effects of destructive neurosurgery and recommendations for less extensive surgery with post operative radiotherapy is partly predicated on aspirations to minimise high morbidity hypothalamic complications in CP patients.

## S38.3

**Management of hypothalamic obesity in patients with craniopharyngioma**

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The presence of a craniopharyngioma, and/or treatment related damage to centres in the hypothalamus that regulate energy balance often results in severe obesity and abnormal eating behaviours. This may be exacerbated by hormonal deficits from coexistent hypopituitarism.
Decreased activity of sympathoadrenal activity, deregulated parasympathetic activity, marked hyperinsulinaemia and elevated leptin disproportionate to fat mass are evident. Plasma levels of alpha-melanocyte stimulating hormone are reduced. Delayed suppression of ghrelin after food intake, and in part the hyperinsulinaemia, along with hyper-responsiveness of GLP1 release to glucose may reflect dysregulation of vagal neural circuits mediating gastro-intestinal function. Intentional and non-exercise associated physical activity decreases. Disruption of the co-ordination of circadian rhythms including the sleep wake cycle and the presence of obstructive sleep apnoea may also be contributory.
Efforts to optimise diet and levels of physical activity and pituitary hormone replacement, including the minimisation of hydrocortisone dose, adequate sex steroid replacement, and use of growth hormone and CPAP may improve overall health and well-being, but have minimal effect on weight and eating behaviour. The long acting somatostatin analogue octreotide lowers insulin levels, but does not produce significant weight loss, nor does low dose dexamphetamine, although they may help to stabilise weight. Dexamphetamine or modafinil may be helpful when daytime somnolence is problematic. Metformin improves metabolic state and may also help to ameliorate weight gain.
Bariatric surgery, in particular the Roux-en-Y gastric bypass (RYGB), has been shown to be a safe and highly effective treatment modality; an illustrative case and review of the evidence will be provided.
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# Adrenal insufficiency

## S39.1

Abstract unavailable.

## S39.2

**Molecular pathomechanisms of steroidogenetic adrenal disorders**

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Adrenal steroid hormones are vitally important. Deficient adrenal steroid biosynthesis causing a lack of glucocorticoid and / or mineralocorticoid hormones leads to hypoglycaemia, hypotension, salt loss and death. Concomitant changes in adrenal androgen biosynthesis are responsible for virilisation in females or undervirilisation in males. Because of the importance of adrenal steroids for overall survival and reproduction, defects in steroidogenesis and steroid action are rare and have serious consequences. The steroidogenic enzymes belong to the cytochrome P450 enzymes and hydroxysteroid dehydrogenases. Cytochrome P450 enzymes can be subdivided into mitochondrial type 1 enzymes and endoplasmic type 2 proteins. The hydroxysteroid dehydrogenase are either aldo-keto reductases or short-chain dehydrogenases. Expression of the genes that mediate steroidogenesis is tightly controlled by numerous transcription factors such as NR5A and GATA family members. The enzymatic activity is further affected by posttranslational modifications or protein-protein interaction with cofactors, e.g. responsible for electron transfer. Naturally occurring sequence variations in steroidogenic enzymes are responsible for various forms of isolated or complex adrenal insufficiency. By studying the molecular genetics of these rare defects *in vitro* and in silico, the underlying molecular mechanisms can be described. Hereby, protein residues involved in heme positioning and binding, substrate access and product release, electron transfer and cofactor binding as well as regions inhibiting proteolysis have been detected. These analyses give further insights in the structure-function relationships of steroidogenic enzymes what is of fundamental importance for the understanding of adrenal diseases, disorders of sexual differentiation or reproduction.

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## S39.3

### New developments in the immunology of Addison's disease

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Autoimmune Addison's disease (AAD) is an endocrine disease resulting from the immune system's destruction of hormone producing cells in the adrenal cortex. Autoantibodies against the steroidogenic enzyme 21-hydroxylase (21OH) is the hallmark of this form, and found in about 85 percent of patients in European populations. Risk factors are both genetic and presumably environmental. Genes associated with AAD are MHC class II especially the DR locus, MHC class I and MIC-A, but other immune genes such as PTPN22 and CTLA-4 also contribute. Recently, T cells reactivities against 21OH have been observed in patients with AAD, all of which had autoantibodies against 21OH. Moreover, the high-risk genotype DR3/DR4(0404) was frequently observed among those showing T cell proliferation. Autoantibodies potentiated the proliferative response and epitope mapping revealed that the peptide 21OH(342–361) was responsible for much of the reactivity. The adrenocortical cell itself may participate in the autoimmune destruction by expressing toll-like receptors and producing chemokines such as interferon inducible protein 10 thereby attracting lymphocytes to the adrenal cortex.
We hypothesized that the hormone producing cells in the adrenal cortex under proinflammatory conditions, such as viral infections, play a critical part in the induction of autoimmune Addison's disease in genetically susceptible individuals.
Declaration of interest
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# New familial endocrine cancer syndromes: pathophysiology and counselling

## S40.1

### DICER1 mutations characterize a novel syndrome with endocrine features

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DICER1 is a microRNA processing-RNase III-type endoribonuclease and is crucial for embryogenesis and early development. Nearly 50 different heterozygous germ-line DICER1 mutations have been reported world-wide in individuals who developed, as children or young adults, pleuropulmonary blastoma, cystic nephroma, ovarian sex cord stromal tumors (especially Sertoli–Leydig cell tumor), multi-nodular goiter, embryonal rhabdomyosarcoma (of cervix and other typical sites), Wilms Tumor (WT) and other rare phenotypes, including pituitary blastoma. The latter is a very rare tumor with an apparently specific presentation: infants less than age 24 months with Cushing's syndrome or diabetes insipidus; the three investigated to date all carry DICER1 mutations.
In addition, single cases of primitive neuroectodermal tumor, pulmonary sequestration, juvenile intestinal polyps, adult-onset pleomorphic sarcoma, ciliary-body medulloepithelioma and pineoblastoma have been reported in DICER1 mutation carriers.
More recently, somatic mutations in DICER1 have been identified in nearly 30% of non-epithelial ovarian tumor, notably 60% in Sertoli–Leydig cell tumors, including 4/4 from individuals with germline DICER1 mutations. Somatic mutations involved codons encoding metal-binding sites within the RNase IIIb catalytic centers, critical for microRNA interaction and cleavage. Mutations were also found in non-seminomatous testicular germ-cell tumor (1/14), embryonal rhabdomyosarcomas (2/5), and ovarian/endometrial carcinoma (1/266). The mutant DICER1 proteins showed reduced RNase IIIb activity but retained RNase IIIa activity.
In an extension of this work, we have recently identified somatic DICER1 mutations, both within and outside the RNaseIIIb domain, in a small percentage of apparently sporadic WT. Notably, all three WT with germ-line DICER1 mutations possessed likely pathogenic somatic mutations. Since these mutations do not obliterate DICER1 function but alter it in specific cell types, the traditional two-hit model of carcinogenesis does not seem to apply.
The prevalence of endocrine presentations (goitre, androgenisation in young women and infants with pituitary dysfunction, especially Cushing syndrome) indicates that endocrinologists should consider germ-line DICER1 mutations when evaluating children and adolescents with these distinctive clinical presentations.
Declaration of interest
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## S40.2

### The paediatric patient with paraganglioma syndrome

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Pheochromocytomas and paragangliomas (PCC/PGL) are rare tumours of the adrenal gland or derived from sympathetic and parasympathetic paraganglia occurring sporadically or as part of a familial cancer syndrome. Up to 20% of them are diagnosed in children, and they are not genetically well characterized. As most are functional tumors, children more often present with signs and symptoms related to hypertension. In fact, sustained hypertension is found in more than 60–90% of paediatric pheochromocytoma cases, whereas it is reported in only 50% of adult cases.
Originally, it was suggested that 10% of PCCs are hereditary, but current advances in molecular genetics have shown that germline mutations occur in up to 30–40% of PCC/PGLs. There are several features to take into account to suspect a hereditary tumor syndrome. Among them, the family history, the presence of multiple tumours or the age of onset are important clues to estimate the risk of a hereditary tumor syndrome. In this regards, 40% to 59% of PCCs patients younger than 18 years have germline mutations in some of the PCC/PGL susceptibility genes, and this proportion increases to 70% for those presenting before 10 years of age. According to data available, VHL is the most commonly (42%) mutated gene, followed by SDHB, SDHD, RET, and NF1. In some cases PCC/PGL presenting during childhood represents an early manifestation of an adult disease caused by predisposing germline mutations. Therefore, genetic testing should be performed in every child with PCC/PGL, because a proper characterization of the underlying mutation is a key factor in estimating the risk for development of malignant disease and contralateral tumour, which may also guide the follow-up and clinical management.
Declaration of interest
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## S40.3

### Li-Fraumeni Syndrome: A paradigm of genetic testing to clinical surveillance for pediatric endocrine tumors

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Li Fraumeni syndrome (LFS) is a prototypic cancer predisposition syndrome, characterized by multiple, early-onset malignant tumours including breast cancer, bone and soft tissue sarcomas, brain tumors and adrenocortical carcinoma (ACC). LFS is most commonly attributed to germline mutations in the TP53 tumor-suppressor gene. p53 activity is tightly regulated by multiple post-translational

mechanisms, disruption of which may lead to tumorigenesis. ACC occurs at disproportionately high rates among p53-mutation carriers, suggesting tissue-specific manifestations of p53 deficiency. Additionally, p53-associated ACC demonstrates a strong predominance in infants and children. Several of the p53 alleles associated with pediatric ACC retain significant wild-type activity and demonstrate incomplete penetrance, a finding distinct from other LFS-component tumors. This presentation will evaluate current knowledge of the relationship between p53 and adrenocortical carcinogenesis, with specific focus on disease-specific alleles, tumorigenesis in the context of adrenal development and potential therapeutic approaches to p53-associated ACC. In addition, evaluation of the implementation of a clinical surveillance protocol for early tumor detection will be discussed. The example of LFS as a paradigm of translation from molecular testing to clinical intervention will be highlighted in this session.
Declaration of interest
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## The endocrinology of adipose tissue

### S41.1

**Physiological and neuronal determinants of brown adipose tissue-mediated thermogenesis in small mammals and humans**
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Brown adipose tissue (BAT) is a thermogenic organ. Its tremendous thermogenic potential is conferred by uncoupling protein 1 (UCP1), which dissociates ATP synthesis from energy substrate oxidation and thereby insures heat production. BAT represents a key thermogenic effector implicated in thermoregulatory thermogenesis. The physiological control of BAT activity and capacity is ensured by the sympathetic nervous system (SNS), which densely innervates brown adipocytes. SNS-mediated BAT thermogenesis is essentially governed by hypothalamic and brainstem neurons. BAT is not only controlled by brain thermoregulatory circuits but also by brain energy balance pathways including the brain melanocortin pathway, whose major role in energy balance tends to support a genuine involvement of SNS-mediated BAT thermogenesis in energy homeostasis. BAT could be involved as a thermogenic effector not only in small mammals but also in humans. The use of molecular imaging procedures such as positron emission tomography / computed tomography (PET/CT) scanning have revealed noticeable BAT depots in the cervical, clavicular, paraspinal areas in adult humans. Moreover, there is recent evidence pointing to the presence of brown adipocytes in the human epicardial adipose tissue. The detection/prevalence of these depots was reported to increase with exposure to low temperature, to be higher in women than in men, and to decrease with age and body fat mass. The purpose of this short article is to provide an overview of the recent advances made in our understanding of the physiological and neuronal determinants of BAT thermogenesis in laboratory rodents and adult humans.
Declaration of interest
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### S41.2

**'Obesity and insulin resistance: the cross-talk macrophage-adipocyte view'**
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H Girona, Girona, Spain.

Background
Adipocytes and macrophages resident in adipose tissue produce and secrete a variety of biologically active mediators (adipokines) that contribute to the development of insulin resistance, type 2 diabetes mellitus and cardiovascular disease. Malfunction of adipocytes may play an important role in the development of low-grade inflammatory state associated with obesity.
Objectives
Describe alternative ways to adipokines with potential impact on the development of this inflammatory state associated with obesity.
Methods
Review of recent contributions in the field.
Results
There are new circulating markers that can help identify the development of inflammation and insulin resistance in obese subjects. The study of some components of the immune system and of the complement system helps to better understand their relationship with carbohydrate metabolism. Examination of the bacterial flora composition sheds new ways in this regard as well as the shortening of telomere length in cells from adipose tissue. Finally, a detailed study of microRNAs present in adipose tissue is a relatively unexplored field of knowledge.
Conclusions
The exploration of these tools could provide new therapeutic approaches in insulin resistance associated with inflammation.
Discussion
The balance between different inflammatory factors and anti-inflammatory systemic level should be further explored to better understand the different eukaryote and prokaryote biological processes involved in a complex phenotype such as insulin resistance.
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### S41.3

Abstract unavailable.

## Novel insights into regulation of puberty

### S42.1

**Central networks controlling the timing of puberty**
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Attainment of mammalian reproductive capacity depends on an increased pulsatile release of gonadotropin releasing hormone (GnRH) from hypothalamus neurons. After reaching the pituitary gland GnRH stimulates the synthesis and release of gonadotropins, which in turn promote gonadal function. The pubertal activation of GnRH secretion is brought about by coordinated changes in transsynaptic and glial-neuronal communication, which involve an increase in neuronal and glial excitatory inputs to the GnRH neuronal network and a decrease in transsynaptic inhibitory influences. Coordination of this regulatory neuronal-glial network appears to require gene networks hierarchically arranged. One level of coordination is provided by subordinate genes encoding proteins required for dynamic cell–cell communication. A second level is provided by subordinate genes engaged in intracellular signaling. A third and higher level of control involves the transcriptional regulation of these genes by a handful of genes that acting as 'central nodes' sustain the functional integration of the network. The existence of functionally connected genes controlling the pubertal process is consistent with the concept that puberty is under genetic control, and that the genetic underpinnings of both normal and deranged puberty are polygenic rather than specified by a single gene. It is unclear, however, how such inherited, permanent changes in DNA sequence can dynamically coordinate the expression of gene sets controlling the pubertal process. Using high-throughput approaches and computational methods for global analysis of DNA methylation and chromatin modifications, as well as quantification of mRNA and protein expression, we have obtained evidence suggesting that the activity of gene networks controlling puberty is coordinated by an epigenetic mechanism of transcriptional repression, which prevent the premature unfolding of the pubertal process.
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## S42.2

**Re-wiring the hypothalamus with new peptides to control puberty**

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Puberty is a tightly regulated process by which an individual attains reproductive capability. An intricate network of central and peripheral factors has been described to play a role in this process; however, the mechanism triggering puberty onset remains largely unknown. Recently, the neuropeptides kisspeptin (encoded by Kiss1) and Neurokinin B-NKB- (encoded by TAC3 in humans and Tac2 in rodents) have been placed as essential gatekeepers of puberty onset. Studies in humans and rodents have revealed that loss-of-function mutations in the genes encoding either Kiss1/NKB or their receptors, Kiss1r/neurokinin 3 receptor (NK3R), lead to the absence of sexual maturation and infertility. Kisspeptin, NKB and dynorphin A are co-expressed in neurons of the arcuate nucleus (Arc), so called KNDy neurons. Importantly, these neurons also co-express NK3R. Compelling evidence suggests a stimulatory role of NKB (or the NK3R agonist senktide) on LH release in a number of species and exogenous administration of senktide is able to rescue puberty onset in undernourished female rats. This effect is likely mediated by autosynaptic inputs of NKB on KNDy neurons to induce the secretion of GnRH in a kisspeptin-dependent manner with the coordinated action of other neuroendocrine factors such as dynorphin, glutamate or GABA. Thus, we have proposed a model in which NKB feeds back to the kiss1 neuron to shape the pulsatile release of kisspeptin, and hence GnRH, in a mechanism also dependent on the sex steroid level. Investigating how the GnRH pulse generator activates during puberty onset and remains functional in adulthood is a primary goal in recent neuroendocrinology of reproduction.

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## S42.3

**Novel insights into regulation of puberty: lessons from human genetics**

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The human pubertal development is a very complex biological process that can be influenced by multiple factors including the genetic ones. A growing list of genes has been implicated in the pathogenesis of the congenital isolated hypogonadotropin hypogonadism (IHH) pointing to the complexity of the genetic basis of this condition. These genes encode peptides, which are involved in the development and migration of GnRH neurons or in regulation of synthesis, secretion and action of GnRH. Among several distinct genes associated with IHH, two neuropeptides are the main regulators of the GnRH secretion: Kisspeptin and its receptor KISS1R and neurokinin B (TAC3) and its cognate receptor TACR3. Inactivating mutations have been identified in KAL1, GNRHR, FGFR1, KISS1 and KISS1R, PROK2 and PROK2R and more recently in TAC3 and TACR3 genes in patients with congenital IHH.

Central precocious puberty (CPP) has a striking predominance among girls, and in most cases is considered idiopathic. However, a familial history of early sexual maturation in girls with idiopathic CPP suggests a genetic basis for this condition. We hypothesized that gain-of-function mutations of KISS1 or of KISS1R genes might be associated with CPP. The new heterozygous mutation R386P in the GPR54 gene was identified in a Brazilian girl with CPP, the first evidence of non-constitutively dominant activating mutation of GPR54 related to the development of precocious puberty in humans. Functional study of this unique GPR54 mutation demonstrated a prolonged activation of intracellular GPR54 signaling pathways in response to kisspeptin. We also investigated the presence of potential variants in KISS1 gene and the new heterozygous P74S mutation was found in a boy with CPP. The capacity to stimulate signal transduction was significantly greater for P74S compared to the wild type after pre incubation of wild type and mutant kisspeptins with human serum, indicating that the mutant was more resistance to degradation.

Most of the identification of the molecular defects came from well characterized human phenotypes. The several emerging next-generation sequencing technologies for whole genome analyses surely will allow the identification of new genes responsible for congenital pubertal disorders.

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# Graves' orbitopathy (GO)

## S43.1

**Immunopathogenesis of Graves' orbitopathy**

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Graves' orbitopathy (GO) is an inflammatory autoimmune disorder of the orbit. The close clinical and temporal relationships between Graves' hyperthyroidism and GO suggest that both conditions derive from a single systemic process and share the thyrotropin receptor (TSHR) as autoantigen. Autoimmunity directed against the TSHR on orbital fibroblasts sets in motion connective tissue remodeling within the orbit that leads to the various clinical expressions of the disease. Hyaluronic acid (HA) accumulation, expansion of orbital adipose tissues and the presence of inflammatory cells and pro-inflammatory cytokines appear to be the salient histologic features of the disease. Orbital fibroblasts express functional TSHR and are considered to be the target cells. Several laboratories have explored the impact of TSHR activation in these cells on signaling and cellular functions relevant to the orbital tissue changes. Both HA synthesis and new fat cell development are enhanced by activation of orbital fibroblast TSHR, whether effected through ligation of the receptor by TSH or monoclonal antibodies directed against the receptor (TRAb), or by the introduction of an activating mutant TSHR. The phosphoinositide 3-kinase/Akt signaling cascade appears to be an important effector pathway in this process, with input from adenylyl cyclase/cAMP and other signaling pathways. Circulating TRAb in patients with Graves' hyperthyroidism are heterogeneous and have differing potency and affinities. Stimulatory TRAb activate various TSHR signaling cascades in thyrocytes, resulting in the over-production of thyroid hormones. It appears likely that they similarly activate orbital fibroblast TSHR to modulate HA synthesis and adipogenesis. While IGF1 and other growth factors may act in an autocrine or paracrine fashion to impact TSHR signaling, little evidence supports a role in GO for circulating autoantibodies directed against IGF1R. Future therapies may involve the inhibition of TSHR signaling in orbital fibroblasts, perhaps using small molecular ligands that antagonize TSHR effector pathways.

Declaration of interest
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## S43.2

**How to restore euthyroidism in the presence of Graves' orbitopathy? Is there a best way?**

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Around 50% of patients with Graves' disease (GD) have Graves' orbitopathy (GO). However, only 5–10% have overt active disease which merits more than symptomatic treatment. Clearly, the best treatment is prophylaxis related to avoiding tobacco smoking and to obtain and maintain euthyroidism.

Whether antithyroid drugs (ATDs), radioiodine (RAI), or thyroidectomy should be chosen for therapy of GD, whether or not there is presence of active GO, is a matter of debate and randomized controlled trials (RCTs) are scarce. Thus, much is based on 'expert' opinion. ATDs, independent of whether the titration or the block-repacement regimen is used, and thyroidectomy (as long as euthyroidism is maintained) do not influence the natural history of GO. RAI can cause progression or de novo GO in around 15% of GD patients, smokers being particularly at risk. This can at large be prevented by prophylactic glucocorticoid therapy.

In patients with mild or inactive GO, the choice of therapy for GD is independent of GO. In patients with active GO the choice of therapy is mainly based on 'expert' opinion modified by the wishes of the patient and not based on RCTs. Questionnaire surveys indicate that RAI, in this situation, is only used by a small minority of 'experts'. Preliminary data suggest that biological therapy with rituximab (monoclonal anti-CD20 antibodies), aiming at B cell depletion may be advantageous for both GD and GO and thereby offer a shift in paradigm.The recently investigated low-cost administration of the trace-element selenium, also

holds promise. Importantly, a too strong focus on the risk of GO may unintentionally overrule taking availability of therapy options, co-morbidity, perceived risk of overall side-effects, cost-benefit, and the wish of the patient into consideration.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## S43.3

### Progress in immunosuppressive treatment of GO (selenium, steroid dosage, rituximab)

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Management of Graves' orbitopathy (GO) is a challenge, and 30–50% of patients are eventually dissatisfied with medical treatment outcome. GO natural history is poorly understood, but some patients experience a progression of GO over time. Thus, prevention of development/progression by abolishing risk factors (e.g. smoking, thyroid dysfunction) is important. The recent results of a randomized clinical trial (RCT) carried out by the EUGOGO group showed that selenium (sodium selenite for 6 months) prevented progression of mild GO to more severe GO. Selenium, probably acting through antioxidative and immunoregulatory pathways, also caused a significant improvement of preexisting GO compared to placebo. Thus, selenium is a useful tool for prevention of progression and for management of mild GO. Accordingly, a 6-month course of selenium is advisable for patients with mild GO.

Glucocorticoids, preferentially given intravenously, represent the first-line treatment for moderate-to-severe and active GO. The most common regimen employs a cumulative dose of 4.5 g methylprednisolone, subdivided in 12 weekly intravenous infusions. However, evidence concerning the optimal dose in terms of efficacy and side effects is missing. The EUGOGO group has just completed an RCT comparing three different cumulative doses (2.5, 5.0, 7.5 g). Preliminary analysis suggests that the highest dose is more effective (particularly on eye motility) but associated with more major side effects. Thus, for the time being, it is reasonable to say that the dose should be tailored in each patients based on the activity and severity of GO, evaluating potential benefits and risks.

The frequently unsatisfactory response to glucocorticoids underscores the need for novel and more pathogenic treatments. Given the role played by B lymphocytes in GO pathogenesis, rituximab, an agent that depletes CD20-positive B lymphocytes, might be such a drug. Results in the field of GO are preliminary, based on small, uncontrolled studies. But they are promising by showing beneficial effects, mostly in GO patients resistant to conventional glucocorticoid treatment. Two ongoing RCTs (rituximab vs glucocorticoids, rituximab vs placebo) should hopefully provide useful information on the efficacy and safety of this expensive drug.

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# Genome wide studies in reproduction

## S44.1

### Genome-wide association study of endometriosis identifies a locus at 7p15.2 with pleiotropic effects

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Endometriosis is a common gynaecological disease associated with severe pelvic pain and sub-fertility. We conducted a genome-wide association (GWA) study in 3,194 surgically confirmed endometriosis cases and 7060 controls of European ancestry from Australia and the UK using 540,000 genetic markers (SNPs). Polygenic predictive modelling showed significantly increased genetic loading among the 1364 (43%) cases with moderate-severe (rAFS III/IV) endometriosis. The strongest association signal was observed on chromosome 7p15.2 for SNP rs12700667 for all endometriosis ($P = 2.6 \times 10^{-7}$, OR = 1.22 (1.13–1.32)) and for moderate-severe disease, ($P = 1.5 \times 10^{-9}$ (OR = 1.38 (1.24–1.53)). We replicated rs12700667 in an independent US cohort of 2392 self-reported surgically confirmed endometriosis cases and 2271 controls ($P = 1.2 \times 10^{-3}$, OR = 1.17 (1.06–1.28)), resulting in a genome-wide significant $P$ value of $1.4 \times 10^{-9}$ (OR = 1.20 (1.13–1.27)) for all endometriosis in our combined datasets.

Rs12700667 is located in a 48 kb genomic segment of high LD, and marked one of 13 loci associated with waist-hip ratio adjusted for BMI (WHRadjBMI) in an independent GWA dataset of 77,167 individuals using ~2.8 million genotyped and imputed SNPs (top SNP rs1055144: $P = 1.49 \times 10^{-8}$). $P$-values for 5/12 other WHRadjBMI-associated SNPs varied from 0.01 to 0.81 for all endometriosis and from 0.05 to 0.77 for rAFS III/IV cases. The probability of finding a common genetic locus associated with two traits by chance at the given significance levels was, allowing for multiple testing of 6 SNPs, very small: $P = 2.2 \times 10 - 4$. Alleles of SNPs at 7p15.2 which are associated with increased risk of moderate-severe endometriosis are associated with decreased WHRadjBMI ('pear-shape'), consistent with hypotheses of hormonal regulation for both, suggesting that the locus has pleiotropic effects on the two phenotypes. Further results will be discussed.

Declaration of interest

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## S44.2

### Cytogenetic analysis and genome-wide association study (GWAS) in chinese women with premature ovarian failure (POF)

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Premature ovarian failure (POF) is defined as cessation of menstruation before the expected age of menopause. The disorder is considerable heterogeneous with a wide spectrum of causes- genetic, autoimmune, metabolic, infectious, and iatrogenic. However, etiology remains to be elucidated in most cases.

Chromosomal abnormalities have long been recognized as frequent causes of POF. However, few large cohorts have been studied. Ethnic background is usually not well characterized. We investigated 472 Chinese females with POF to determine the prevalence of cytogenetic anomalies in this ethnic group.

Chromosomal abnormalities were detected in 11.2% (53/472). 94.3% involved the X chromosome; 1 involved autosomes (t(13q;14q)), and 2 were 46,XY and 45,X/46,XY. We detected 15 X terminal deletions; 8 isochromosomes; 1 ring X and 1 inv X; 1 non-mosaic isodicentric, and 1 complex X arrangement. Eight X-autosome translocations were detected. Aneuploidy was found in 15 cases. Our result confirms a major role for X chromosome abnormalities in POF, highlighting the importance of routinely assessing for chromosomal abnormalities.

We sought to identify additional genetic loci associated with POF by performing the first large-scale GWAS. GWAS using Affymetrix SNP 6.0 chip was conducted in an initial discovery set of 391 Chinese POF patients, compared to 895 unrelated Chinese female controls. A replication study was then performed in an independent set of 400 cases and 800 controls. Suggestive significant associations were observed at 8q22.3. Replication of eight SNPs was confirmed. No specific candidate gene was found in the immediate region of 8q22.3. This GWAS, involving by far the largest sample of POF cases accumulated to date, revealed heretofore unrecognized association between POF and a novel genetic locus or region of unknown nature on 8q22.3. We speculate existence of a long-distance regulatory region that has relevance to the control of ovarian differentiation or oogenesis.

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## S44.3

**Genetics of (In)fertility**

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Since menarche is the landmark for the beginning of fertility and menopause marks the end of a women's reproductive lifespan genetics of fertility as well as infertility will be discussed taking recent GWAS data on both menarche and menopause into account.
Recently, 30 new loci for age at menarche were identified in a meta-analysis of 32 genome-wide association studies (GWAS) in women of European descent. In addition to two known loci the new loci included four genes previously associated with body mass index, three in or near other genes implicated in energy homeostasis and three in or near genes implicated in hormonal regulation of the ovary. Similarly, in a recent meta-analysis of 22 GWAS 13 newly genetic loci involved in the occurrence of natural menopause were identified. Candidate genes located at these newly associated loci include genes implicated in DNA repair and immune function. The finding that the innate immune response can be upregulated in response to DNA damage suggests that interplay between the two main pathways we identified (DNA repair and inflammation) may contribute to variation in age at natural menopause. Surprisingly there were only very few genes identified which might be involved in folliculogenesis. Three of the 17 regions can be linked to hormonal regulation, an additional route to follicle pool exhaustion.
In summary, our findings demonstrate a pivotal role of genes that regulate DNA repair and immune function in regulating age at menopause, indicating that the process of ageing is involved in both somatic and germ line aging. During this lecture the old dogma that regarding the cause of menopause will be challenged and evidence will be provided to support the theory that ageing of the soma initiates the cessation of ovarian function rather than the ovary itself.
Declaration of interest
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# Bone quality and bone strength

## S45.1

Abstract unavailable.

## S45.2

**Finite element analysis in osteology**

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Osteoporosis is a metabolic bone disease characterized by low bone mass leading to fractures that are associated with high mortality, morbidity and growing health care costs. Diagnosis of osteoporosis relies on dual energy X-ray absorptiometry (DEXA) to measure bone areal density, which is constantly reported as a poor surrogate of bone fracture risk.
Bone tissue is a composite of collagen, mineral and water, which multi-scale organization is increasingly well understood with the help of X-ray computer tomography. Its mechanical properties are assessed with various techniques at various levels and are altered by aging and disease.
Finite element analysis (FEA) is a widely applied engineering method to compute the strength of mechanical structures such as airplanes, bridges or cellular phones subjected to various loading scenarios. The increasing resolution of X-ray computer tomography opens new perspectives for finite element analysis of human bones from the constituent up to the organ level.
In the laboratory, desktop micro-computed tomography systems allow evaluation of trabecular architecture using micro finite element models of bone samples by converting directly each image voxel into a finite element. These microFE models bring new insight in bone mechanics, but often require high performance computing.
In a clinical environment, high resolution peripheral computed tomography provides detailed anatomy of bones such as the distal radius and permits to extract cortical thickness, volume fraction, orientation of trabecular bone in order to generate accurate patient-specific finite element models. Quantitative computed tomography delivers bone mineral density distributions and can also be exploited as a template for simplistic patient-specific finite element models. These models are carefully validated by experiments, require a standard PC for computation and can be used to make patient-specific predictions of bone strength that are significantly more accurate than DEXA.
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## S45.3

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# Chronic inflammation and insulin resistance

## S46.1

**AIMing at metabolic syndrome: towards development of novel therapies for modern metabolic diseases via macrophage-derived AIM**

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Metabolic syndrome is a cascade of metabolic diseases starting with obesity and progressing to atherosclerosis and is often fatal because of serious cardiovascular problems such as heart/brain infarction and hemorrhage. Accumulating evidence has revealed a critical involvement of inflammatory responses triggered by lesional macrophages in its pathogenesis. Importantly, we found that macrophages are associated with the progression of these diseases not only in the induction of inflammation but also in the production of apoptosis inhibitor of macrophage (AIM), which we initially identified as a soluble factor expressed by macrophages[1]. At atherosclerotic plaques, AIM is highly expressed by foam macrophages and inhibits apoptosis of these cells. This results in the accumulation of macrophages, causing inflammatory responses within the lesion, and ultimately disease progression[2]. In adipose tissue, macrophage-derived AIM is incorporated into adipocytes through CD36-mediated endocytosis, thereby reducing the activity of cytosolic fatty acid synthase. This unique response stimulates lipolysis, resulting in a decrease in adipocyte size, which is physiologically relevant to the prevention of obesity[3]. The lipolytic response also stimulates inflammation of adipocytes in association with the induction of metabolic disorders subsequent to obesity[4]. Thus, AIM is involved in the progression of metabolic syndrome in both advancing and inhibitory fashions. Regulation of AIM could therefore be therapeutically applicable for metabolic syndrome.
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## S46.2

**Macrophage regulation of adipocyte biology**

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During the progression from the lean to the obese state, adipose tissue undergoes hyperplasia as well as hypertrophy in an attempt to cope with the increased demand for triglyceride storage. This requires a high degree of plasticity at both

the cellular and at the tissue level. Even though adipose tissue as a whole seems to be a relatively static tissue containing many adipocytes that turn over relatively slowly, these cells are embedded in an environment that can rapidly adapt to the needs of expanding and newly differentiating adipocytes.
Subclinical inflammation is frequently associated with obesity. We want to better define the acute inflammatory response during fasting. Representatives of immune-related proteins in circulation and in tissues were analyzed as potential markers for adipose tissue inflammation and modulation of the immune system. Lipopolysaccharide treatment or high fat diet leads to an increase in circulating serum amyloid (SAA) and α1-acid glycoprotein (AGP), while adipsin levels are reduced. Mouse models that are protected against diet-induced challenges, such as adiponectin overexpressing animals or mice treated with PPARgamma agonists, displayed lower SAA levels and higher adipsin levels. An oral lipid gavage increased circulating SAA concurrent with the elevation of FFA levels. Moreover, prolonged fasting increased circulating SAA and was associated with an increased number of Mac2 positive, crown-like structures and an increase in the capillary permeability in adipose tissue. This fasting-induced inflammatory response in adipose tissue was associated with increased VEGF expression and an increase in several M2-type macrophage markers. This fasting-induced switch to M2 type macrophages is impaired in metabolically challenged animals. Therefore, one of the underlying reasons for the 'metabolic inflexibility' observed under those conditions is the prevailing lack of 'immunological fitness' of adipose tissue.
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## S46.3

**The inflammatory-adipokine hypothesis and stem cell abnormalities**
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Increased body weight and adipose tissue accumulation amplifies the risk of developing various age-related diseases, such as cardiovascular disease, type 2 diabetes mellitus, musculoskeletal disorders. Adipose tissue-derived secreted pro-inflammatory adipokines generate a chronic low-grade inflammation which contributes to the pathogenesis of obesity-linked complications. Recent advancements in tissue-resident adult stem/progenitor cell research have revealed that inflammation, enhanced telomere shortening, oxidative stress, may occur in these immature and regenerative cells during chronological aging. Particularly, the alterations in key signaling components controlling their self-renewal capacity may result in their dysfunctions, growth arrest and senescence or apoptotic death during the aging process. These molecular events may culminate in a progressive decline in the regenerative functions and the number of tissue-resident adult stem/progenitor cells, and age-related disease development. In the microenvironment, inflammation may favor adipose derived stem cell (ASC) differentiation into endothelial cells while inhibiting their differentiation into adipocytes. On the other hand it has been shown that ASCs possess anti-inflammatory properties and promote endothelial cell differentiation and microvascular regeneration.
The chronic low-grade inflammation and oxidative stress cold lead to muscle-to-fat conversion of muscle satellite cells (SCs), thus explaining the increase in intermuscular adipose tissue depots that occurs under some pathological conditions (i.e. primary myodystrophies, obesity, hyperglycaemia, high plasma free fatty acids, hypoxia, age-related sarcopenia, etc.) or simply because of a sedentary lifestyle or during aging. Several pathways and factors (PPARs, WNT growth factors, myokines, GEF-GAP-Rho, p66shc, mitochondrial ROS production, PKCβ) could be implicated in the adipogenic conversion of SCs.
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# Cushing's syndrome

## S47.1

Abstract unavailable.

## S47.2

Abstract unavailable.

## S47.3

**Learnings from ERCUSYN: the first 500 patients**
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The European Registry on Cushing's syndrome (ERCUSYN) is a project aimed at gathering data at EU level on clinical features, diagnostic procedures and therapeutic strategies in CS patients. The creation of this large European database is expected to provide comprehensive information on all stages of the disease from the first diagnosis to long-term follow-up. This ultimately should result in earlier recognition of CS and all its co-morbidities, and assist clinicians in considering and treating all possible manifestations of the disease, thus improving long-term prognosis.
The ERCUSYN database currently comprises data on 630 patients from 50 participating centres in 28 European countries. An initial analysis of baseline demographic and clinical data in 481 patients has been recently published. It showed differences in clinical presentation depending on gender and etiology, confirmed a long delay between onset of symptoms and diagnosis of CS, with a high number of specialists consulted who often missed the correct diagnosis. Furthermore, morbidity at diagnosis resulted high, with low bone mass, especially in men. Quality of life was impaired and less than half the cohort was actively working at the time of diagnosis of CS. Thus, there is great potential for improvements in reducing the time to diagnosis, which would have obvious consequences for patients and for the health care systems. In this presentation, updated results from the ERCUSYN database will be shown and future perspectives will be discussed.
In conclusion, ERCUSYN represents the largest collaboration of endocrine centres in Europe and has potentials to provide new insights into the diagnostic and therapeutic challenges of CS and to improve the care of patients with CS.
Acknowledgements
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# Pathogenesis of primary aldosteronism

## S48.1

**Genomic profile in primary aldosteronism**
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Primary aldosteronism has recently been found as a common cause for essential or secondary hypertension. As much as 1/10 may be explained by this disease. Diagnosis is crucial, since a adrenalectomy may cure the patient.
The underlying cause in the majority of sporadic cases have been unknown until recently, while classically three forms of familial hyperadlosteronism (FH) exist: familial hypertension type I, II and III.
FHI, which occurs in childhood, is caused by a chimeric gene product combining the promotor of the 11-β-hydroxylase gene with the coding region of the aldosterone synthetase gene. This derangement is glucocorticoid sensitive.
In FHII there is no phenotypical difference towards classical sporadic PA, and genetic linkage exist to chromosome 7p22, as well as close to the MEN1 gene locus on 11q13.
FHIII is found in families with severe hypertension in early childhood, resistant to aggressive antihypertensive therapy. Although several genes have been proposed in FHIII, no one has been identified as the cause. A possible candidate is the recently identified mutation in the potassium channel KCNJ5, possibly explaining up to 40% of sporadic cases of PA. Interestingly, a family with early onset hypertension and bilateral hyperplasia and a mutation on KCNJ5 adds to the list of hereditary forms of PA.
Mutations in KNCJ5 (G151R or L168R) were found in 40% of aldosterone-producing adenomas which were associated with hyperplasia.

The talk comprise an overview of these genetic deragnements associated with PA.
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## S48.2

**Integrating genetics and genomics in primary aldosteronism**

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Primary aldosteronism (PA) is the most common form of endocrine hypertension. The two main causes of PA are aldosterone producing adenoma (APA) and bilateral adrenal hyperplasia (BAH). Efficient and timely screening for PA is of major importance, given the severe cardiovascular outcome of aldosterone excess that is independent of blood pressure levels. Yet, the pathogenic mechanisms leading to aldosterone hypersecretion and cell proliferation are largely unknown. Several studies have addressed the molecular changes underlying APA formation. Transcriptome analyses have highlighted the contribution of different signaling pathways converging upon increased expression of CYP11B2, coding for aldosterone synthase. Less is known with regard to the mechanisms underlying nodulation. Recent evidence indicates a link between APA formation and peritumoral tissue remodelling and ZG hyperplasia, which are major features of adrenals with APA. Both APA and adjacent ZG present characteristics of stem/precursor cells; the re-expression of genes involved in foetal adrenal development could underlie excessive cell proliferation and APA formation.
Recently, a few recurrent somatic mutations of the KCNJ5 gene, coding for the potassium channel Kir3.4, have been implicated as a cause of APA, while inherited mutations of the same gene were identified in familial hyperaldosteronism type 3. Somatic KCNJ5 mutations are found in ~34% of unselected patients with APA; germline KCNJ5 mutations, however, are not similarly causative for BAH. Mutations all lie near or within the selectivity filter of the Kir3.4 channel; they result in a loss of channel selectivity, with increased sodium conductance leading to membrane depolarization. This leads to opening of membrane voltage-dependent calcium channels which is followed by activation of the calcium signaling pathway. While it has been formally established that this cascade of events leads to increased aldosterone production, it remains still unclear whether and how KCNJ5 mutations are responsible for increased cell proliferation.
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## S48.3

**Potassium channels in primary aldosteronism**

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Potassium channels regulate the membrane voltage of aldosterone-producing glomerulosa cells in the adrenal glands. They are required for the unique $K^+$ sensitivity of these cells and are targets of angiotensin II signaling. Several $K^+$ channels show high levels of expression in the adrenal cortex and are believed to be important for the control of hormone secretion, e.g. KCNJ5, TASK1, TASK3, KCNMA1, and KCNQ1.
In a pioneering study, Lifton and co-workers have described somatic mutations of the $K^+$ channel KCNJ5 in aldosterone producing adenoma. Recent follow-up studies have confirmed the concept that pore-region mutations turn KCNJ5 into a $Na^+$-permeable channel. Interestingly, such mutations can be found in more than 30% of aldosterone producing adenomas.
For TASK1 and TASK3, no human phenotype has been described so far. However, knockout mice for TASK1 and TASK3 have highlighted the functional relevance of these channels: Before puberty, inactivation of the TASK1 gene resulted in autonomous aldosterone production and caused an aberrant expression of aldosterone synthase in zona fasciculata cells that normally produce only glucocorticoids. After puberty, male mice were able to compensate for the defect of TASK1 and their aldosterone synthase regained regular localization in glomerulosa cells. In female TASK1 knockout mice, dyszonation of the aldosterone synthase and hyperaldosteronism persisted after puberty.
In TASK3 knockout mice, plasma renin activity was suppressed and the aldosterone/renin ratio, an indicator of autonomous aldosterone production, was elevated. TASK3 knockout animals were unable to adapt aldosterone production to dietary salt intake in a normal way. The inappropriately high aldosterone levels under high dietary salt resulted in arterial hypertension.
These novel data corroborate the concept that $K^+$ channels play a critical role in adrenocortical cells. Dysfunction of adrenal $K^+$ channels can lead to developmental deficits, hyperaldosteronism and adenoma formation and maladaptation of aldosterone secretion to dietary salt intake.
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# Genetic breakthroughs in reproductive pathology and physiology

## S49.1

Abstract unavailable.

## S49.2

Abstract unavailable.

## S49.3

**RANK ligand mediates hormone induced mammary epithelial proliferation and carcinogenesis**

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RANK and RANKL are essential for mammary gland development in mice. We demonstrated that RANK signalling promotes proliferation and impairs mammary epithelial differentiation in mice. RANK and RANKL expression is detected in breast cancer cells. MMTV-RANK acini show hallmarks of transformation in a RANKL dependent manner. After multiple pregnancies MMTV-RANK females spontaneously develop adenocarcinomas, and after DMBA (dimethylbenz(a)anthracene) and MPA (medroxiprogesterone) treatments show a shorter latency and a higher incidence of preneoplastic lesions and adenocarcinomas as compared to wild-type (WT) mice. Reciprocally, selective pharmacologic inhibition of RANKL after progesterone and carcinogen treatment attenuates mammary tumor formation in MMTV-RANK mice and prevents mammary tumor formation in WT mice. Anti RANKL treatment also decreases tumor formation and lung metastasis in MMTV-neu mice. The reduction in tumorigenesis upon RANKL inhibition was preceded by a reduction in preneoplasias and correlated with rapid and sustained reductions in hormone induced mammary epithelial proliferation. These results show that increased RANKL signalling promotes breast cancer initiation in mice and that RANKL is the main mediator of the protumorigenic effects of progesterone. Importantly, these results suggest that increased RANKL signaling may be a risk factor for mammary tumor development and that RANKL inhibitors may be effective for breast cancer treatment.

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## Circadian regulation of metabolism

### S50.1

**Basic mechanisms of circadian regulation in the brain**

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Circadian clocks regulate the adaptation of the organism to the rotation of the earth around its axis. In mammals the circadian timing system is comprised of numerous cellular oscillators throughout the brain and the body. In the hypothalamic suprachiasmatic nucleus a circadian pacemaker is synchronized to the external light/dark cycle via direct innervation along the retinohypothalamic tract. From the SCN, peripheral clocks are synchronized by neuronal, endocrine and behavioral means. Close interaction between different tissue oscillators in brain and periphery is essential for maintaining plasticity of circadian regulation under different environmental conditions. For example, SCN and adrenocortical clocks together regulate the entrainment of glucocorticoid secretion to the light/dark cycle.
In this talk I will summarize the knowledge on the regulation of molecular and endocrine rhythms by different tissue oscillators and present some of our recent approaches to unravel the communication pathways within the mammalian circadian timing system.
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### S50.2

Abstract unavailable.

### S50.3

**Circadian rhythms in adipose tissue**

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Circadian mechanisms regulate metabolism in adipose tissues. The genes encoding the circadian regulatory proteins oscillate over a 24 h period in human and murine adipose tissue depots,. This expression profile is entrained by photic stimuli, temporally restricted food access, and/or nuclear hormone receptor ligands, such as glucocorticoids. In mice, deletion or mutation of the circadian regulatory genes, clock and PPAR gamma coactivator 1 (PGC1), is associated with increased risk of obesity and/or abnormal glucose metabolic function. Bioinformatic analyses of transcriptomic data indicate that $>20\%$ of the adipose-tissue expressed genes exhibit pronounced circadian or diurnal rhythmicity. These *in vivo* findings can be observed in *in vitro* models. For example, dexamethasone exposure synchronizes the expression profile of circadian protein encoding genes in primary cultures of undifferentiated and adipocyte-differentiated human adipose-derived stem cells. Similar observations regarding the synchronization of circadian regulatory genes have been reported in murine 3T3-L1 pre-adipocyte cultures. In addition, multiple circadian regulators directly modulate both adipogenic and osteogenic differentiation in these *in vitro* models. The expression profiles of the circadian regulatory genes correlate directly with those of adipogenic markers in human adipose tissue mRNAs. Furthermore, polymorphisms in circadian regulatory genes have been correlated directly with obesity risk in human subjects. There is a growing body of endocrine and epidemiological evidence linking circadian dysregulation to the incidence of obesity, hypertension, cardiac disease, and the metabolic syndrome. Consequently, there will be a potential role for targeting circadian mechanisms in the treatment of clinical endocrine and metabolic disorders of adipose tissue using behavioral and/or pharmacological interventions.
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## Recent management of pheochromocytoma/paraganglioma syndrome

### S51.1

**Recent progress in biochemical testing for pheochromocytoma**

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Background
Since signs and symptoms of pheochromocytomas are unspecific, diagnosis is crucially dependent on biochemical evidence of excessive catecholamine production. Diagnostic workup was previously based on measurement of urinary catecholamines. Subsequently it was shown that pheochromocytomas contain high amounts of catechol-*O*-methyltransferase (COMT), the enzyme that converts epinephrine to metanephrine and norepinephrine to normetanephrine. This metabolism occurs in a continuous fashion within tumour cells and independently of variations of catecholamine release, providing diagnostic advantages to measuring the metanephrines over the parent catecholamines.
Role of metanephrines: Determination of plasma free metanephrines by liquid chromatography–tandem mass spectrometry (LC–MS/MS) is now regarded as gold standard. Due to the cost- and time-intensive technique the method is limited to specialized centers. Immunoassays may be an alternative process. The diagnostic value of plasma free and urinary metanephrines measured by either RIA or enzyme immunoassays (EIA) was recently demonstrated by us and others. In our study, urinary fractionated metanephrines demonstrated a slightly lower sensitivity and specificity than their plasma counterparts. Urinary metanephrines largely reflect sulfate-conjugated metabolites, which are formed in gastrointestinal tissue. Thus, they are not related only to pheochromocytoma.
Influence of confounding variables: Non-selective alpha-blockers may be associated with higher levels of norepinephrine and normetanephrine. Beta-blockers, to a much lower extent, may be responsible for elevated levels of metanephrines. We found, that coffee elevated normetanephrine. Furthermore, physical exercise led to relevant changes of metanephrine and normetanephrine and should therefore be avoided prior to sampling. Although effects of age, sex and BMI were small, these variables should be considered when interpreting biochemical results. In contrast, supine rest significantly decreased both metanephrine and normetanephrine when compared to standing rest. Metanephrine and normetanephrine were not significantly influenced by time of day, menstrual cycle or venepuncture. Of note, adjusted thresholds may be required for patients with hereditary syndromes.
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### S51.2

**Prevalence of SDH gene mutations in pheochromocytoma**

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Hereditary pheochromocytoma/paraganglioma (PHEO/PGL) syndrome is a rare disease. It has been demonstrated that about 25% of the apparently sporadic PHEO/PGL are due to a germ line mutation in one of the susceptibility genes, such as, VHL, RET, NF1, TMEM127, MAX and the genes encoding the four

subunits of the mitochondrial enzyme succinate dehydrogenase (SDH) as well as the gene encoding one SDH assembly factor, SDHAF2. SDH is located in the inner mitochondrial membrane and is formed by four subunits: SDHA–B–C–D. It is an enzyme involved in the tricarboxylic acid cycle and in the mitochondrial electron transport chain.

Beside the pivotal role played by mitochondria in modulating programmed cell death and other fundamental processes within cells, the first incontestable examples of causality between mitochondrial dysfunction and tumorigenesis were only discovered when mutations in SDH were found to be the initiating events of familial PHEO/PGL.

Germ line mutations in SDHD, SDHAF2, SDHC and SDHB genes are responsible for the occurrence of different syndromes named PGL1, PGL2, PGL3 and PGL4 respectively. Also mutations of SDHA can predispose to functioning PHEO/PGL (PGL5 ?). PGL1 presents benign, non-secreting, multiple head and neck PGLs (HN-PGL) not frequently associated with PHEOs and/or abdominal secreting PGLs. PGL2 presents only HN-PGLs. PGL3 is mainly characterized by single HN-PGL but recently abdominal secreting PGL has also been reported. PGL4 presents PHEO or abdominal/thoracic PGL, which, at variance with tumors developed in PGL1 and PGL3, are malignant in up to 30% of cases, leading to metastatic disease. PGL1 and PGL2 are parent of origin diseases and only carriers of the mutation in the paternal allele can develop tumors of the paraganglia. Overall, SDH mutations accounts for 3–5% of PHEO/PGL presenting as apparently sporadic.

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## S51.3

**Treatment of malignant pheochromocytoma**

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Effective treatments of malignant pheochromocytoma have not been established. Although 131-I-MIBG radiotherapy, chemotherapy with a combination of cyclophosphamide, vincristine and dacarbazine (CVD), and molecular target therapies have been used for unresectable tumors, those are usually palliative and rarely curative. However, excessive catecholamine impairs the activities of daily living by causing various symptoms (e.g. palpitation, paroxysmal hypertension and hypotension and severe constipation) and even more fatal arrhythmia and heart failure. Therefore, controlling chronic overproduction of catecholamine is one of the most important therapeutic targets to maintain hemodynamics stable and to improve performance status of the patients. Although molecular target therapies such as tyrosine kinase inhibitors and the mammalian target of rapamycin (mTOR) inhibitor are promising and under clinical trial in the EU and North America, CVD is the most representative therapy available in Japan and only a limited numbers of case series have been reported. In our 14 patients treated with CVD, no patients showed complete biochemical and tumor responses. However, partial biochemical and/or tumor response was achieved in five patients (35.7%). No significant biochemical or tumor response was seen in four patients (28.6%), while deterioration in biochemical and tumor responses was seen in five patients (35.7%). These effects were demonstrated 1–5 months after CVD (average: 3 + 2 months) and lasted for 12–60 months (average: 32 + 21 months). The patients with biochemical response were associated with an improvement in diabetes mellitus and hypertension and were free from various symptoms and cardiac complications associated with catecholamine excess. In addition, adverse effects associated with CVD were transient and mild. Our results suggest that CVD still could be a beneficial treatment in relieving excessive catecholamine and related complications as well as in reducing tumor volume although the effects are limited in its magnitude and duration.

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# Thyroid & Pregnancy

## S52.1

**Outcome of the controlled antenatal thyroid screening study**

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Fetal brain development depends on thyroid hormone and children born to mothers with low thyroid hormone levels have decreased cognitive function. Nineteen per cent of children born to mothers known to have a high TSH during gestation had an IQ $<80$ compared to 5% of children born to mothers known to have normal TSH levels in pregnancy ($P<0.001$; Haddow *et al*. 1999).

We conducted a randomized trial of antenatal hypothyroidism screening with selective treatment and assessment of childhood cognitive function.

Pregnant women were randomized at about 12 weeks' gestation into a screen or control group. Blood was collected for thyroid stimulating hormone (TSH) and free thyroxine ($FT_4$) measurements before 16 weeks. In the screen group measurements were made immediately; control group serum was stored and measurements made shortly after delivery. Women with TSH levels $>97.5$th centile and/or $FT_4$ levels $<2.5$th centile were designated positive and women in the screen group were prescribed levo-thyroxine. IQ measurements were performed on children of women with positive results aged three by psychologists unaware of whether the women were in the screen or control group.

Out of 21 846 women tested, 390 in the screen group and 404 in the control group tested positive. Nineteen percent of women required levo-thyroxine dose adjustment. The mean IQ's in the screen and control positive groups were 99.2 and 100.0; a difference of 0.8 (95% CI $-1.1$ to 2.6, $P=0.403$, intention-to-treat analysis) respectively, and the proportions with IQ $<85$ were 12.1 and 14.1% respectively; a difference of 2.1% (95% CI $-2.6$ to 6.7%, $P=0.39$). An on-treatment analysis showed no significant difference.

Antenatal screening and treatment for hypothyroidism from about 12 weeks of pregnancy showed no benefit in childhood cognitive function assessed at age three. (Funded by The Wellcome Trust UK and Compagnia di San Paulo Turin).

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## S52.2

**The case for thyroid screen in pregnancy**

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Screening is defined as 'the presumptive identification of unrecognized disease or defect by the application of tests, examinations, or other procedures which can be applied rapidly. Screening tests sort out apparently well persons who probably have a disease from those who probably do not'. Ideally all the following criteria should be met: The Condition should be an important health problem; well known epidemiology and natural history of the condition. The test should be a simple, safe, precise and validated with agreed reference range. The treatment should be effective, there should be agreed evidence based policies covering which subjects need treatment and the appropriate treatment to be offered. The Screening Programme is effective in reducing mortality or morbidity (evidence from high quality Randomised Controlled Trials); is clinically, socially and ethically acceptable to health professionals and the public. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment). The opportunity cost of the screening programme should be economically balanced in relation to expenditure on medical care as a whole (cost benefit and/or cost effectiveness analyses). In the case for thyroid screening in pregnancy, most of the above mentioned points are satisfied. In particular, everyone agrees that overt thyroid dysfunctions need to be treated, and a cost-effective analysis demonstrated that universal screening is cost-effective even assuming that only overt hypothyroidism has adverse obstetrical outcomes. The problem is still represented by the halo of uncertainty that surrounds clinical entities such as subclinical hypothyroidism and isolated hypothyroxinemia. The lack of high quality Randomized Controlled Trials in these two conditions, does not allow, by now, to universally endorse a screening for thyroid disease in pregnancy.

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## S52.3

**Treatment of hyperthyroidism in pregnancy**

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Graves' disease is the most common cause of autoimmune hyperthyroidism in pregnancy accounting for 0.1 to 1% (0.4% clinical and 0.6% subclinical) of all pregnancies. It may be diagnosed for the first time in pregnancy, may present as a recurrent episode in a woman with past history of hyperthyroidism, or in a women on antithyroid drugs (ATD). Less common non autoimmune causes include multinodular goiter, toxic adenoma, and factitious hyperthyroidism. More frequent than Graves' disease as the cause of hyperthyroidism is the syndrome of gestational hyperthyroidism defined as 'transient hyperthyroidism, limited to the first half of pregnancy characterized by elevated $FT_4$ or adjusted $TT_4$ and suppressed or undetectable serum TSH, in the absence of serum markers of thyroid autoimmunity'.

Poor control of thyrotoxicosis is associated with miscarriages, PIH, prematurity, low birth weight, intrauterine growth restriction, stillbirth, thyroid storm and maternal congestive heart failure. Antithyroid drugs are the mainstay of treatment for hyperthyroidism during pregnancy. Side effects occur in a 3–5%, of patients taking thionamide drugs, mostly allergic reactions such as skin rash. The greatest concern in the use of ATD in pregnancy is related to teratogenic effects. Exposure to MMI may produce several congenital malformations, mainly aplasia cutis and the syndrome of 'methimazole embryopathy' that includes choanal or esophageal atresia, and dysmorphic faces. Although very rare complications, they have not been reported with the use of PTU. Report from the Adverse Event Reporting System of the Food and Drug Administration (FDA) has called attention to the risk of hepatotoxicity in patients exposed to PTU and an advisory committee recommended to limit the use of PTU to the first trimester of pregnancy. Hepatotoxicity may occur at any time during PTU treatment, without warning; however it may be appropriate to monitor liver function tests regularly while PTU is administered. In women with hyperthyroidism due to Graves' disease or a toxic nodule goiter, ATDs are initiated, or adjusted in those women on treatment, at conception. PTU is preferred in the first trimester. Following the first trimester, PTU should be switched to methimazole.

The combination of ATD plus levothyroxine is not recommended in the management of hyperthyroidism in pregnancy. Adrenergic β blocking agents, such as propranolol 20–40 mg every 6–8 h may be used for controlling hypermetabolic symptoms. In the vast majority of cases the drug could be discontinued in 2 to 6 weeks. Long term treatment with propranolol has been associated with intrauterine growth restriction, fetal bradycardia and neonatal hypoglycemia. Thyroidectomy should be considered in cases of allergies to both ATDs, women requiring large doses of ATDs and the occasional patient who is not adherent to drug therapy. If surgery is indicated, second trimester is the optimal time. A determination of serum TRAb titers is mandatory at the time of surgery in order to assess the potential risk of fetal hyperthyroidism. Preparation with β-blocking agents and a short course of potassium iodine solution (50–100 mg a day) are recommended.

# Genetic polymorphism in reproduction

## S53.1

**Genetic variation of the androgen receptor: from gene regulation to prostate cancer**

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In addition to symptoms of androgen deficiency induced by low serum testosterone concentrations (i.e. classical hypogonadism), also variable phenotypes of androgen insensitivity exist in humans, mainly owing to defective, mutated androgen receptors. A more subtle modulation of androgen effects has been related to the CAG repeat polymorphism (CAGn) in exon 1 of the androgen receptor gene: *in vitro*, transcription of androgen-dependent target genes is attenuated with increasing length of triplets. As a clinical entity, the CAG repeat polymorphism is, in healthy males, compensated for by an increased release of LH and, hence, testosterone. In men with an affected hypothalamic–pituitary–gonadal signal chain, however, it can relate to variations of androgenicity in (apparantly) eugonadal men in various tissues and psychological traits: the longer the CAGn, the less prominent is the androgen effect when individuals with similar testosterone concentrations are compared. A strictly defined threshold to hypogonadism might then be replaced by a continuum originating fom genetics, hormone concentrations as well as symptom specificity. In addition, effects of externally applied testosterone can be markedly influenced by the CAGn and respective pharmacogenetic implications are likely influence indications as well as modalities of testosterone treatment of hypogonadal men.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## S53.2

Abstract unavailable.

## S53.3

**Genetic complexity of FSH receptor function**

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FSH acts via binding to its specific receptors, the FSHR, possessing a large number of SNPs. The FSHR SNPs at nucleotide position 919 and 2039 in exon 10 are very common (heterozygosity: 0.469) and result in the aminoacid transition Thr/Ala at codon 307 and Asn/Ser at codon 680 respectively. In addition a G/A SNP is found in the promoter region at position −29, with the G allele covering 75% and the A allele 25% of the alleles in Caucasians. In turn, the FSHB gene, encoding the α subunit of the gonadotropin, possesses an interesting SNP in the promoter region (SNP, rs10835638; G/T) 211 bp upstream from the FSHB mRNA transcription start-site, located within a highly conserved region among placental mammals.

Several studies demonstrated that the FSHR SNPs affect ovarian response to FSH in women undergoing assisted reproduction techniques (ART). The FSHR Ser680 genotype is less sensitive to the FSH action *in vivo*, compared to the FSHR Asn680 genotype. A recent meta-analysis including data available from different ethnic group have confirmed that the marker Ser680 is associated with a poor response during ART, concluding that FSHR genotyping could provide important information to customize the dose of FSH during controlled ovarian hyperstimulation and reinforcing the critical role of FSHR SNPs in assisted reproduction treatment. The FSHR polymorphism G/A at position −29 could modulate ovarian response to FSH as well, since it controls the transcription rate of the mRNA. Data in fertile and infertile men are less clear-cut. Studies *in vitro* were so far unable to demonstrate the molecular and cellular effects of the SNPs in the FSHR.

Concerning, the FSHB gene studies in males demonstrated that the SNP in the promoter is significantly associated with serum FSH and testosterone levels and that the -221T allele has an increased prevalence in infertile men.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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# Vitamin D

## S54.1

**Vitamin D is a multifunctional hormone Roger Bouillon Clinic and laboratory of experimental medicine and endocrinology**

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The vitamin D endocrine system (D-endo) is essential for calcium and bone homeostasis. Absence of a functional VDR or CYP27B1 creates a severe rachitic bone and growth plate phenotype in humans and mice as in severe vitamin D deficiency. The intestine is the key target for VDR as a high calcium intake or selective VDR rescue in the intestine restores a normal bone and growth plate phenotype. Selective absence of VDR in osteoblasts does not create a bone phenotype when calcium intake is normal.

VDR is ubiquitously expressed and about 3% of the mouse or human genome is regulated by D-endo. The native immune defense system is activated by D-endo but VDR or vitamin D deficiency leads to increased sensitivity to autoimmune diseases such as inflammatory bowel disease or autoimmune diabetes after exposure to predisposing factors. VDR deficient mice do not have a spontaneous increase in cancer but are more prone to oncogen, chemocarcinogen or UV-B induced tumors. A wealth of observational studies in men also links a poor vitamin D nutritional status to increased risk of all major cancers. D-endo is also related to the cardiovascular system as VDR or CYP27B1 KO mice develop high renin hypertension, cardiac hypertrophy and increased thrombogenicity. Observational studies in men also link poor vitamin D status to all aspects of the metabolic syndrome and increased risk of cardiovascular diseases. The muscle development of VDR KO mice is delayed and their fertility is impaired.
Whether the same spectrum of activity is also operational in humans is not yet established but vitamin D deficiency is frequently associated with an increased prevalence of diseases expected on the basis of the VDR KO phenotype. Prospective and intervention studies will be presented as to define the spectrum of activities and the optimal vitamin D status for global health.
Declaration of interest
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## S54.2

**Vitamin D and primary hyperparathyroidism**

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The occurrence of vitamin D (vitD) insufficiency (plasma 25OHD < 50 nmol/l) and deficiency (25OHD < 25 nmol/l) in primary hyperparathyroidism (PHPT) varies worldwide in prevalence, severity and symptoms. Epidemiological studies support that the clinical presentation of PHPT is more severe in patients with coexisting vitD deficiency. Most PHPT patients in Europe only have slightly decreased plasma 25OHD levels compared with gender-, age- and season matched controls, and severe symptomatic vitamin D deficiency is rare. However, in the patients low plasma 25OHD appear to be associated with higher levels of PTH, calcium and bone turnover and lower femoral neck and forearm BMD. Low 25OHD has also been associated with higher left ventricular mass index.
The decrease in plasma 25OHD in PHPT has been explained by i) a stimulated renal 1α-hydroxylation of 25OHD to 1,25(OH)2D induced by PTH and hypophosphatemia, ii) an increased 24-hydroxylase activity with hydroxylation of 25OHD and 1,25(OH)2D to more polar metabolites, iii) increased body weight in PHPT patients, and iv) development of secondary and later tertiary hyperparathyroidism because of long standing severe vitD deficiency.
In cases where the combination of both PHPT and hypovitaminosis D is diagnosed vitamin D repletion is an option. However, only limited evidence exists for this treatment. In case series and cohort studies vitD treatment lowers preoperative PTH levels and may reduce bone turnover in some cases. Furthermore, high vitD status may decrease the risk of postoperative hypocalcaemia and secondary hyperparathyroidism. However, there are no randomised controlled studies to prove any beneficial effects on calcium and bone metabolism or on muscle function, CNS symptoms or quality of life. Furthermore, serum and urinary calcium may increase in some patients. It is unknown, whether routine vitD supplementation should be offered preoperatively to all PHPT patients or as part of long term medical follow-up.
Declaration of interest
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## S54.3

Abstract unavailable.

# The fatty liver as an endocrine disease

## S55.1

**Pathogenesis of fatty liver disease**

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Non-alcoholic fatty liver disease (NAFLD) is gaining increasing recognition as a component of the epidemic of obesity worldwide. The spectrum of NAFLD ranges from simple fatty liver with benign prognosis, to a potentially progressive form, non-alcoholic steatohepatitis (NASH), which may lead to liver fibrosis and cirrhosis, resulting in increased morbidity and mortality. All features of the metabolic syndrome, including obesity, type 2 diabetes, arterial hypertension, and hyperlipidemia are associated with NAFLD/NASH. Despite being potentially severe, little is known about the natural history or prognostic significance of NAFLD. Excessive accumulation of triglycerides in hepatocytes is the hallmark of NAFLD. Despite the existing correlation between fatty liver and insulin resistance it remains unclear whether insulin resistance causes the excessive accumulation of TG in liver or whether the increase in TG or of metabolic intermediates may play a causal role in the development of hepatic or systemic insulin resistance. Studies have reported that the accumulation of intra-hepatic lipids precedes the state of insulin resistance while others showed that hepatic TG themselves are not toxic and may in fact protect the liver from lipotoxicity by buffering the accumulation of deleterious fatty acids. Such findings suggest that hepatic steatosis is not necessarily associated to insulin resistance. In agreement with this concept, some identified population of obese humans can stay free of insulin resistance and are metabolically healthy despite morbid obesity. These findings suggest that not all lipids are detrimental for insulin sensitivity and that specific lipid species, when present in the proper location and time, may trigger signals that modulate adaptation to stress. In the recent years, we have determined the metabolic impact of modulating liver *de novo* fatty acid synthesis (lipogenesis) via the transcription factor ChREBP on the outcome of hepatic steatosis and/or insulin resistance in mice and humans with NAFLD.
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## S55.2

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## S55.3

**Fatty liver disease in childhood**

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Nonalcoholic fatty liver disease (NAFLD) is a multifactorial condition, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) with or without fibrosis. NAFLD affects both adults and children who present with particular risk factors, including obesity, sedentary lifestyle and/or a predisposing genetic background. The escalation of the prevalence of NAFLD in children worldwide is a worrying phenomenon because this disease is closely associated with the development of both cirrhosis and cardiometabolic syndrome in adulthood. The etiopathogenesis of primary NAFLD in children is unknown; however, considerable knowledge about the mechanisms of liver damage that occur during disease progression has been gathered over the past 30 years. Understanding the pathogenetic mechanisms, together with the histological pattern, provide the basis to characterize potential early predictors of the disease, suitable noninvasive diagnostic tools and design novel specific treatments and possible management strategies. Despite a few clinical trials on the use of antioxidants combined with lifestyle intervention for NAFLD that showed encouraging results, to date, no treatment guidelines exist for children with NAFLD.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## Pituitary development transcription factors: stem cells and beyond

### S56.1

**The GPS niche of stem cells in the pituitary**

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The pituitary is an anatomically well protected endocrine gland in permanent contact with the central nervous system. Although it was known that pituitary has plasticity in response to the physiological demands of the body, there were few clear data about the post-natal mechanisms in charge of this plasticity. The discovery of a group of cells expressing stem cell markers precisely organized in the outer zone of the adenopituitary open up new possibilities to explain the constant pituitary renewal. Moreover, the organization and location of these cells is conserved from rodents (mouse and rat) to human pituitary suggesting an essential role of this structure called the GPS niche. The GPS cells express the tyrosine-kinase receptor RET together with two of its co-receptors GFRa2 and GFRa3 (***G***). But also they retain expression of PROP1 (***P***) a key transcription factor during pituitary embryonic development, while they co-express markers of pluripotency such as SOX2, OCT4 and KLF4 (***S***). Opposite GPS cells do not express any pituitary hormone or transcription factors associated to differentiation as for example Pit-1. The data obtained so far indicate that more than one type of cells can exist around the GPS. The precise characterization of markers together with functional studies suggest a true niche of stem cells. The GPS niche could be implicated in disease as data obtained in human cranyopharingiomas suggest.
But if new cells are being recruited from the niche to the adenopituitary to replace those which are old or non-functional, we should explain how unfit cells are isolated and marked for removal. Our recent data in rodent somatotrophs indicate that the RET receptor, when is not activated by the ligand through another co-receptor GFRa1, is intracellularly processed by Caspase 3 triggering a mechanism of arrest and cell death. Thus, RET over-activates the Pit-1 promoter, Pit-1 excess directly activates the ARF promoter increasing ARF expression which in turn stabilizes p53 leading to cell death. New data in somatotroph adenoma cultures indicate that the pathway is conserved in humans.
In summary, the data gathered at present highlight the importance of GPS cells in pituitary cell renewal and further our understanding on the mechanisms leading to pituitary hyperplasia, pituitary adenomas, and related tumors such as craniopharyngiomas.

### S56.2

**LHX3: an insightful model of pituitary hormone deficiency**

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Our goal is to better understand the molecular and cellular nature of paediatric combined pituitary hormone deficiency diseases in order to facilitate improved diagnosis, treatment, and family counselling. LIM-homeodomain transcription factors regulate many aspects of development and mutations in the genes that encode these regulatory proteins are associated with several diseases. The LHX3 LIM-homeodomain protein is critical for pituitary gland organogenesis and some nervous system development. Loss of function of the LHX3 gene can cause complex paediatric syndromes involving combined pituitary hormone deficiency, limited neck rotation, hearing loss, and other symptoms. In recent studies, we have described patients with mutations in the LHX3 gene and we have investigated the aberrant mechanisms underlying loss of function of the gene or protein. Using gene knock-in technology to alter the mouse genome to mimic a human mutation in the LHX3 gene, we have generated a mouse model of paediatric combined hormone deficiency disease. This resource is allowing analyses of the developmental aspects of the disease using cellular and proteomic approaches and has also permitted studies of the influences of genetic background on the varied phenotypic outcomes of human transcription factor gene mutations. To understand the mechanism by which the human LHX3 gene is itself regulated, we have used transgenic approaches to characterise genomic promoter and enhancer elements that guide temporal and spatial expression of the gene in the endocrine and nervous systems. We are also investigating the mechanism by which LHX3 proteins interact with chromatin-associated regulatory complexes to modulate anterior pituitary gene expression. Supported by NIH HD42024 to SJR.
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### S56.3

**Transcriptional and epigenetic mechanisms for cell fate choice in pituitary**

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All secretory cells of the anterior and intermediate pituitary derive from the oral ectoderm that forms Rathke's pouch. Pituitary progenitors present in the pouch as well as similar progenitor/stem cells that remain along the cleft of the adult gland, are marked by expression of Sox2. Sox2-positive progenitors can self-replicate or enter differentiation. Before any differentiation marker is expressed, progenitors first exit the cell cycle under the control of the cell cycle inhibitor p57kip2; this mechanism appears to be restricted to progenitors and the related p27kip1 prevents cell cycle re-entry of differentiated cells. Initial events involved in differentiation and determination of cell fate are still poorly understood but transcriptional regulators driving terminal differentiation of pituitary lineages have been identified. Thus, Pit1 is required for terminal differentiation of somatotropes, lactotropes and thyrotropes whereas SF1 is required to complete differentiation of gonadotropes. Similarly, we identified Tpit as the transcriptional regulator for terminal differentiation of both POMC-expressing lineages, the corticotropes and melanotropes. The requirement on the same factor Tpit for terminal differentiation of both lineages does not explain the cell fate choice between these lineages. The combinatorial action of other transcription factors together with Tpit is one model that may direct unique cell fate choices. Interestingly, we have identified a factor that is expressed slightly before Tpit, only in melanotropes and that behaves as a cell fate switch and selector gene since its knockout results in a switch from melanotrope to corticotrope identify. Current evidence suggests that epigenetic changes including chromatin remodeling are required in order to change the outcome of Tpit-driven gene expression and cell identity. Genome-wide alteration of chromatin organization thus sets the stage for transcriptionnally driven terminal differentiation.
Declaration of interest
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## What's new in congenital adrenal hyperplasia (CAH)?

### S57.1

**Congenital adrenal hyperplasia: counselling from birth to the next generation**

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Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders causing deficient cortisol synthesis. The commonest cause, 21-hydroxylase deficiency, accounts for about 90–95% of cases. Other entities such as deficiencies of 11β-hydroxylase, 17α-hydroxylase, 3β-hydroxysteroid dehydrogenase type 2, and P450 oxidoreductase are considerably rarer. The differential diagnosis has to be established as different forms of CAH will require different therapeutic approaches and have different health risks. For over a decade, CAH newborn screening is clinical practice in an increasing number of countries. It is beneficial to reduce morbidity; however the impact on mortality is a matter of

debate. Newborns detected early enough can be commenced on medication and generally avoid hospitalisation. Improved steroid analysis employing rapid and comprehensive mass spectrometry profiling as first or second tier test reduces false positive rates. Such improvements have also simplified the differential diagnosis of CAH other than 21-hydroxylase deficiency. The drug of choice for glucocorticoid replacement in paediatric life is hydrocortisone. This therapy aims to replace cortisol, to normalise androgens into age and sex-specific ranges and to avoid normalisation of 17-hydroxyprogesterone concentrations. No supporting evidence exists on superiority on different timings of glucocorticoid application. Glucocorticoids should be minimised to avoid iatrogenic side-effects and adjusted to requirement during infancy, childhood and adolescence. This is often only achieved if mineralocorticoid and salt replacement are meticulously monitored. Additional growth promoting and puberty suppressing treatment are not routine therapy. Co-morbidities in CAH have been described in adults and to some degree in children. However, comprehensive data on the general health status during childhood and adolescence do not exists. These are warranted to identify the onset of co-morbidities and develop paediatric preventive health care provision strategies in CAH to improve prevention of long-term co-morbidities. This presentation will discuss diagnostic strategies, therapeutic management and onset of long-term problems in CAH.

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## S57.2

**CAH and adulthood**

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CAH is the commonest inborn endocrine disorder and associated with significant morbidity. The health status of CAH adult patients has recently been reported by the United Kingdom Congenital Adrenal Hyperplasia Adult Study Executive, CaHASE (Arlt *et al. JCEM* 2010 **95** 5110–21). Compared to the health survey for England, metabolic abnormalities were common in adult patients with CAH: obesity (41%), hypercholesterolemia (46%), insulin resistance (29%), osteopenia (40%), and osteoporosis (7%). HR-QOL (SF-36) showed significant impairment similar to scores in heart failure. The CAH patients were taking different glucocorticoid therapies at various doses ($n=196$): hydrocortisone ($n=25$M, 26W), prednisolone ($n=21$M, 67W), dexamethasone ($n=15$M, 22W), or combination therapy ($n=4$M, 16W). The CaHASE group have hypothesised that steroid dose mediates some adverse metabolic outcomes. ANOVA and univariate regression analysis only showed weak correlations ($r<0.2$) between prednisolone equivalent dose and SBP and DBP, HDL-cholesterol and HOMA-IR. However, using principal component analysis (PCA), it was identified that disease control factors, BP and mutation severity are associated with both the choice and total dose of glucocorticoid prescribed. Studies, independent of CaHASE, have examined the development of a modified release formulation of hydrocortisone, Chronocort, for the treatment of adults with CAH. Chronocort, in dexamethasone suppressed normal individuals, is capable of recreating the physiological rise in overnight cortisol levels and in adult patients with CAH improved control of morning 17-hydroxyprogesterone compared to immediate release hydrocortisone. In conclusion, health status in adults with CAH is significantly impaired and Chronocort represents a foundation for future drug development in the pursuit of physiological cortisol replacement.

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I fully declare a conflict of interest. Details below.
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## S57.3

Abstract unavailable.

# Best Clinical Practice

## S58.1

**Towards a European Guideline for the treatment of hyponatremia**

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The growing complexity of medical practice has increased the demand for well conceived guidelines. Such guidance is also urgently needed in endocrinology, but difficult, as well-conducted trials are scarce. As a first step towards the systematic development of 'Best Practice in European Endocrinology' a guideline for diagnosis and treatment of hyponatremia was initiated.
Hyponatremia is the most frequent electrolyte disorder and of prognostic significance. Furthermore, with the availability of V2-receptor antagonists a new treatment option has become available which poses a challenge to optimal diagnosis and targeted treatment.
The European Society of Endocrinology together with the Europeans Society of Intensive Care Medicine and the European Renal Best Practice Initiative of ERA-EDTA has, therefore, jointly initiated this guideline. A key technique is the PICOM methodology for selecting the relevant papers by defining the patient population (P), the intervention/exposure (I), the adequate comparator (C) and the relevant outcome (O) respectively. Furthermore the methodology of the respective studies needs to be carefully addressed (M). Careful development of the PICOM questions is key in the guideline development, as it is at the basis of the literature search. After defining the PICOM questions a comprehensive literature search was completed with a first screening based on titles and abstracts, followed by a second screening to eliminate editorials letters and comments without primary data. In the next screening phase the working group members of the guideline committee performed comprehensive data extraction of the relevant papers using a standardized data extraction form. This allows a summary of all findings in tables and a grading of the evidence applying GRADE methodology.
It is expected that at the time of presentation a first summary of the evidence can be presented. This effort represents an important first exercise to implement structures for the continuous development of 'Best Practice in European Endocrinology'.

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## S58.2

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## S58.3

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# Obesity and thyroid function

## S59.1

**Obesity and thyroid function**

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While it is well known that hyperthyroidism leads to weight loss, and hypothyroidism is associated with weight gain, there has been an increasing focus on the relationship between thyroid function and body weight in recent years. A moderate elevation of TSH concentrations, which is associated with triiodothyronine ($T_3$) values in or slightly above the upper normal range, is frequently found in obese humans. These thyroid hormone alterations in obesity seem rather a consequence than a cause of obesity since weight loss leads to a normalization of elevated thyroid hormone levels. The underlying pathways are not fully understood. White adipose tissue, the largest energy store in the body,

actively produces various hormones, cytokines, and chemokines, which together exert important roles in homeostasis and also in thyroid hormone regulation. The adipokine leptin seems to be the most promising link between body weight and TSH levels since leptin stimulates the hypothalamic–pituitary–thyroid axis.
High serum $T_3$ levels in obesity and overfeeding suggest a role for $T_3$ in metabolic adaptation to these situations: increased basal metabolic rate and thermogenesis in obesity and as a consequence the availability of accumulated energy for conversion into fat is diminished. Since rapid weight loss is associated with a decrease of TSH and $T_3$, the resulting decrease in REE may contribute towards the difficulties maintaining weight loss.
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The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S59.2

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## S59.3

**Type 2 deiodinase in muscle during health and disease**
A. Boelen
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One of the major routes for metabolism of thyroid hormones is by deiodination through the iodothyronine deiodinases, an enzyme family consisting of three members, types 1 (D1), 2 (D2) and type 3 (D3). The outer ring of thyroxin ($T_4$) can be deiodinated by D2, leading to the formation of active hormone, triiodothyronine ($T_3$). D2 is expressed predominantly in the brain and pituitary, but also in brown adipose tissue and muscle. Within the cell, D2 is localized in the endoplasmic reticulum and it is negatively regulated by thyroid hormone both pre- and post-transcriptionally.
Human skeletal muscle D2 was believed to be involved not only in local $T_3$ production but also in systemic production of $T_3$ in the basal condition. However, this concept was recently challenged as skeletal muscle D2 is expressed only at very low levels under euthyroid conditions in healthy persons. In addition, muscle D2 mRNA expression was reported to be modulated by fasting and by insulin suggesting that muscle D2 is involved in energy metabolism. Finally, recent studies have reported alterations in muscle D2 expression during inflammation. Both acute and chronic inflammation increase D2 mRNA expression in skeletal muscle while bacterial sepsis is associated with decreased D2 expression. Septic patients often develop muscle dysfunction which is probably due to decreased mitochondrial activity and content. This may point to a role for D2 in muscle dysfunction during sepsis as thyroid hormones are involved in mitochondrial function and biogenesis. In summary, D2 expression varies in skeletal muscle according to the type of inflammation. The changes in deiodinase expression are likely to result in altered local $T_3$ availability which may affect muscle function. Our future studies will focus on the question whether the observed changes in thyroid hormone metabolism in muscle during inflammation are clinically relevant in terms of muscle (dys)function.
Declaration of interest
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# Performance enhancing hormones in sports

## S60.1

**The spectrum of hormones in sports doping**
F. Lanfranco
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Physical activity exerts an important influence on the endocrine system, modulating synthesis and secretion of several hormones. On the other hand, several hormones are able to influence physical performance and body composition. Thus, a two-way relationship between exercise and hormones exists. The use of performance-enhancing compounds dates back to the initial Olympiad in ancient Greece where herbal remedies and animal extracts were used by the athletes before competition. In the past decades, hormone abuse has become a widespread habit among professional and - most of all and more frequently – recreational athletes. Anabolic androgenic steroids (AAS), GH and erythropoietin (EPO) are the most frequently abused hormonal substances in sports. The use of AAS as performance enhancing drugs dates back at the 1936 Olympic Games in Berlin, shortly after testosterone was first isolated and synthesized. GH was introduced to the world of sport by the Underground Steroid Handbook, a leaflet published in 1982 and used in the 1988 Olympic Games. Recombinant human EPO has been imputed to be abused by athletes in aerobic sports early after its marketing as an erythropoiesis-stimulating drug.
AAS are chemically modified analogs of testosterone which act on the skeletal muscle as a primary target tissue. The anabolic effect of testosterone is dose dependent, and significant increases in muscle size and strength occur only with supra-physiological doses. GH is used as a drug of abuse in sports, although there are no proper scientific studies demonstrating GH to be performance enhancing in normal subjects. EPO stimulates an increase in hemoglobin thus increasing the oxygen-carrying capacity of the blood.
The adverse effects of hormone abuse are numerous and affect several organs and body systems leading to increased mortality. Thus, hormone abuse among athletes and specific subsets of the general population represents a major public health issue which requires further research in sports endocrinology and widespread educational programs.
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## S60.2

**GH and testosterone: do they work?**
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Perceived anabolic benefits of androgens and GH have fueled their abuse among both competitive and recreational athletes. While both hormones increase muscle mass, whether they enhance exercise capacity in healthy adults is less clear. The yearning to boost performance however continues to bolster their inappropriate use in sports, despite many adverse effects. These include acne, excessive hair growth, prostate hypertrophy, behavioural, psychiatric, cardiovascular side effects, hepatic and sexual dysfunction for androgen use and oedema, carpal tunnel syndrome, arthralgias, as well as a state mimicking acromegaly with increased risk for diabetes, cardiomyopathy and malignancy, in the case of GH. As GH and testosterone interact to promote anabolic effects, many athletes abuse both of these hormones. Testosterone significantly and dose-dependently increases muscle mass and strength in healthy adults. The effect of testosterone on aerobic exercise capacity is less clear. GH increases lean body mass, although fluid retention contributes to this effect. Our recent data indicate that GH does not enhance muscle strength, power, or aerobic exercise capacity, but improves anaerobic exercise capacity. When GH and testosterone abused together, there is not only potentiation of their effects on muscle mass and function, but also increased risk to develop side effects. As both these hormones are effective in improving body composition and certain aspects of performance, they are tempting targets for abuse among athletes. Whether they live up to the expected, that's another question.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S60.3

**Erythropoietin and its derivatives**
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Erythropoietin (EPO) is a glycoprotein hormone endogenously produced by the kidney, whose main physiological role is the stimulation of red blood cells

production. Since the beginning of the nineties, recombinant human EPO (rhEPO) has been manufactured by pharmaceutical companies and was immediately misused by athletes to increase their muscle oxygenation process, enhancing then tremendously their endurance performances. It is why EPO has been put on the list of the forbidden substances of the International Olympic Committee (IOC), and then of the World Anti-doping Agency (WADA). Nowadays, not only EPO, but all the erythropoiesis-stimulating agents are part of the forbidden peptide hormones from the WADA list. The darbepoietin alpha (NESP for Novel Erythropoiesis Stimulating Protein), the methoxy polyethylene glycol-epoetin beta (CERA for Continuous Erythropoietin Receptor Activator) and the pegninesatide (Hematide) are also cited as example of forbidden compounds when practicing sports competitions. There are nowadays also many biosimilar epoietins on the market, mainly derived from epoietin alpha, widely used by the cheating athletes.

The analytical differentiation of endogenously produced erythropoietin from its recombinant form by using isoelectric focusing (IEF) and double blotting is one of the most efficient tool in the detection of doping with rhEPO. However, because of the use of these various analogous products, it is not always easy to detect them by the standard IEF-method. The different modes of application, by using for example micro doses of EPO biosimilars, make the detection window thinner and the efficiency of any method less robust than expected. Because of the lack of sensitivity of the direct detection of the doping substance, the new individual and longitudinal follow up of the athletes, called the biological passport, will certainly provide the proper answers to question of the abuse of EPO in top level sport.

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## New insights in thyroid cancer diagnosis

### S61.1

**BRAF(T1799A) can be detected in the blood of papillary thyroid carcinoma patients and correlates with disease status**

M. Zatelli
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The worldwide incidence of thyroid cancer has been increasing during the last years and is a public health concern because treatment of thyroid cancer implies high health costs and may raise overtreatment issues. On the other hand, effective medical treatment for advanced disease escaping radiometabolic treatment and not amenable for surgical approach is still lacking. Current methods of detection, decision making, prognostic indications, and monitoring rely on clinical, morphologic, and histopathologic characteristics. Therefore, precocious diagnosis is still a mainstay for thyroid cancer treatment in the early stages.

The activating BRAF(T1799A) mutation occurs in 45–80% of papillary thyroid cancers and is believed to cause the malignant transformation of the follicular cell. Detection of this mutation has been used to increase the diagnostic sensitivity of fine-needle aspiration biopsies of thyroid nodules suspected for malignancy and to provide a better staging, allowing detection of minimal disease metastatic to locoregional lymphnodes. In addition, BRAF(T1799A) mutation has been associated with unfavourable outcomes in retrospective studies.

Previous studies on melanoma have shown that circulating BRAFT1799A is indeed detectable and could potentially be used as a marker of prognosis and/or a surrogate for assessment of clinical response. The mutation has been more frequently found in metastatic tissue than in primary tumors. Therefore,the serum assay may be used in monitoring for disease progression. Accordingly, recent studies showed that detection of circulating BRAFT1799A might allow diagnosis of PTC with a blood test, identifying patients with minimal residual disease to be addressed to radioiodine remnant ablation, radioactive iodine therapy, or other adjuvant treatment measures. On the other hand, BRAFT1799A positivity in blood did not correlate with disease status/stage at the time of presentation and did not predict out come. Large-scale prospective studies are needed before circulating BRAFT1799A detection can become an accepted clinical tool for thyroid cancer screening and management.

Declaration of interest
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### S61.2

Abstract unavailable.

### S61.3

Abstract unavailable.

## Early Development and Treatment of PCOS

### S62.1

**Early hormonal abnormalities in children born to women with PCOS**

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Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic condition with a strong familial component suggesting a genetic susceptibility. During pregnancy, PCOS mothers offer an altered intrauterine environment which may also be implicated in the origin of this syndrome, and could determine endocrine/metabolic disturbances in their offspring (boys and girls) that may persist later in life. We have evaluated this possibility studying sons and daughters of PCOS mothers from early infancy to early adulthood. In daughters of women with PCOS (PCOSd), we have observed higher serum AMH concentrations and increased ovarian volumes during early infancy and adolescence compared to daughters of control mothers. In addition, these girls exhibit higher postprandial insulin serum concentrations and lower serum adiponectin levels compared to carefully matched controls, even before the onset of puberty. During puberty, we have studied PCOSd using the GnRH analog test, showing increased basal and peak testosterone concentrations and higher peak LH and 17-OHP levels, at a time when some early clinical signs of hyperandrogenism are mildly evident. During this period, 45% of PCOSd in Tanner IV and 60% in Tanner V have biochemical hyperandrogenism compared to control girls. However, it is not known how many PCOSd ultimately develop PCOS in the early reproductive period. After menarche (1–3 years) using the AES criteria, hyperandrogenism is the main diagnostic feature for PCOS present in these girls, while menstrual irregularity, PCO morphology and AMH levels are secondary elements that may aid in the diagnosis of this syndrome. In sons of women with PCOS, we have also observed increased serum AMH concentrations and higher BMIs, well before the onset of puberty. During adulthood, they remain overweight and show higher postprandial insulin concentrations and lower insulin sensitivity (adjusted for BMI) compared to controls. In summary, our studies have addressed the timing and sequence of the onset of metabolic and reproductive perturbations in the offspring of PCOS patients. Considering the early onset and the nature of these alterations, PCOS sons and daughters constitute a high-risk group for metabolic and reproductive disease.

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### S62.2

**Early development and pubertal prevention of polycystic ovary syndrome**

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PCOS (defined by NIH, Rotterdam or AES criteria) is traditionally viewed as an ovarian disorder that appears in adolescence and may lead to metabolic

complications in adulthood. Emerging evidence, however, indicates that PCOS is primarily a disorder of adipose-tissue hyperexpansion that may originate in early life, develop across childhood and puberty, and advance into end-stage disease (including ovulatory dysfunction and ovarian androgen excess) by adolescence. Prepubertal markers pointing to PCOS risk include a viscerally adipose body composition, high levels of circulating triglycerides, insulin, IGF1, DHEAS and leptin, and low levels of adiponectin and SHBG.
The novel concept on the ontogeny of PCOS does not only harbour the most common (obese and non-obese) phenotypes of PCOS, but implies also a potential to prevent PCOS by preventing the hyperexpansion of adipose tissue in childhood and puberty. This potential has now been tested in a subgroup of non-obese girls that can be identified early as being at high risk for PCOS, namely low-birthweight girls with precocious pubarche. Treatment with metformin across late prepuberty and puberty was associated with postpubertal reductions in visceral, hepatic and total-body adiposity and also with a reduction in the prevalence of PCOS (5 vs 47% by NIH or AES criteria, after 8 year of study). Welcome epiphenomena of such early metformin intervention were an increment of adult stature (by ∼4 cm towards normal) and a delay of menarche (by ∼1 year towards normal).
Another implication of the novel concept is that intervention in adolescent girls with PCOS should not aim primarily at silencing the ovaries (with an oral contraceptive) but rather aim at reducing adipose-tissue hyperexpansion and low-grade inflammation (for example, with a low-dose combination of metformin, flutamide and pioglitazone) thereby allowing for spontaneous resumption of ovulation and also for waning of androgen excess.
Declaration of interest
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## S62.3

**Hyperandrogenism in obese girls: what is the significance?**
C. McCartney
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Polycystic ovary syndrome (PCOS) is marked by (primarily ovarian) hyperandrogenemia and ovarian dysfunction. In many cases, PCOS first manifests during adolescence, and both peripubertal hyperandrogenemia and obesity are considered risk factors for the development of PCOS. Many, but not all, pubertal girls with obesity – including those in early puberty – exhibit relative hyperandrogenemia. However, the causes of peripubertal obesity-associated hyperandrogenemia, and mechanisms by which hyperandrogenemia could support progression to PCOS, are unknown.
Early data suggest that adrenal androgen responsiveness to ACTH is exaggerated in overweight peripubertal girls; this may partly reflect the effects of hyperinsulinemia. However, LH is the proximate stimulus for ovarian androgen production, and LH appears to be a better predictor of free testosterone than insulin in obese girls. Studies in adults with PCOS and in animal models suggest that excess androgens decrease GnRH pulse generator sensitivity to sex steroid negative feedback, augmenting GnRH pulsatility. These relationships – LH stimulating androgen production and androgens increasing GnRH (and LH) secretion – may constitute a vicious cycle that links peripubertal hyperandrogenemia and the development of full-blown PCOS.
How obesity and hyperandrogenemia affect the normal pubertal sequence of GnRH secretion is unclear. Early puberty is characterized by sleep-associated increases of LH (and by inference GnRH) pulse frequency, while LH pulse frequency decreases during sleep in late puberty. Early data suggest that these wake-sleep changes may partly reflect differential sensitivity of the GnRH pulse generator to negative feedback suppression depending on sleep status. We have proposed a working model in which relative hyperandrogenemia during puberty – as may occur in obesity – disrupts these relationships. Indeed, both early and late pubertal girls with obesity – especially those with hyperandrogenemia – exhibit blunted wake-sleep changes of LH pulsatility. Such alterations may increase LH release and limit FSH production, worsening hyperandrogenemia and promoting progression toward PCOS.
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# Emerging ideas about phosphate metabolism

## S63.1

Abstract unavailable.

## S63.2

**FGF23 and tumor-induced osteomalacia**
S. Fukumoto
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Tumor-induced osteomalacia (TIO) is a paraneoplastic syndrome characterized by hypophosphatemia associated with renal phosphate wasting and low to low normal serum level of 1,25-dihydroxyvitamin D (1,25(OH)2D). FGF23 was identified as a causative factor for TIO which inhibits proximal tubular phosphate reabsorption and decreases serum 1,25(OH)2D by modifying the expression levels of vitamin D-metabolizing enzymes. This disease is cured by complete resection of responsible tumors. However, it had been clinically often difficult to diagnose and treat patients with TIO. After the identification of FGF23, it was shown that FGF23 levels are elevated in virtually all patients with TIO and rapidly decrease after complete removal of responsible tumors. Therefore, measurement of FGF23 levels seems to be useful in the diagnosis and follow up of patients with TIO. The causative tumors for TIO are often small and difficult to find. Several imaging studies including as magnetic resonance imaging, positron emission tomography and octreotide scintigraphy have been used for the search of responsible tumors for TIO. However, none of these imaging studies indicate that the detected tumors are producing FGF23 and responsible for TIO. We have conducted systemic venous sampling for FGF23 in several patients with suspected TIO and reported that this maneuver is useful for the detection of responsible tumors at least in some patients. Furthermore, it is possible that the inhibition of FGF23 activity could be a new therapy for patients with TIO whose tumors cannot be found or removed by technical reasons. In this symposium, I would like to discuss about the diagnosis and treatment of TIO with special emphasis on FGF23.
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## S63.3

**FGF23 in chronic renal failure**
J. Silver
Hadassah Hospital, Jerusalem, Israel.

FGF23 secretion is increased in response to 1,25(OH)2 vitaminD3 (1,25D) and an oral phosphorus (P) load. Patients with chronic kidney disease (CKD) are unable to excrete their dietary P which contributes to an increased secretion of the two major phosphaturic hormones, PTH and FGF23. The increased FGF23 would normally bind to its receptor, klotho-FGFR1 in the renal tubules to increase P clearance and decrease the synthesis of 1,25D. Increased FGF23, P and PTH levels are all associated with an increased mortality in CKD. Physiologically, FGF23 acts on its receptor klotho-FGFR1 in the parathyroid to decrease PTH expression and parathyroid cell proliferation but in advanced CKD there is a decrease in the expression of klotho-FGFR1 both in the parathyroid in the kidney. This is associated with high levels of both FGF23 and PTH. In turn, the high levels of PTH act on bone cells to increase FGF23. We recently showed that PTH acts directly on osteoblast like cells to increase FGF23 expression. Remarkably, parathyroidectomy in rats both prevents and corrects the high FGF23 due to short-term experimental uremia. In patients a decrease in PTH leads to a decrease in serum FGF23. Therefore, in CKD, the high levels of PTH are necessary to maintain the high levels of FGF23. Experimental CKD is associated with low renal klotho levels and the administration of klotho has been shown to bind to TGFβ1 and improve renal function. Dialysis patients' course is often complicated by left ventricular hypertrophy (LVH) and cardiac failure. FGF23 acts directly on cardiomyocytes to cause LVH and the progression to LVH in CKD correlates with the high levels of FGF23. Moreover, administration of an FGFR blocker attenuated LVH in experimental CKD. Therefore, FGF23 expression is increased by P, 1,25D and PTH and it has important actions on the kidney, parathyroid and heart.

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## Endoplasmic Reticulum Stress, Obesity and Metabolic Homeostasis

### S64.1

Abstract unavailable.

### S64.2

Abstract unavailable.

### S64.3

Abstract unavailable.

## Somatostatin receptors in pituitary

### S65.1

**Imaging somatostatin activity in live pituitary**
S. Schulz
Jena University, Jena, Germany.

Fluorescence resonance energy transfer (FRET) using genetically encoded biosensors has proven to be a powerful technique to monitor the spatiotemporal dynamics of cAMP signals stimulated by Gs-coupled receptors in living cells. In contrast, real-time imaging of Gi-mediated cAMP signals under native conditions remains challenging. Here, we describe the use of transgenic mice ubiquitously expressing the highly sensitive cAMP sensor Epac1-camps for cAMP imaging in living pituitary slices and primary pituitary cells. This technique can be widely used to assess the contribution of various pituitary receptors, including individual Gi protein-coupled somatostatin receptors, to the regulation of cAMP levels under physiologically relevant settings.
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### S65.2

**Is somatostatin receptor expression in adenomas related to pharmacological response?**
S. Fougner
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The clinical response to somatostatin analogue (SMS) treatment in acromegaly is highly variable. In unselected, previously untreated patients, biochemical control is achieved in 20–50% of patients, and mean overall tumour shrinkage has been reported to 25–40%.
The somatostatin analogues exert their effects through binding to the somatostatin receptor (SSTR) localized in the cell membrane. The available analogues bind with high affinity to SSTR2, less to SSTR5 and lower to SSTR3. It can therefore be expected that the adenoma expression of SSTR2 is important for the SMS response. Indeed, the first studies of SSTR2 mRNA from adenoma extracts demonstrated a correlation to *in vivo* octreotide response either in an acute test or long-term treatment. This has later been confirmed in studies of SSTR2a protein expression and long-term SMS treatment. Two studies of 18/20 patients have shown that the percentage GH or IGF1 reduction during treatment is larger in adenomas with a high proportion of SSTR2a positive cells in adenoma immunohistochemistry. Another study found that patients with $<50\%$ GH reduction during preoperative treatment did not express SSTR2a in immunohistochemistry, in contrast to octreotide responders. However, the studies also demonstrated patients with low SSTR2a positivity and good octreotide response, and patients with high SSTR2a positivity and very poor octreotide efficacy. This suggests that not only adenoma SSTR2 expression determines the clinical efficacy of SMS. One could expect an impact of SSTR5, but the research of SSTR5 expression and SMS response has not been conclusive. However, the densely granulated somaototroph adenomas respond significantly better to SMS treatment compared to sparsely granulated adenomas, but they also display lower SSTR2a protein expression. Despite a lower SSTR2a protein expression in gsp positive adenomas, they do not respond better to long-term SMS treatment. This can imply additional mechanisms important for SMS response in these adenomas.
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### S65.3

**Somatostatin receptors and filamin: a developing story**
S. Najib[1,2], N. Saint-Laurent[1], D. Fourmy[2], S. Schulz[4], R. Luque[5], J. Castano[5], M. Delisle[1,3], S. Pyronnet[1,2], C. Susini[1,2] & C. Bousquet[1,2]
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The G protein-coupled (GPCR) somatostatin receptor sst2 transduces the majority of somatostatin actions, including inhibition of hormone and growth factor secretion, cell survival and angiogenesis. Sst2 agonists such as octreotide are widely used for the treatment and diagnostic of human neuroendocrine tumors that frequently over-express sst2. To initiate its inhibitory signalling pathways, sst2 engages, in addition to G protein subunits, different protein partners such as phosphatases and kinases. We have reported an original mechanism for sst2 to restrain the phosphoinositide 3-kinase (PI3K) activity, involving a ligand-regulated direct interaction between sst2 with the PI3K regulatory p85 subunit. We also identified the scaffolding protein filamin-A (FLNA) as a critical player regulating the dynamic of this complex. A pre-existing sst2–p85 complex, which was shown to account for a significant basal PI3K activity in the absence of ligand, is disrupted upon sst2 activation. FLNA was identified as a competitor of p85 for direct binding to two juxtaposed sites on sst2. Switch of sst2 binding preference from p85 towards FLNA is determined by changes in tyrosine phosphorylation of p85- and FLNA-binding sites on sst2 upon activation. It results in disruption of the sst2–p85 complex and subsequent inhibition of PI3K. Knocking-down FLNA expression, or abrogating FLNA recruitment to sst2, reversed inhibition of PI3K and of tumor growth induced by sst2. FLNA is an actin-binding and scaffolding protein for numerous cytosolic and transmembrane signalling proteins including GPCRs. Our results demonstrate that FLNA is critical to maintain the stability of sst2 at the membrane in close proximity with its signalling effectors including actors of the lipid PI3K family. Strikingly, in GH-secreting pituitary adenomas expressing sst2, resistance to octreotide correlates with decreased expression of FLNA, further emphasizing the critical role of FLNA in sst2 functions.
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## HPA axis, stress metabolism and adaptation

### S66.1

**Neuroendocrine and (epi)genetic impact on HPA function**

E. de Kloet
Leiden University Medical Center, Leiden, The Netherlands.

The HPA axis facilitates adaptation to stress and is essential for metabolism and health, but dysregulation of the HPA axis compromises resilience and promotes vulnerability to stress-related diseases. This includes diseases of the brain where an aberrant stress hormone action targeting susceptibility pathways causes emotional and cognitive disturbances, and affects the onset and progression of neurodegenerative processes, while impairing recovery and neurogenesis. This action exerted by the hormone involves genes, but also epigenetic modulations triggered by early life experience may permanently alter behaviour and HPA axis activity. The focus is on the action of the HPA axis endproduct cortisol and its dual high- and low affinity receptor systems: mineralocorticoid (MR) and glucocorticoid receptors (GR). Both receptor types are co-expressed in abundance in limbic brain regions and serve together as a master switch in the control of neural network responses that underly activation and suppression of HPA axis activity. Imbalance in MR:GR driven pathways caused either by genetic receptor variants or by experience-related factors compromises processing of stressful information and dysregulates the HPA axis. Therapies are therefore envisioned to rebalance the HPA axis for protection or repair from damaging signaling pathways. Here I will discuss indepth our recent data on i) functional implications of genetic receptor variants as presented recently by MD Klok and RH de Rijk (Translational Psychiatry (2011) 1, e62), and ii) the work of NP Daskalakis (Thesis Leiden University, 2011) regarding the role of genetic predisposition in the programming effects of adverse early life/adolescent experiences on the HPA axis.

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### S66.2

Abstract unavailable.

### S66.3

**Modulation of cortisol action**

M. Jangani, A. Berry, L. Matthews, S. Farrow, R. Donn & D. Ray
University of Manchester, Manchester, UK.

Variation in glucocorticoid (Gc) sensitivity underlies metabolic disease, and affects therapeutic response in inflammation. Using an in-vitro screening approach we identify two new mechanisms capable of regulating Gc sensitivity. The first involves interferon induced factor 16 (IFI16). IFI16 potentiated both transactivation, and Gc repression of NFκB. IFI16 did not affect glucocorticoid receptor (GR) expression, ligand dependent repression of GR expression, or the ligand dependent induction of GR phosphorylation. Co-immunoprecipitation revealed an interaction, suggesting IFI16 modulation of GR function is mediated by protein cross-talk. Transfection analysis with GR mutants showed that the ligand binding domain of GR binds IFI16, and is the target domain for IFI16 regulation.

The second mechanism involves Merm1, a histone methyltransferase, which potentiates GR transactivation through its SAM and methyltransferase domains. Merm1 is required for maintenance of an active chromatin methyl mark (H3K79me2) at the GREs and facilitates GR binding. Activated GR promotes H3K4me3, and represses H3K79me2 marks at GREs, in a Merm1 dependent manner, suggesting coordinated histone modification whereby one change influences the other. Inflammation causes glucocorticoid resistance, which is replicated in-vitro by the combined action of inflammatory cytokines TNFα, and IFNγ. These cytokines suppress Merm1 protein, impair GR recruitment to GREs, and inhibit acquisition of the H3K4me3 mark. Glucocorticoid sensitivity was rescued by restoring Merm1 expression.

In conclusion, IFI16 is a novel modulator of GR function, acting through the GR ligand binding domain, and Merm1 regulates chromatin structure so affecting GR recruitment, GR function, and mediates cytokine-induced cellular glucocorticoid resistance.

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## Central regulation of energy homeostasis

### S67.1

**CNS control of energy and glucose metabolism**

C. Könner
Institute for Genetics, Cologne, Germany.

The tight coordinated regulation of food intake and energy expenditure represents the prerequisite for stable body weight control. Dysregulation of energy homeostasis, i.e. obesity represents a major and steadily growing health burden to western societies. The CN coordinates both the control of energy homeostasis, i.e. the balance of food intake and energy expenditure as well as peripheral fuel metabolism. Anatomical lesion experiments, pharmacological inhibition of signaling pathways and, more recently, the analysis of conditional mouse mutants with modifications of hormone and nutrient signaling in defined neuronal populations have broadened our understanding of how these complex neurocircuits interact and how their activity is regulated. The hypothalamus receives information from the periphery of the body about the fuel sources available either via hormonal signals such as leptin and insulin as well as directly by nutrient components such as amino acids, glucose and fatty acids. The presentation will focus on the genetic analysis of interacting neurocircuits involved in these processes.

Declaration of interest

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### S67.2

**Gut hormones: history, physiology and therapeutic opportunities**

Dr Keval Chandarana

In recent decades, a great deal of investigation has been focused on the role of gut hormones in energy homeostasis. The gastrointestinal tract is the largest endocrine organ in the body producing hormones that have important sensing and signalling roles in regulating body weight and energy expenditure. Dietary modifications are the first-line treatment for obesity however dieting results in only moderate weight-loss and maintenance of weight-loss is poor. Compensatory gut hormone changes appear to contribute to the failure of weight-loss through dietary means. In contrast, bariatric surgery is an efficacious treatment modality for obesity, resulting in durable weight loss and amelioration of obesity-associated co-morbidities, particularly type 2 diabetes mellitus. Moreover, the metabolic benefits of bariatric surgery occur independently of weight loss. An increasing body of evidence suggests that alterations in circulating gut hormones mediate the weight-loss and metabolic benefits of bariatric surgery. Logically, strategies aimed at modulating circulating gut hormone concentrations or targeting their receptors are being developed as potential pharmacotherapies for obesity.

### S67.3

Abstract unavailable.

## European network for the study of adrenal tumours (ENSAT)

### S68.1

**Genetics for pheochromocytoma and paraganglioma in 2012**

M. Robledo
Spanish National Cancer Center, Madrid, Spain.

Pheochromocytomas (PCCs) and paragangliomas (PGLs) are rare tumours of neural crest origin, previously referred to as 'the 10 percent' tumor due in part to the frequency of inherited forms. During the last decade the scientific community has been witness to a fast increase of knowledge of the genetics of these tumours. In this regards, the use of different strategies and new platforms has allowed to identify up to date thirteen PCC/PGLs susceptibility genes. Currently the proportion of familial PCC/PGLs may exceed 40%, which renders PCC/PGL as one of the most inherited tumor entities.

There is no doubt about the importance of detecting a mutation in any of the known genes related to the disease, not only for the affected patient but also for the relatives. Keeping in mind the genetic heterogeneity we face, it is critical the consideration of clinical, biochemical and immunohistochemical features to set up efficient algorithms able to guide a rational genetic screening in each patient. It is worth highlighting that at least 10% of patients, negative for the genetic study of the so far disease-related genes, are actually good candidates to be carriers of mutations in other susceptibility loci not yet identified.

Despite the powerful tools currently available, the identification of new susceptibility genes is not a straightforward issue. Only after a thorough patient's selection, either due to the phenotypic features or other OMIC data, the discover of new genes will be feasible. The participation of International Consortium is mandatory to this end.

Declaration of interest
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### S68.2

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Abstract unavailable.

### S68.3

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Abstract unavailable.

## Current progress in the management of thyroid cancer

### S69.1

**TSH suppression in differentiated thyroid cancer: how low should we go?**

D. Cooper
The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

Suppression of serum thyrotropin (TSH) is a cornerstone of thyroid cancer treatment, due to the tropic effects of TSH on thyroid cells. However, when deciding on the degree of TSH suppression during initial and long-term management, it is necessary to consider the aggressiveness of the cancer, as well as the potential for adverse effects induced by iatrogenic subclinical or overt hyperthyroidism. More aggressive TSH suppression is indicated in patients with high-risk disease or recurrent tumor, whereas less aggressive TSH suppression is reasonable in low-risk patients. In patients with high-risk differentiated thyroid cancer and an equally high risk of adverse effects (e.g. osteoporosis, cardiovascular disease), long-term treatment with levothyroxine therapy should be individualized and balanced against the potential for adverse effects.

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### S69.2

Abstract unavailable.

### S69.3

Abstract unavailable.

## Young Active Researchers Symposium (YAR)

### S70.1

**New mechanisms underlying the role of the mTORC1 pathway in the hypothalamic control of energy balance**

D. Cota
INSERM, Bordeaux, France.

Similar to individual cells, hypothalamic neural circuits profit from intracellular pathways known to work as fuel sensors to maintain energy balance.

The mammalian target of rapamycin complex 1 (mTORC1) signaling cascade is among the latest intracellular fuel-sensing pathways to be implicated in the hypothalamic regulation of energy balance.

mTORC1 activity is found in both NPY/AgRP- and POMC-producing neurons of the arcuate nucleus. Activation of mTORC1 and phosphorylation of its downstream targets are critical intracellular steps mediating the anorectic actions of both nutrients, such as amino acids, and hormonal signals like leptin. Interestingly, mTORC1 activity in the mediobasal hypothalamus varies as a function of the cell type and of the particular stimulus employed, as opposed to responding in a uniform manner to nutritional and hormonal changes. Furthermore, a link exists between intracellular fatty acid metabolism and mTORC1 and recent studies have shown that high-fat feeding and diet-induced obesity are conditions leading to the impairment of mTORC1 activity in the hypothalamus.

Here we will give an overview of the known functions of this pathway in the context of energy balance regulation and we will further present downstream mechanisms that we have recently identified to be engaged by mTORC1 for the control of food intake and body weight.

Taken together, these findings lead to conclude that mTORC1 is a highly conserved fuel sensor, which integrates signals from both stored and immediately available fuels and whose activity in the hypothalamus triggers adaptive feeding responses.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S70.2

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Abstract unavailable.

## S70.3

**DNA methylation of the body weight-regulating proopiomelanocortin gene: functional and ontogenetic aspects**

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Obesity is a polymorphic chronic disease with epidemic prevalence. Furthermore, heritability of the weight phenotype is high. However, within the catabolic leptin–melanocortin signalling pathway, which is pivotal for body weight regulation, gene mutations are rare. This indicates that other, non-genetic heritable factors might play a role in the development of obesity, such as epigenetic mechanisms. In a candidate gene approach, we analyzed the functional relevance and ontogenesis of the DNA methylation of the proopiomelanocortin (POMC) gene, which has a key role in hypothalamic body weight regulation. In vitro luciferase reporter gene analyses revealed DNA methylation-dependent promoter activity of both CpG islands (CGIs) of POMC. Regarding the ontogenesis, postnatally stable POMC DNA methylation patterns with interindividual conservation and non-tissue specificity were detected for both CGIs in mice, applying bisulfite sequencing. In addition, it was observed that the POMC DNA methylation patterns develop prenatally between the blastocystal stage and the early organogenesis.
Overall, these results indicate that stochastic variances arising in the course of the POMC DNA methylation development might influence the POMC activity and consequently alter the risk to develop obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S70.4

**Mineralocorticoid receptor antagonism counters metabolic dysfunctions induced by high fat diet in mice**

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Drospirenone (DRSP) is a potent antimineralocorticoid with progestative and moderate antiandrogenic properties. We have previously shown that DRSP exerts a potent antiadipogenic activity in murine and human preadipocytes through specific antagonism of the MR. In the present study responses to DRSP were investigated in a model of diet-induced obesity for 12 weeks. Female 10-week-old C57bl6 mice were fed with normal chow or a high fat diet (HF, containing 45% fat by calories). Mice were concomitantly treated for 12 weeks with either vehicle, DRSP (3 and 30 mg/kg per day) or the MR antagonist Potassium Canrenoate (kCan 20 mg/kg per day). Glucose tolerance tests were performed after 3 and 12 weeks of treatment. High levels of MR mRNA were detected in all examined depots of adipose tissue. Mice fed HF showed significantly increased body weight and fat mass, increased expression of MCP-1 and adipogenic markers and a reduced glucose tolerance. DRSP (3 mg/kg per day) significantly improved glucose tolerance and reduced gonadal, renal and subcutaneous fat mass in mice fed HF; similar responses were observed with KCan, though to a lesser extent. Importantly, DRSP reversed HF-induced up-regulation of leptin, LPL, AcetilCoA and MCP-1 mRNA expression. HF diet-induced increase in adipocyte size was also completely reversed by DRSP, and to a lesser extent by kCan. Finally, DRSP induced the appearance of paucilocular UCP1 positive adipocytes in gonadal and subcutaneous adipose tissue of mice fed HF.
In conclusion, we show that DRSP can reverse adipocyte dysfunction induced by HF in an MR-dependent manner. Finally, DRSP treatment caused a dramatic morphological switch of white to brown adipocytes. This could explain the improvement in the metabolic profile of treated mice, suggesting a novel role for MR in controlling fat metabolism and white to brown transdifferentiation. MR blockade therefore has promise as a novel therapeutic option for the prevention of metabolic syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## S70.5

**A four-week fat overfeeding study in constitutional thin women: a human model of weight gain resistance**

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Introduction
Constitutional thinness (CT) is a rare condition of natural low body weight, with normal menstruation, no fear of weight gain and no hormonal abnormalities except for an anorexigenic hormonal profile. CT can be considered as the opposite of obesity and its resistance to weight loss. Therefore, we hypothesised they would have a resistance to weight gain.
Methods
10 female Controls (BMI 18.5–25 kg/m$^2$) and 10 CT women (BMI $<$ 17.5 kg/m$^2$) underwent a 4-week fat overfeeding (700 kcal) intervention. Body weight, food intake, body composition, energy expenditure and the profile of appetite regulatory hormones after test meals were monitored prior to and after dietary intervention.
Results
After one month of fat overfeeding, the mean body weight remained the same in the CT group ($-0.300$ kg, $P=0.258$ vs baseline) showing a resistance to weight gain, whereas it normally significantly increased in the control group ($+1.3$ kg, $P=0.0248$). After one month ad libitum food intake, the CT group body weight significantly dropped down ($-0.850$ kg, $P=0.0245$ vs the end of the overfeeding period), showing a rapid escape of the body weight gain, whereas the control group maintained its body weight gain ($+1.5$ kg, $P=0.02$ vs baseline). Resting energy expenditure significantly increased in the CT group only ($P=0.0307$). In the CT group, post meal PYY plasma levels ($P=0.008$) and post meal exposure time to GLP-1 significantly increased ($P=0.04$), emphasising the baseline anorexigenic profile. Conversely, in the control group, total and acylated ghrelin plasma levels increased significantly ($P=0.016$ and $P=0.028$ respectively) in an orexigenic profile.
Conclusion
This data is the first to demonstrate a resistance to weight gain in constitutional thinness population in response to 4-week fat overfeeding, associated with an increase in resting energy expenditure and an emphasised anorexigenic hormonal profile.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Endocrine Nurse Symposium

## EN1.1

**Puberty induction in males and females**

Caroline Brain
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Induction of puberty is indicated in children with primary or secondary gonadal failure, and where there is pubertal arrest or delay for whatever reason.
Timing of induction will vary depending upon age at presentation/diagnosis, but as far as possible this should coincide with expected pubertal changes within the normal population range.
Optimization of final height, breast and uterine development, and fertility are important outcome considerations and are dependent upon the underlying diagnosis (e.g.Turner syndrome, Klinefelter's Syndrome, survivors of childhood cancer).
Indications for pubertal induction in both sexes fall into two main diagnostic categories;

- Hypogonadotrophic hypogonadism
  Hypopituitarism (primary & Secondary)
- Hypergonadotrophic hypogonadism
  Primary Gonadal failure (dysgenic/syndromic/autoimmune/toxicity)

Specific genetic diagnoses may require disclosure and counciling prior to pubertal induction and ideally require a multidisciplinary team approach.
Protocols for induction of puberty must be individualized as response to sex hormone replacement is very variable and may depend upon the underlying diagnosis.
Puberty may be induced using preparations given by oral, transdermal or intramuscular (Male) routes. Titration of dose is made depending upon individual clinical response with emphasis on mirroring normal pubertal development.
Appropriate monitoring and transition to adult service as appropriate, is an integral part of the induction process.
*The role of the Endocrine clinical nurse specialist is crucial to this process and for optimum transition to long term adult follow-up.*

## EN1.2

**The role of the endocrine nurse in puberty induction**

Meg Keil

The adolescent/young adult who requires pubertal induction presents unique challenges to the health care team. Nurses serve as a key resource for patients and their families during the process of pubertal induction. Health promotion is the keystone of nursing practice and a developmental model is an appropriate framework to tailor nursing assessment and intervention to the adolescent/young adult's developmental level. The nurse's role in health promotion during pubertal induction may include: psychosocial and physical assessment, advocacy, case management, counseling, and patient education. Perhaps the most important component of the initial assessment prior to initiation of pubertal induction is for the nurse to address the patient and/or family member's anxiety or fears in order to identify potential barriers to initiation of and/or compliance with treatment. Typical patient/family concerns include: body image, short stature, final height, social interaction, physical and emotional changes, what to expect during treatment, possible side effects, fertility, and selection and cost of treatment. The endocrine nurse also provides education and advice to patients and their families regarding: normal puberty, effects of pubertal delay, diagnostic testing, goals and expectations of treatment, options for sex steroid replacement, possible side effects of treatment, clinical monitoring of treatment, and sexuality. In addition, the role of the endocrine nurse includes providing reinforcement of medical information discussed with endocrine health care team and facilitation of communication with the primary health-care provider, school nurse, and insurance provider. Part of counseling the patient/family includes referral to appropriate resources such as patient support groups, social work, genetic counseling, and patient education literature/web sites. Future studies are needed to identify effective intervention models for health promotion that include outcome measures such as quality of life, patient satisfaction, and cost effectiveness.

## EN1.3

Abstract unavilable.

## EN1.4

**Choosing the right sex steroid replacement option for patients: the role of the endocrine nurse**

Shashana Shalet (Endocrine Advanced Nurse Practitioner)

In the UK the expansion of nurses' autonomy and scope of practice over recent years means that the clinical nurse specialist (CNS) can effectively provide a quality service to endocrine patients. The role of the endocrine CNS in enabling patients to choose the right sex steroid replacement option does not differ much from the Doctor's role. The CNS does, however, often have more time to provide the necessary education to the patient required to make an informed choice and thereby potentially increasing compliance with their chosen therapy. The CNS is able to review the patient and their medications on a more frequent basis which again can support compliance with treatment.
A sound knowledge base of the available treatment options and the ability to focus those options down to meet the exact needs of the individual patient is a key element in providing this service. By understanding the benefits and risks of individual medications, being knowledgeable around the necessary precautions as well as creating a trusting relationship with the patient through clear communication the role of the endocrine CNS is to support the informed choice process. It is important that there are clear and structured clinical guidelines that support this process and ensure the safety of both the patient and the CNS.

## EN2.1

**Diagnosis and management of Cushing's syndrome**

Richard Feelders
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Cushing's syndrome (CS) is characterized by chronic overproduction of cortisol resulting in significant morbidity and, when untreated, an increased mortality. CS is divided into adrenocorticotropin (ACTH)-dependent CS and ACTH-independent CS. ACTH-dependent CS, the majority of cases, can be caused by a corticotroph pituitary adenoma and, more rarely, by ectopic ACTH production. ACTH-independent CS is usually caused by an unilateral adrenal adenoma and less frequent by an adrenal carcinoma and bilateral adrenal hyperplasia.
The diagnosis CS is biochemically established by demonstration of increased urinary cortisol excretion, insufficient cortisol suppression by dexamethasone and absence of the cortisol diurnal rhythm. In case of ACTH-dependent CS and a negative MRI of the sellar region, bilateral inferior petrosal sinus sampling (BIPSS) is performed to differentiate between a non-visible pituitary adenoma and ectopic ACTH production. If BIPSS shows no central to peripheral ACTH gradient the ectopic cause can often be visualized with CT and somatostatin receptor scintigraphy. In patients with ACTH-independent CS, CT or MRI of the abdomen is performed to detect an adrenal neoplasia.
Surgery is the primary treatment for all causes of CS. The remission rate in patients with pituitary-dependent CS varies between 60 and 80% after transsphenoidal adenomectomy. Patients with persistent or recurrent pituitary-dependent CS can be treated with radiotherapy and/or medical therapy with neuromodulatory drugs (dopamine agonists, somatostatin analogs), adrenal blocking drugs (ketoconazole, metyrapone) or glucocorticoid receptor antagonists (mifepristone). Ectopic ACTH production is frequently accompanied by severe hypercortisolism which may necessitate bilateral adrenalectomy.

## EN2.2

**The role of the endocrine nurse in the diagnosis and management of the patient with Cushing's syndrome**

W Geilvoet

Cushing's syndrome is a rare disease and difficult to diagnose. A number of different physical and psychological signs and symptoms can be present. Lots of patients have symptoms many years before diagnosis. Endocrine nurses can play an important role for these patients during diagnosis, medical or surgical treatment and in the postoperative phase.
During the diagnostic phase, nurses need to know why patients must undergo certain endocrine function tests, which diagnostic medication needs to be administrated and how to inform the patient. During the operative phase, the endocrine nurse will inform the patient about the pre-operative work up, the surgery and post-operative observations. But it is also important to pay attention

to the psychological disturbances patient may suffer from like depression, anxiety and even psychoses.
After surgery, psychological en physical recovery may take a long time. Even after long-term remission of Cushing's syndrome, patients report more negative illness perceptions compared with patients with other acute or chronic conditions. There are some patients who will need a rehabilitation program to work on a better physical condition and to get psychological support during reducing the glucocorticoid replacement therapy to a 'normal level'. In this phase, the endocrine nurse gives education about the recovery process, helps the patient with coping strategies, adherence to medication and self-management. The self-management aspects that come with glucocorticoid replacement therapy, like dose adaptation during medical emergencies, recognising and preventing life threatening situations, like an acute adrenal crisis needs to be highlighted repeatedly.
Not only information and instructions given by nurses to the patient and his family are important, but also education for nurses in the ward or outpatient clinic is recommended. Endocrine nurses can make the difference for these patients.

## EN2.3

**Diagnosis and management of prolactinoma**

M Andersen
Odense University Hospital, Odense C, Denmark.

Prolactinomas are the most prevalent pituitary adenomas. The incidence of prolactinomas varies with age and sex. The ratio between women and men has been estimated to 10:1 before the age of 50 years. Hypogonadism and infertility are important clinical problems associated with prolactinomas. There may be mass effects including visual field defects in a patient with a macroadenoma.
Hyperprolactinaemia is a relatively frequent finding in women. It is important to consider medication and primary myxoedema that may cause hyperprolactinaemia and, when appropriate, a pregnancy test must be performed. There are pitfalls regarding the biochemical diagnosis of hyperprolactinaemia. However, if hyperprolactinaemia is unequivocal, an MR-scan of the pituitary is indicated.
Prolactin is the most important acute blood sample in a patient with a pituitary adenoma and visual field defects. Because, generally, dopamine-agonists are first-line therapy for prolactinomas. Cabergoline, bromocriptine and quinagolide are the dopamine-agonists, we use. We want to normalize prolactin levels to obtain eugonadism and we aim at reducing tumour size. Surgery and radiotherapy may be used for the few resistant prolactinomas.
When fertility is an issue, the woman has to be informed about the possible risks of prolactinoma growth during pregnancy and we have to consider which dopamine-agonist should be chosen. In all patients the safety of dopamine-agonists is important. During the last 5 years, there have been reports on cabergoline and the risk of valvular heart disease. The authorities have requested that patients on cabergoline are referred to echocardiography follow-up. There are ongoing studies trying to elucidate the risk of heart disease in patients with prolactinomas. Dopamine-agonist withdrawal may be considered in patients who achieve normoprolactinemia and complete tumour disappearance. Patients with prolactinomas and hypopituitarism should receive standard hormone replacement therapy. However, gonadal replacement should be delayed. An assessment of the bone mineral density (BMD) is generally indicated.
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The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## EN2.4

Abstract unavilable.

## EN3.1

Abstract unavilable.

## EN3.2

**The role of nurse prescribing in the care of endocrine patients**

Shashana Shalet (Endocrine Advanced Nurse Practitioner)

In the UK, the number of nurses able to legally prescribe medicines is rapidly increasing. The skills of nurse independent prescribing (NIP) offer an effective way for the Clinical Nurse Specialist (CNS) to be more responsive to the changing, and unpredictable, needs of patients by enabling timely and appropriate symptom control in a single, seamless consultation. The structure of the Nurse-led clinic often allows for a longer consultation time enabling the CNS to provide a greater depth of information to the patient around the different medication options, the potential side effects and benefits of that medication. The NIP role promotes expansion and innovations in practice that can meet the changing needs, and costs, of effective service delivery.
The endocrine CNS is able to develop a therapeutic relationship with the patient that encourages informed decisions about which medical therapy is most suitable and the consultation is completed by prescribing the correct medication to the correct patient at the correct time. It enables the timely dose titration of medications with the NIP qualification providing validation for these changes with other members of the multi-disciplinary team. A final factor in the role of the NIP in endocrinology is the amount of time saved when the CNS does not have to seek out a medical prescriber's signature, this time can then be better spent providing direct care to patients. Each NIP's work is closely monitored by both National and local guidelines that dictate prescribing must only be within their area of expertise.

# JOE/JME Prize Presentation Sponsored by *Journal of Endocrinology*

## JP1

### Melanocortin receptor accessory proteins (MRAPs) in adrenal gland physiology and beyond

LF Chan
Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London.

The melanocortin receptor (MCR) family consists of five G protein-coupled receptors (MC1R-MC5R) with diverse physiological roles. MC1R controls pigmentation, MC2R is a critical component of the hypothalamic-pituitary-adrenal axis, whilst MC3R and MC4R have a vital role in energy homeostasis and MC5R is involved in exocrine function.

The melanocortin receptor accessory proteins, MRAP and its paralogue MRAP2, are small single-pass transmembrane proteins that have been shown to regulate MCR expression and function.

In the adrenal gland, MRAP is an essential accessory factor for the functional expression of the MC2R/ACTH receptor. The importance of MRAP in adrenal gland physiology is demonstrated by the clinical condition familial glucocorticoid deficiency, where inactivating MRAP mutations account for approximately 20% of cases. MRAP is highly expressed in both the zona fasciculata, and the undifferentiated zone. Expression in the undifferentiated zone suggests that MRAP could also be important in adrenal cell differentiation and/or maintenance. In contrast, the role of adrenal MRAP2, which is highly expressed in the fetal gland, is unclear.

The expression of MRAPs outside the adrenal gland is suggestive of a wider physiological purpose, beyond MC2R mediated adrenal steriodogenesis. In vitro, MRAPs have been shown to reduce surface expression and signaling of all the other melanocortin receptors (MC1,3,4,5R). MRAP2 is predominantly expressed in the hypothalamus, a site that also expresses a high level of MC3R and MC4R. This raises the intriguing possibility of a central nervous system role for the MRAPs.

# Meet the Expert Sessions

## MTE1

**Adrenal incidentaloma and subclinical Cushing's syndrome**

P. Stewart
University of Birmingham, Birmingham, UK.

The widespread use of abdominal CT/ MRI has resulted in a new and common diagnosis for the clinical endocrinologist – the management of patients with adrenal incidentalomas. Defined as an adrenal mass discovered incidentally in the work-up or treatment of clinical conditions not related to suspicion of adrenal disease, incidentalomas cover a spectrum of underlying adrenal pathologies with a common pathway of discovery. Because of the risk of malignancy, they raise uncertainty, confusion and concern in doctors and patients alike and consume significant resource. We will define the scale of the problem, discuss diagnostic challenges as they relate to functionality of the tumours and ascertaining whether the lesions are benign or malignant. The natural history and suggested follow-up and treatment of patients based on published NIH clinical guidelines will be addressed; such guidelines perhaps over-inflate the real risk of malignancy and a more 'risk-averse' approach to management is now required. Our new biomarker research based on analyzing the urinary steroid metabolome may improve the diagnosis and follow-up of such cases. One particular area of contention is the term 'sub-clinical' Cushing's reported in up to 15% of all cases; the suggestion being that this may account for underlying obesity, low BMD and cardiovascular morbidity. However diagnostic criteria vary considerably, have usually failed to comply with Endocrine Society guidelines with false positive results generating considerable uncertainty. In the absence of any test with 100% sensitivity and specificity, the issue is likely to be one of diagnosing mild Cushing's syndrome where there is a limited evidence base that reversal of the condition significantly alters clinical features.

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The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## MTE2

**Approach to the patient with Cushing's disease after pituitary surgery failure**

M. Tóth
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The treatment of choice for ACTH-producing pituitary adenoma is selective transsphenoidal adenomectomy. The remission rate after transsphenidal surgical intervention for Cushing's disease ranges from 69 to 98% (average 83%). If the first pituitary surgical intervention fails, the following questions should be answered: i) Is the localization diagnosis correct without doubt (pituitary or ectopic source of ACTH- overproduction)? ii) Is it reasonable to recommend repeat pituitary surgery? The answers to these questions depend on the results of inferior petrosal sinus sampling (IPSS) and histology.

If the diagnosis of ACTH-producing pituitary adenoma is without doubt, in cases of microadenomas (both visible and invisible on MRI) repeat pituitary surgical intervention seems to be the best choice. In the best hands, remission after repeat surgery may occur in 50–60% of patients contrary to the 75–90% remission rate of the first surgery. Regarding the extent of repeat pituitary surgery, selective adenomectomy, hemihypophysectomy or total hypophysectomy may be performed. Scarce literature data suggest that IPSS performed before reoperation has much inferior diagnostic information than the sampling performed before the first intervention. This observation may probably be explained by altered anatomic situation and venous drainage caused by the initial surgery.

If repeat pituitary surgery does not achieve remission, then radiosurgery or stereotactic radiotherapy, bilateral adrenalectomy, and/or medical therapy are the therapeutic options. Medical therapy for the treatment of Cushing's disease may target the ACTH secretion of the pituitary tumor (cabergoline, pasireotide) or block the steroid biosynthesis of the adrenals (ketoconazole, metyrapone, mitotane).

Bilateral laparoscopic adrenalectomy may be a therapeutic option in most severely hypercortisolemic patients. Nelson's syndrome is potentially the most severe complication of bilateral adrenalectomy developing in 20–30% of adrenalectomized patients. The newest series of Nelson's syndrome represent that the modern 'Nelson's syndrome' is different from the entity originally described.

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The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## MTE3

Abstract unavailable.

## MTE4

**Challenges of transsphenoidal pituitary surgery**

P. Cappabianca
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The endoscopic endonasal approach, initially reserved only for sellar lesions, is a continuous evolving speciality of modern neurosurgery, which requires precise anatomical knowledge, technical skills and integrated appreciation of the pathology dealt with. Nowadays, it represents a minimally invasive approach to deal with several diseases interesting mostly the entire skull base – namely the suprasellar, retrosellar and parasellar spaces – obviating brain retraction. The endoscopic endonasal approach offers some advantages arising from the use of the endoscope itself: a superior close-up view of the relevant anatomy and an enlarged working angle with an increased panoramic vision inside the surgical area.

Most pituitary adenomas can be managed and removed through a standard transsphenoidal approach either microscopic or endoscopic. Nevertheless, several cases, present some features such as dumbbell shape or para/suprasellar extension and/or fibrous or rubbery consistency – high likely in case of recurrence tumors –, that somehow hinder such route. More recently, the introduction of the endoscope in the extended endoscopic endonasal approach has definitely afforded its widespread so that, nowadays this technique can be considered suitable for removal of lesions extending beyond the sellar area such as parasellar, suprasellar and/or retrosellar spaces.

We have been employing the endoscopic endonasal technique since 1997 on more than 1000 patients aiming to remove first sellar and, recently, skull base lesions applying the so-called extended endonasal approach. Namely, in the management of invasive pituitary adenomas this technique allowed us to use two surgical corridors the conventional endosellar extra-arachnoidal and a suprasellar trans-arachnoidal.

We, though, report our experience throughout a step-by-step depiction of the surgical techniques to access the different compartments, detailing the anatomy as seen from the endonasal perspective, focusing on the 'dangerous landmarks', describing possible complications and the techniques to manage this kind of lesions.

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The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## MTE5

**Meet the expert: discussion of common but difficult thyroid cancer cases**

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[2]University School of Medicine, Washington, District of Columbia, USA.

Endocrinologists today face an ever increasing variety of presentations of thyroid cancer related to the well documented increasing frequency of thyroid cancer patients worldwide. The overwhelming majority of these tumors are small microcarcinomas with excellent prognosis for which management is quite straightforward. But many patients are seen for whom optimal steps for

management are unclear, constituting therapeutic dilemmas that are typically not well addressed in current guidelines of our professional societies. This session will discuss several patient scenarios, ranging from the misleadingly innocent microcarcinoma to the more aggressive thyroid cancer that requires more innovative management. For the microcarcinoma, because there are potential adverse effects of overly aggressive management, questions arise such as whether more conservative approaches are sufficient such as: (i) subtotal rather than total thyroidectomy; (ii) if central compartment dissection is really necessary; (iii) if radioiodine ablation can be avoided; and (iv) how much TSH suppression is required? Yet, management cannot be overly cavalier because a significant percent of patients with microcarcinomata already have lymph node metastases at presentation that imply future recurrences, and a small number may have, or will develop, distant metastases. Other cases will be discussed that require more aggressive surgery, local or regional ablation interventions, and higher dose radioiodine therapy such as that given by dosimetry. It will be proposed that these varied potential clinical presentations call for a risk-stratified approach to management that would minimize harm to the patient and yet optimize outcomes. Clinical parameters such as age, sex, tumor size, multifocality, vascular or capsular invasion, extrathyroidal extension, lymph node metastases, histologic variants of PTC, or the presence of mutational markers that might require more aggressive management will be discussed, to place them into a risk-adapted algorithmic approach intended to achieve minimal morbidity and optimal outcomes at less cost to the patient and society.

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The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## MTE6

**Clinical management of adrenocortical carcinoma**

M. Terzolo
S. Luigi Hospital, University of Turin, Orbassano, Italy.

A thorough pre-operative hormonal workup is advised following the recommendations of the European Network for the Study of Adrenal Tumors. Demonstration of endocrine activity may serve to the following purposes: i) prove adrenocortical origin of the mass; ii) suggest malignancy, in the event of androgen hypersecretion; iii) provide tumor markers whose assessment may be exploited during follow-up to detect tumor residual or recurrence after surgery; iv) avoid life-threatening post-operative adrenal insufficiency. Hypercortisolism may indeed escape clinical detection, thus preventing use of post-operative steroid replacement. Assessment of tumor dignity and visualization of potential metastases needs an adequate imaging study. Computerized tomography (CT) is the technique of choice but magnetic resonance imaging may be equally effective. CT characteristics suggesting malignancy are: a) large mass size, usually $>4$ cm; b) elevated mass density ($>10$ Hounsfield Units) on unenhanced scan; c) rapid washout of contrast medium on enhanced scan. Recent data demonstrate that 18F-FDG PET helps to differentiate suspicious CT scan lesions.

As to treatment, recent studies challenged the dogma that there is no role for laparoscopy, although data on the comparison between open and laparoscopic adrenalectomy remain conflicting. The crucial issue is to select an experienced surgeon. Adiuvant therapy with mitotane has recently received increased interest after publication of a multicentric study on a large series of ACC patients that showed a significant reduction in the risk of recurrence and death for the mitotane treated patients. However, controversy still exists on the value of post-operative mitotane and a randomized controlled trial is currently ongoing. Results of medical treatment of advanced disease with the available regimens are overall disappointing and also the first experiences with targeted therapies are not encouraging, but new trials are ongoing and hopefully will allow definition of a more personalized treatment.

Declaration of interest

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## MTE7

**Pregnancy and pituitary disorders**

Z. Karaca
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The pituitary gland is altered by both anatomical and physiological changes during pregnancy. The most striking finding is the physiological enlargement of the pituitary gland as a result of lactotroph hyperplasia. Hormonal evaluation of pregnant women requires special consideration due to the emergence of the placenta as a new source of hormone production, changes in binding globulin levels and resistance to hormones such as glucocorticoids.

Pituitary adenomas may adversely affect pregnancy by their hormone production and pose a potential risk of tumour growth. Prolactinomas are the most common pituitary adenomas encountered during pregnancy since the disease is common among women of reproductive age and fertility can be achieved easily after medical treatment. Acromegaly is the second most common pituitary adenoma seen in relation to gestation after prolactinomas. Medical therapy can be ceased safely after confirmation of pregnancy in both GH and PRL secreting pituitary adenomas. The risk of tumour growth during gestation is low in most cases of acromegaly and prolactinomas. Cushing's disease is rare and most cases with Cushing's syndrome are caused by adrenal adenomas, unlike in non-pregnant women. Cushing's disease adversely affects pregnancy, therefore prompt diagnosis and treatment according to gestational period are essential. Other hormonal and non-functional tumours are rarer and have been presented as case reports.

Sheehan's syndrome and lymphocytic hypophysitis are other pituitary disorders associated with pregnancy. They lead to hypopituitarism, sometimes with delays in diagnosis and difficulties in differential diagnosis. Pregnancy is not common among patients with hypopituitarism or pituitary adenomas due to altered gonadotroph functions. Ovulation induction is essential for fertility achievement, but the replacement of other deficient pituitary hormones, including GH, also plays an important role in the preparation of the uterus for implantation. Due to increased fecundity by improved assisted reproductive technologies and treatment methods, the number of pregnant women with pituitary disorders seen in clinical practice is increasing which necessitates special attention.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## MTE8

Abstract unavailable.

## MTE9

**Diagnosis and management of SIADH**

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Hyponatraemia is the commonest electrolyte abnormality in clinical practice; epidemiological data would suggest that SIAD is the commonest cause of hyponatraemia, and it is particularly important in patients undergoing neurosurgery, in whom SIAD is common. Recent data has shown that even mild hyponatraemia (plasma sodium 125–135 mmol/l) is associated with gait instability, falls, fractures, osteoporosis and increased mortality. This has led to the recognition that correct diagnosis and treatment of hyponatraemia may offer the opportunity to reduce morbidity an mortality and to reduce hospital stay. SIAD must be differentiated from other causes of hyponatraemia and the key diagnostic criteria are plasma osmolality $<275$ mOsm/kg, urine osmolality $>100$ mOsm/kg, euvolaemia, urine sodium $>30$ mmol/l and exclusion of hypothyroidism, glucocorticoid deficiency and diuretic use. It is particularly important to exclude acute ACTH/cortisol deficiency in patient with subarachnoid haemorrhage or traumatic brain injury. Fluid restriction is the traditional treatment for SIAD and still has a role, particularly in hospital patients with transient hyponatraemia, but there is a lack of an evidence base, and it is difficult

for patients to maintain without close supervision. Randomised controlled trials have shown that the vasopressin receptor antagonists (vaptans) are superior to placebo in the management of hyponatraemia dur to SIAD and other causes; they cause an aquaresis, but in contrast to loop diuretics, no natriuresis. Tolvaptan is now available in oral form in Europe though cost remains a prescribing consideration, particularly in long term hyponatraemia

## MTE10

**Elastosonography in the evaluation of thyroid and parathyroid lesions**

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Finding of a firm nodule during palpation of the thyroid gland is one of the clues for malignancy. In addition to palpation, as a new imaging technique, tissue stiffness can be evaluated by ultrasound elastography (USE); thereby helping us further to detect firm thyroid nodules, even the non-palpable ones. Tissue stiffness is detected by USE through measuring the amount of distortion that occurs when the lesions is subjected to external pressure. The power of USE in detecting malignant thyroid nodules was also reported in several studie, in which a high power for this new technique was reported. A recent meta-analysis evaluating most of these reports revealed high sensitivity and specificity for USE.
Studies conducted with USE could mainly be divided into two groups. The first group included thyroid nodules only undergoing surgery and having histopathological diagnoses. The second group included nodules having cytological diagnoses as well as those with histopathological diagnoses. However, all of those studies carry two major limiting factors: (i) small sample size and/or (ii) selection bias. As a result, like initial historical studies evaluating the power of each new ultrasound technique, these preliminary USE studies might overshoot the real sensitivity and specificity. Therefore, we planned to discuss the diagnostic power of USE in detecting malignant thyroid nodules in this MTE session.
Parathyroid adenomas were demonstrated to be elastographically stiff lesions. In contrast, almost half of the parathyroid hyperplasias were soft in elastographic evaluations in our hands. USE seems to be a valuable new assisting technique that might enhance the power of the preoperative localization studies of parathyroid lesions. Moreover, the USE image of a parathyroid lesion may further assist in determining the type of operation that is going to be applied. However, this issue should further be studied to investigate whether parathyroid elastography can also improve postoperative outcomes. We will try to discuss the ultrasound elastographic features of parathyroid lesions as well in this session.
Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## MTE11

**Diagnosis and treatment considerations in autoimmune polyendocrine syndrome type 1**

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Autoimmune polyendocrine syndrome type I (APS-1) is a rare autosomal recessive disorder which starts in early childhood, typically associated with chronic mucocutaneous Candidiasis. Patients with APS-1 later develop autoimmunity against endocrine tissues such as the adrenal cortex and the ovaries, and non-endocrine tissues such as the liver and melanocytes. The disease is caused by mutations in the autoimmune regulator (AIRE) gene that encodes a 54 kDa protein expressed in thymic medullary epithelial cells and in certain peripheral lymphoid cells.
APS-1 patients display autoantibodies directed against proteins in the affected tissues. These autoantibodies are often predictive for the development of organ failures such as adrenal insufficiency or premature ovarian failure where autoantibodies reactive to 21-hydroxylase and side-chain cleavage enzyme, respectively, can be detected. Recently, the discovery of autoantibodies against IL-22 may provide an explanation to the mucocutaneous Candidiasis, another autoimmune component of the syndrome.
Due to its rarity the diagnosis of APS-1 can easily be missed. Methods to diagnose, the clinical usefulness of autoantibody analyses, the recommended follow-up of these patients and the management of complications will be discussed.
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## MTE12

Abstract unavailable.

## MTE13

**Graves' ophthalmopathy**

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Management of Graves' ophthalmopathy (GO) requires knowledge of smoking behaviour, thyroid function and antibodies, and activity and severity of the ophthalmopathy.
Smokers should be strongly adviced to stop smoking because outcome of Graves' disease is less favourable in smokers compared to nonsmokers.
High serum concentrations of TSH receptor antibodies (TBII) are associated with a more severe and protracted course of GO. Restoration and maintenance of euthyroidism is relevant for the eyes, but how hyperthyroidism should be treated in GO patients remains controversial. Antithyroid drugs and near-total thyroidectomy are apparently neutral with respect to the course of GO, but 131I therapy carries a risk of about 15% for devleoping and/or worsening of GO. The risk can almost be neutralized by a prophylactic course of steroids, but its timing, dose and duration are ill-defined. Steroid prophylaxis should be restricted to patients who are at high risk, i.e. patients who smoke, have active GO, or high TBII.
Mild GO usually requires no treatment, but a recent randomized clinical trial demonstrated improvement of eye changes and prevention of progression to more severe GO upon treatment with selenium (100 microgram sodium selenite twice daily for 6 months). Moderate-to-severe GO if active should be treated preferably with intravenous methylprednisolone pulses (e.g. 0.5 g weekly for 6 weeks, followed by 0.25 g weekly for another 6 weeks), which are more effective and have less side effects than oral prednisone. However, intravenous methylprednisolone pulses exceeding a cumulative dose $>8$ gram have been associated with liver failure and cardiovascular events. In case of steroid failure, the combination of low dose oral prednisone (20 mg daily) with either cyclosporin or retrobulbar irradiation can be tried. Rituximab –although still experimental- might be applied in desperate cases. Very severe GO (dysthyroid optic neuropathy) requires high-dose intravenous methylprednisolone pulses (e.g. 6 pulses of 1.0 g each, administered on alternate days); if visual functions do not improve within two weeks, an urgent surgical orbital decompression is indicated. Complete restoration of appearance and visual functions is obtained in many patients only after various surgical procedures (decompression, squint surgery, blepharoplasty), which should be done in the inactive phase of the disease.
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## MTE14

**Hirsutism**

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Hirsutism, affects 5–8% of the whole female population, results either from an increase in circulating androgen concentrations, an increase in the sensitivity of the pilosebaceous unit to normal androgen concentrations or a combination of these factors. Polycystic ovary syndrome (PCOS) is the underlying cause in vast majority of the patients with hirsutism. PCOS can only be diagnosed after exclusion of some other diseases such as non-classical congenital adrenal hyperplasia (NCAH), idiopathic hyperandrogenemia, Cushing's syndrome, hyperprolactinemia and acromegaly. In approximately 1–8% of the women with hirsutism, NCAH due to 21-hydroxylase deficiency may be diagnosed. Basal or ACTH stimulated 17-OHP concentration greater than 10 ng/ml was considered as the hormonal criteria for 21-OH deficiency. Some hirsute patients do not have evidence of detectable androgen excess or endocrine imbalance, as in women with 'idiopathic hirsutism'. A number of patients have hyperandrogenemia with normal ovarian morphology and regular cycles and called as idiopathic hyperandrogenemia. A correct etiological diagnosis is essential in order to exclude life threatening conditions such as androgen secreting tumors or the life long consequences of some disorders associated with hirsutism such as PCOS and NCAH. Thus certain tests must be conducted to ascertain properly the etiology of hirsutism. However, there is no universal consensus regarding the least required tests for the differential diagnosis of hirsutism. Specific causes of hirsutism such as Cushing's syndrome, adrenal/ovarian tumors should be treated by surgical excision of the tumor. In the other patients pharmacological approach is the mainstay of the therapy. The options are anti-androgens, combined oral contraceptive pill with or without anti-androgen agents, gonadotrophin-releasing hormone agonists and rarely insulin sensitizers. Patients should be aware that most of the drugs used in the management of hirsutism are contraindicated in women desiring pregnancy and simultaneous treatment of infertility and hirsutism is difficult.

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## MTE15

**Pituitary tumours in adolescents and adults**

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Pituitary tumours are usually benign, but aggressive local growth may occur. Malignancy with demonstrable extracranial metastases is rarely seen. Tumour formation is probably the result of genetic changes involving tumour suppressor gene inactivation and oncogene activation. Pituitary adenomas that occur in a familial setting account for 4–5% of the tumours, they can be part of endocrine-related tumour syndromes. In 2006, Vierimaa *et al.*[1] reported the results of a comprehensive genetic study that identified mutations in the aryl hydrocarbon receptor-interacting protein (AIP) gene as being associated with the familial presentation of somatotropinomas and prolactinomas. Prolactinomas are the most frequent pituitary adenomas in adolescents as well as adults. It is important to consider possible macroprolactinaemia, when prolactin measurements are performed. Usually macroprolactin consists of a monomer of prolactin and IgG, macroprolactin is not bioactive. The impact of failure to recognize macroprolactinaemia is significant. There can be no doubt that the diagnosis of acromegaly is often delayed and even overlooked. GH-hypersecretion may, however, rarely be found in patients with known pituitary adenomas, clinically silent or in prolactinomas.

Temozolomide is the first chemotherapeutic agent to show substantial response rates in aggressive pituitary tumours. Temozolomide is an alcylating agent with great ability to cross the blood–brain barrier, it was first used for malignant gliomas. The lesion at *O*6-guanine is the critical methyl adduct produced by temozolomide and responsible for the greatest cytotoxicity. This lesion is repaired in the presence of *O*6-methylguanine-DNA methyltransferase (MGMT). Future studies will evaluate the role of temozolomide in the management of benign aggressive pituitary adenomas.

(1) Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, *et al.* Pituitary adenoma predisposition caused by germline mutations in the AIP gene. *Science* **312** (5777) 1228–30, 2006.

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## MTE16

**Management of non-functioning pituitary adenomas**

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Non-functioning pituitary adenomas (NFAs) are benign pituitary neoplasms arising from the adenohypophyseal cells. They are not associated with clinical evidence of hormonal hypersecretion and have a prevalence of 22 cases per 100,000 people. At presentation, the majority is macroadenomas and their clinical manifestations are the result of pressure effects to surrounding structures.

Management options include observation, surgical removal combined or not with external irradiation, radiotherapy alone or medical treatment.

Studies assessing the natural history of presumed NFAs, have shown probability of enlargement 19% for microadenomas and 44% for macroadenomas at 48 months. Surgery remains the treatment of choice in patients with macroadenomas and achieves improvement of vision in around 80% of the cases. Hypopituitarism (partial or complete) is present in a significant number of patients post-operatively (30–83%) and its rate increases following radiotherapy. Regrowth rates in subjects treated solely by surgery range between 6–46% (the risk is higher if there is large tumour remnant) and in those managed by surgery and adjuvant radiotherapy between 0–36%. Up to 20% of the relapses have been detected 10 years post-operatively necessitating long-term surveillance. Reliable markers of tumour relapse at a pathological and/or molecular level are lacking.

The management of patients presenting with pituitary apoplexy (conservative or surgical) is dictated by the presence of pressure effects and careful monitoring for recurrence is required post-operatively (11.1% at a mean follow up of 6.6 years). The value of medical treatments (dopamine agonists, somatostatin analogues) remains to be assessed.

Recent data from the Oxford series suggest that the quality of life of patients with NFAs following treatment (surgery combined or not with radiotherapy) is not compromised to a significant extent.

Finally, the impact of NFAs on the long-term mortality is not as yet, clear.

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## MTE17

**Multiple endocrine neoplasia type 2**

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Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant hereditary cancer syndrome caused by missense gain-of-function mutations of the RET proto-oncogene, encoding a receptor tyrosine kinase, on chromosome 10. It has a strong penetrance of medullary thyroid carcinoma (MTC) and can be associated with bilateral pheochromocytoma and primary hyperparathyroidism. MEN 2 is divided into three varieties depending on clinical features: MEN 2A, MEN2B and FMTC (familial MTC). The specific RET mutation may suggest a predilection toward a particular phenotype (MEN 2A, MEN 2B and FMTC) and clinical course of the MTC, with strong genotype–phenotype correlations. Offering RET testing is best practice for the clinical management of patients at-risk of MEN 2, and MEN 2 has become a classic model for the integration of molecular medicine into patient care. Recommendations on the timing of prophylactic thyroidectomy and extent of surgery are based on classification of RET mutations into risk levels according to genotype–phenotype correlations. By earlier identification of patients with hereditary MTC, the presentation changed from clinical tumours to preclinical disease resulting in a high cure rate of affected patients with much better prognosis.

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## MTE18

**Normocalcemic primary hyperparathyroidism**

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Normocalcemic primary hyperparathyroidism (NC-PHPT) is a condition characterized by normal albumin-adjusted total serum calcium or ionized calcium and persistently elevated serum PTH. Therefore, to recognize this entity it is necessary to measure PTH in normocalcemic individuals. The question arises why would someone measure PTH in a normocalcemic subject? A pro-active approach to women evaluated in their early postmenopausal years for parameters of skeletal health as well as the attitude of measuring PTH in all subject undergoing evaluation for low bone mineral density may at least partially answer this question. In the diagnostic workup vitamin D status should be firstly evaluated. Indeed vitamin D insufficiency (25OHD levels below 30 ng/ml) is a rather common condition and often account for elevated PTH levels. Patients with low 25OHD should be replaced with vitamin D and reevaluated. Occasionally these patients will become hypercalcemic, thus unmasking the more typical hypercalcemic PHPT. If PTH remains elevated following vitamin D repletion, all other causes of secondary hyperparathyroidism, such as liver disease, renal disease, significant hypercalciuria (urinary calcium $>350$ mg/24 h), and gastrointestinal disease associated with malabsorption, or other metabolic bone disease that could affect PTH levels (e.g. Paget's disease) should been considered and ruled out. Patients taking drugs which might affect PTH levels (lithium carbonate, thiazide diuretics) or calcium metabolism (estrogens, loop diuretics, bisphosphonates, and anticonvulsants) should be reevaluated after drug withdrawal. The natural history of NC-PHPT is unknown, but observational studies provided evidence that some individuals may develop hypercalcemia or evidence of disease progression with development of kidney stones, decline of bone mineral density, fractures, marked hypercalciuria. The current evidence indicates that NC-PHPT is a real clinical entity, which has been recognized at the 3rd International Workshop on the Management of Asymptomatic PHPT as a variant of PHPT.

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The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## MTE19

Abstract unavailable.

## MTE20

**The diagnosis and treatment of secondary osteoporosis in children**

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There is increasing awareness that children with chronic illnesses, particularly those with glucocorticoid use and neuromuscular disorders, have the potential to develop significant bone fragility due to osteoporosis. In such cases, osteoporosis manifests as low-trauma extremity fractures, with vertebral fractures an under-recognized consequence of reduced bone strength. We have studied the Genant semi-quantitative classification for characterizing vertebral fractures in children with chronic illness, and have shown that the strongest predictor of vertebral fractures in children with leukemia at 12 months was the presence of vertebral fractures around the time of diagnosis. We have further shown that even mild (Grade I) vertebral fractures were associated with increased odds for future spine fracture. These observations assign biological relevance to the Genant classification in children.

The use of clearly-defined, DXA-based bone mineral density (BMD) cut-offs for the diagnosis of osteoporosis in children with chronic illness has been challenging to date, in part due to the paucity of data on the relationship between BMD and fractures in children with underlying disorders. We have shown that such children can sustain vertebral fractures with spine BMD Z-scores well within 2 standard deviations (SD) below the mean, an observation which calls into question the use of – 2 SD as a definitive cut-off to define osteoporosis in the pediatric chronic illness setting.

The treatment of secondary osteoporosis in children presents unique issues due to the potential for spontaneous restitution of bone mass deficits and reshaping of vertebral bodies through bone growth, particularly when bone health threats are transient. As such, the identification of candidates for intervention involves not only confirmation of osteoporosis but assessment of the potential for spontaneous recovery. Bisphosphonates have been the most frequently used agents to treat secondary osteoporosis in children; a number of promising novel treatments on the horizon will also be discussed.

Declaration of interest
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## MTE21

**Autoimmune hyperthyroidism: initiation and duration of thionamide therapy**

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Graves' disease is a common autoimmune disorder with autoimmunity against the TSH-receptor being a central pathogenetic element. The disease may present with a number of clinical manifestations, among which hyperthyroidism caused by TSH-receptor stimulating antibodies is the most prevalent. The disease is common in all adult ages and affects women 4–5 times more often than men.

In many countries the preferred initial therapy of the hyperthyroidism is thionomide drugs (TD) that will in most patients lead to euthyroidism and be followed by a gradual remission of the underlying autoimmune abnormality with disapperance of TSH-receptor autoantibodies from blood

The three major problems with TD therapy are:

1. Side-effects to the drugs.
2. A frequent relapse of hyperthyroidism after drug withdrawal
3. Insufficient response to TD in a minority of patients

The choice of drug in specific situations, the initial dosing, and evidence for and against prolonged TD therapy will be discussed.

Interim data from the RISG study (Remission Induction and Sustenance in Graves' hyperthyroidism) investigating prolonged use of TD in a prospective randomized design will be discussed, as will data related to the use of Propylthiouracil for initial therapy of patients with impending thyrotoxic crisis and patients not responding to conventional TD therapy.

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## MTE22

**Clinical management in transgender sex hormone treatment**

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Gender Identity Disorder (GID) is a condition in which a person experiences discongruency between their assigned sex and what they feel their genderidentity is. A person with gender dysphoria experiences persistently uncomfortable feelings about their birth gender (Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) (American Psychiatric Association, 1994). During the 'real life experience' hormonal treatment starts and applicants are required to live socially in the desired gender role before irreversible surgical

reassignment is considered. Cross sex hormonal treatment is desired by transsexual persons to help them successfully live as a member of their identified gender. It is clear that both in adults and adolescents the decision for starting hormonal treatment in transsexualism is not to be made by the endocrinologist. The mental health professionals (psychiatrists and/or psychologists), by preference working in a multidisciplinary Gender team, will guide these persons to make an informed decision about hormonal treatment. Eligibility criteria and readiness as described by WPATH's Standards of Care for GID-7th version, should be evaluated. The goal of treatment in female-to-male transsexual persons is to induce virilization and to stop menses. The prinicipal hormone treatment is a testosterone preparation. In male-to-female transsexual persons oestrogen and anti-androgen treatment is provided. Treatment regimens are currently not standardised and include various forms of oestrogens, progestins, and/or (anti-) androgens as reported by different clinical centres. So far, no randomized intervention trials are available so treatment is largely experience-based.
Appropriate care for transgender persons will lead to better outcome and should avoid unnecessary psychological pain, health risks (e.g. secondary psychiatric conditions or suicide), or self medication with inherent greater risk of complications.
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## MTE23

**Subclinical hypothyroidism**

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Subclinical hypothyroidism (SHypo) is characterized by elevated serum TSH and thyroid hormone levels at the lower limit but within their respective reference range.
It is necessary to distinguish between patients with mildly increased serum TSH levels (5–9 mU/L) and patients with more severely increased serum TSH levels (10 mU/l or higher). About 75% of all SHypo patients have mild disease.
The high prevalence (between 4 and 20% of the adult population) and the various implications of SHypo require the need to establish a correct diagnosis, clinical assessment and treatment of this disorder.
The evaluation of transient and false causes of mild increases in TSH should be excluded before treating SHypo.
Subclinical hypothyroidism may be progressive or reversible. The annual rate of progression to overt disease is particularly increased (4.3%) in women with elevated serum TSH and anti-thyroid antibodies.
Important cardiovascular and metabolic effects may develop in long-term untreated SHypo.
Elderly Subjects with a TSH level of 7 mU/L or greater have a higher risk of heart failure events than euthyroid subjects.
There are some discrepancies in epidemiological data with reference to the risk of coronary heart disease (CHD) in patients with SHypo. A recent meta-analysis analyzed the individual data of 55 287 participants from 11 prospective cohorts. This analysis confirms that the risk of both CHD and mortality due to CHD were significantly increased in participants with TSH levels of 10 mU/L or greater. These results strongly support the association between CHD and SHypo in patients with TSH of 10 mU/L or greater.
Treatment of SHypo is recommended for all patients with SHypo with serum TSH levels of 10 mU/L or greater because these patients may have a significantly increased risk of progression to overt hypothyroidism, are more frequently symptomatic and may have a higher chance of dyslipidemia and cardiovascular dysfunction with an increased risk of CHD events, CHD mortality and CHF.
No consensus exists on the clinical significance and treatment of the mild form of thyroid failure. The available data suggest that treatment of mild SHypo should be personalized. Clinicians should consider the degree of TSH increase, the patients' age, the risk of progression to overt disease, the quality of life, the cognitive, metabolic and cardiovascular risk factors and the presence of associated co morbidities.
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## MTE24

**Approach to the management of the infertile couple**

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Infertility is defined by the World Health Organization (WHO) as the inability of a couple to achieve conception or bring a pregnancy to term after 1 year or more of regular, unprotected sexual intercourse. It remains a major clinical and social problem, affecting 13–15% of couples worldwide. Evaluation usually starts after 12 months, although it might be indicated earlier. The most common causes of infertility are divided into: male factor, related to sperm abnormalities, female factor, such as ovulatory dysfunction and tubal pathology, combined male and female factor or unexplained infertility, where no obvious cause is found. We reviewed randomized studies, meta-analyses and scientific societies recommendations to propose a simplified approach for the management of the infertile couple.
Full history-taking (obstetric, developmental, menstrual, contraceptive, sexual, familial, past/present history) is a necessary tool to identify most of underlying problem.
To exclude male and/or female factor, clinical examination, semen analysis (with the WHO reference values), detection of testicular function (hormonal assay, scrotal ultrasonography), ovarian function (early follicular FSH and LH levels, mid-luteal progesterone, transvaginal ultrasonography), and evaluation of tubal patency by hysterosalpingography should be performed. Chromosomal karyotyping and genetic screening are indicated in selected cases (suspected genetic disorders, sex chromosomal aneuploidy, cystic fibrosis, deletion of Y-chromosome).
The therapeutic strategies can be classified as etiological or empirical, whether or not a causative factor is found; fertility treatments are classified into: medical (including hormones for ovulation/spermatogenesis induction, nutraceutical supplementation); surgical (such as hysteroscopy, varicocelectomy and treatment of obstructive disorders); assisted reproduction techniques (ART). The need for ART, however, should not preclude the search for etiological treatments, any surgical or medical therapy appropriate for improving the fertility potential. Finally, the approach to diagnosis and management of infertility should be balanced taking into account several aspects that include efficacy, timing, costs, compliance and side effects.
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## MTE25

**Pituitary incidentalomas**

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During the past several years the wide application of sensitive brain imaging techniques (CT, MRI) has led to an increasing recognition of asymptomatic lesions in the pituitary. Although their etiology covers a wide range of pathologies, most incidentally discovered pituitary lesions are benign adenomas, ranging in size from micro- ($<10$ mm) to macro- ($>10$ mm) adenomas. Micro-incidentalomas are very common, with a reported incidence in normal individuals of 4–20%. Although the identification of such lesions raises the theoretical risk of hormonal hypersecretion further screening confers minimal benefit and may not be cost-effective. Although, in the absence of clinical stigmata of Cushing's disease or acromegaly, only the measurement of prolactin represents a cost-effective strategy, recent guidelines suggest that a broader hormonal investigation may be more widely recommended. MRI follow-up of micro-incidentalomas is expensive. Since the majority of micro-incidentalomas do not increase in size during follow-up, the suggested need for routine application of repeat scans needs careful evaluation. At variance with pituitary micro-incidentalomas, the incidental discovery of a macro-lesion requires extensive investigation. Most, but not all, macro-incidentalomas demonstrate radiological features consistent of pituitary adenomas. If the lesion causes hypersecretion of prolactin, GH or ACTH, specific therapy is required. If the lesion compresses the optic chiasm, and there is no evidence of prolactin hypersecereti on, surgical removal is obviously indicated. If no hormonal hypersecretion is found, and if the lesion is in some distance from the optic chiasm, routine surgical removal may not be necessary. Indeed, not all tumors demonstrate a significant increase in size requiring surgical

excision. Thus expectant management is a safe option for many patients, given that regular MRI surveillance is recommended. Evaluation for anterior pituitary hormone deficiencies is required in all patients with macro-lesions and, hormone replacement therapy should be offered as required.

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## MTE26

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## MTE27

**Differential diagnosis of Cushing's syndrome**

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The characteristics of Cushing's syndrome (CS) pathophysiology, the heterogeneity of its clinical presentation and the different patterns of ACTH and cortisol secretion independently of the cause make the diagnosis of ACTH-dependent CS a difficult task in some cases. Clinical symptoms are not always specific enough to establish the diagnosis. 1 mg DEX suppression is one of the most used tests for CS screening. Nocturnal salivary cortisol may be used, but its accuracy has to be validated in each laboratory and can be elevated in other diseases. Urinary free cortisol and nocturnal plasma cortisol are used to confirm the diagnosis. Stress, liver or renal disease, and interferences due to several drugs should be excluded. Severe mental disease and other clinical disturbances may also induce functional hypercortisolaemia, also called seudoCushing's syndrome, confounding the real diagnosis. Differentiation of seudoCushing's from mild ACTH-dependent CS is difficult. Combination DEX-CRH and, more recently, DDAVP stimulation, have been proposed, but these tests are not free of pitfalls. Episodic and cyclical disease can complicate the diagnosis, especially in quiescent secretory phases. To establish the origin of ACTH-dependent Cushing could represent a real challenge, since ACTH-secreting pituitary microadenomas can be small enough to be seen in MR studies, and, on the other hand, bronchial carcinoids may functionally behave as pituitary adenomas usually do. IPSS has to be considered before confirming an etiological diagnosis in those cases. PRL measurements can be useful to improve the performance of the procedure. Diagnosis of ACTH-independent CS is based upon consistent cortisol hypersecretion with low or undetectable ACTH levels. When patients present with bilateral or multiple adrenal masses associated to CS, a possible ACTH-independent macronodular hyperplasia with aberrant expression of adrenal receptors may be suspected. A correct diagnosis is essential for an effective therapeutic intervention.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## MTE28

Abstract unavailable.

## MTE29

**Management of bone loss induced by cancer treatment in early breast and prostate cancer**

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Patients with endocrine-related cancers are notably at increased risk for developing osteoporosis as a complication from their adjuvant anticancer treatment, especially aromatase inhibitors (AIs) in early breast cancer (EBC) and androgen deprivation therapy (ADT) in early prostate cancer (EPC). AI-induced bone loss (AIIBL) occurs at a rate at least 2-fold higher than bone loss seen in healthy, age-matched postmenopausal women and they have a more than 30% higher risk of fractures. Recently considered fracture risk factors in EBC are AI therapy, T-score $< -1.5$, age $> 65$ years, family history of hip fracture, history of fragility fracture after age 50, oral corticosteroid use $> 6$mo, smoking, and BMI $< 20\ kg/m^2$. The WHO-FRAX algorithm does not address AIIBL and may underestimate EBC fracture risk. General advice includes exercise, calcium/vitamin D supplements, and baseline BMD monitoring. Bisphosphonates and denosumab can preserve BMD during AI therapy for EBC. Poor compliance may reduce oral bisphosphonate benefits. Overall, bisphosphonate evidence is strongest for zoledronic acid (ZOL; 4 mg q6mo). Potential anticancer activity of ZOL might provide benefits beyond preserving bone mass. Patients initiating AI with T-score $< -2.0$ or $\geq 2$ risk factors should receive appropriate antiresorptive therapy. Bone loss that occurs with ADT is also more rapid and severe than that associated with normal age-related bone loss. ADT also increases fracture risk and the hazard ratios appear to be comparable to the ones reported for AIs in breast cancer. There are a few randomized studies in patients with prostate cancer demonstrating that bisphosphonates, notably ZOL, can prevent bone loss under ADT. The effects of denosumab (60 mg sc q6mo) on bone loss and incidental vertebral fractures have been demonstrated in a large-scale placebo-controlled trial in the setting of ADT-induced bone loss. Specific guidelines are lacking for the treatment and the prevention of ADT-induced bone loss.

Declaration of interest

The author declares that there is a conflict of interest.

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## MTE30

**Treatment of vitamin D deficiency**

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The need for treating vitamin D deficiency arises either when patients present with musculoskeletal symptoms attributable to hypovitaminosis D or when screening of individuals at high risk reveals the presence of vitamin D deficiency, as defined by serum 25(OH)D levels $< 20$ ng/ml. The recommended dietary intakes of vitamin D vary with age and physiological state. The recommended daily intake ranges from 400 IU in infants, 600 IU in children and adults and 800 IU in those over 70 years of age, though higher doses varying from 1000 IU per day in infants and children to 1500–200 IU per day in adults and the elderly being required to raise 25(OH)D levels to 30 ng/ml (the level of sufficiency). The recommended dose increases 2- to 3-fold when the individual is on anti-convulsant drugs, glucocortiocids, anti fungal agents and anti-retroviral therapy. Correction of vitamin D deficiency requires higher daily doses of vitamin D, ranging from 2000 IU for those $< 1$ year of age, 4000 IU for children between 1–18 years of age, and 10,000 units for adults. These doses can either be delivered on a daily basis or an equivalent dose calculated and provided on weekly basis. For infants and children, both the daily dose (2000 IU /day) or a weekly calculated dose (50,000 IU per week for 6 weeks followed by 1500–200 units per day) are acceptable. In adults, the commonest strategy is to give 50,000 units per week for 8 weeks followed by 2000 IU per day or an equivalent weekly or monthly dose. In the elderly additional anticipated benefits include fall prevention and fracture risk reduction. vitamin D therapy targeting serum 25(OH)D levels $\geqq 60$ nmol/l is associated with fall prevention, while levels between 66–74 nmol/l appear to be required to reduce the risk of non-vertebral and hip fractures.

## MTE31

**Cryptorchidism**

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Normal position of the testes at term birth is on the bottom of the scrotum. In 2–9% of newborn boys, the testes have failed to descend normally (cryptorchidism), and at the age of three months, 1–3% of all boys remain cryptorchid. Thereafter spontaneous descent occurs rarely, whereas testicular ascent starts to appear and acquired cryptorchidism becomes a problem almost as often as congenital disorder. Cryptorchidism is associated with an increased risk of testicular cancer and subfertility. Germ cell loss in a cryptorchid testis appears already during the second year, and therefore early orchidopexy is generally recommended for treatment. Early operation (before 1 year) has been reported to improve testicular growth as compared with later operation (at 3 years). Hormonal treatment with either human chorionic gonadotropin (hCG) or gonadotropin releasing hormone agonist is still used in some countries, although the efficacy is rather poor (approximately 20%) and some short-term and long-term potential side effects have been reported after hCG (interstitial bleeding, inflammation, increased apoptosis, reduced adult testicular size). The benefit is the avoidance of surgery. In most cases the reason for cryptorchidism remains elusive, although several rare genetic mutations disturbing hormonal regulation of testicular descent are known. Normal function of hypothalamo-pituitary-testicular axis is necessary, and disorders of synthesis/action of androgens and/or insulin-like peptide 3 can cause cryptorchidism. Environmental endocrine disrupters affecting these systems are suspected to be involved in etiology. Links to impaired semen quality, increased risk of testicular cancer and hypospadias suggest that cryptorchidism can be a part of testicular dysgenesis syndrome (TDS) where fetal maldevelopment of the testis can result in one or several signs depending on the timing and nature of the disrupting agent/event.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## MTE32

**Management of GEP-NET tumors**

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Neuroendocrine gastro-enteropancreatic tumors constitute a diverse group of malignant neoplasms with a common feature of hormone production and release. The incidence and prevalence have constantly increased over the last decades with an incidence of about 6/100 000 and a prevalence of 35/100 000 inhabitants. The majority of patients present a metastatic disease at diagnosis. The most common subtypes of GEP-NETs are small intestinal NETs (carcinoids) followed by pancreatic and rectal NETs. The diagnosis is based on histopathology verification of neuroendocrine features such as positive immunohistochemical staining for CgA, synaptophysin and NSE. The proliferation is determined by the antibody MIB-1 (Ki-67). The biochemical workup includes a general tumor marker such as CgA, which is complemented by peptides and amines related to clinical symptoms (i.e. u-5HIAA, gastrin, glucagon). Localisation procedure include standard radiology, such as CT/MRI, US and endoscopic US. The majority of GEP-NETs express somatostatin type 2 receptors, whereby staging and localisation can be performed by somatostatin receptor scintigraphy with 111Indium-DTPA-Octreotide as well as PET-scanning with 68Ga-DOTATATE. The treatment of neuroendocrine GEP tumors includes surgery as well as debulking procedures such as RFA, embolization (plain or radio-embolization). Peptide receptor radiotherapy is more and more applied using radioactive 177Lutetium-DOTATATE or 90Yttrium-DOTATOC. The medical treatment includes biotherapy such as somatostatin analogs, interferons but also cytotoxic agents, including streptozotocin, 5FU, cisplatinum, etoposide, temozolomide + capecitabine. Most recently pancreatic NETs have been successfully treated with tyrosine kinase inhibitors, sunitinib, as well as the mTOR inhibitor, everolimus (Afinitor). The treatment is based on tumor type, stage and grade. In the future a more personalized treatment will be developed, based on information from molecular genetics and tumor biology.

Declaration of interest

The author declares that there is a conflict of interest.

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# Oral Communications

## Pituitary Clinical I

### OC1.1

**Pasireotide LAR is significantly more effective than octreotide LAR at inducing biochemical control in patients with acromegaly: results of a 12-month randomized, double-blind, multicenter, Phase III study**

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Introduction
Using the criterion of GH $<2.5$ μg/l and normalized IGF1, response rates to currently available somatostatin analogues in medically-naïve patients with acromegaly are 20–25% after 12 m. The broader somatostatin receptor binding profile of pasireotide may potentially improve response rates. This randomized, double-blind 12-m study compared pasireotide LAR with octreotide LAR.
Methods
Patients with acromegaly (GH $>5$ μg/l or GH nadir $\geq 1$ μg/l post-OGTT, and IGF1 $>$ ULN) who were *de novo* with a visible adenoma on MRI or medically-naïve (no previous medical therapy but prior pituitary surgery) received pasireotide LAR 40 mg/28d ($n=176$) or octreotide LAR 20 mg/28 d ($n=182$) for 12 m. At 3 and 7 m, dose titration to pasireotide LAR 60 mg or octreotide LAR 30 mg for suboptimal biochemical response was permitted, but not mandatory. Primary objective: comparison of the proportion of patients in each arm with GH $<2.5$ μg/l and normal IGF1 at 12 m. GH was measured as a 5-point mean (2 h curve).
Results
At baseline, mean GH was 21.9 and 18.8 μg/l in the pasireotide LAR and octreotide LAR arms, respectively; mean IGF1 was 2.6×ULN and 2.8×ULN. 80.1 and 85.7% of pasireotide LAR and octreotide LAR recipients completed 12 m. Dose uptitration was performed in 50.6 and 67.6% of pasireotide LAR and octreotide LAR recipients. Mean GH and IGF1 decreased by 3 m and remained suppressed. The primary endpoint was achieved by 31.3 and 19.2% of pasireotide LAR and octreotide LAR recipients ($P=0.007$); 48.3 and 51.6% had mean GH $<2.5$ μg/l ($P=0.536$); 38.6 and 23.6% had normal IGF1 ($P=0.002$). Pasireotide LAR recipients were 63% more likely to achieve full biochemical control than octreotide LAR recipients. Symptom improvement and tumor volume reduction were similar in both groups. The most common AEs with pasireotide LAR vs octreotide LAR were diarrhea (39.3 vs 45.0%), cholelithiasis (25.8 vs 35.6%), headache (18.5 vs 26.1%) and hyperglycemia (28.7 vs 8.3%). Most AEs were mild or moderate.
Conclusions
In the largest randomized study of a medical therapy in patients with acromegaly, pasireotide LAR was significantly more effective at inducing full biochemical control, as well as normal IGF1, than the current standard medical therapy octreotide LAR, with an acceptable safety profile.
Declaration of interest
The authors declare that there is a conflict of interest.
Funding
This work was supported, however funding details are unavailable.

### OC1.2

**Patients with Cushing's disease achieve normal urinary cortisol with LCI699, a potent 11β-hydroxylase inhibitor: preliminary results from a multicenter, proof-of-concept study**

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Introduction
The clinical features and complications of Cushing's syndrome result from chronic excess of circulating cortisol, typically quantified by 24-h urinary free cortisol (UFC). LCI699 is a potent inhibitor of 11β-hydroxylase. Since 11β-hydroxylase catalyzes the final step of cortisol synthesis, LCI699 is a potential new treatment for all forms of Cushing's syndrome.
Methods
Adult patients with mild-to-severe Cushing's disease (UFC $>1.5\times$ the upper limit of normal (ULN), mean of three collections in 14 days) received oral LCI699 for 10 weeks in an open-label study. LCI699 was initiated at 2 mg bid. Dose escalation was planned every 2 weeks to 5, 10, 20 and 50 mg bid until UFC normalized, in which case the dose was maintained until day 70, when treatment stopped. Dose reduction for tolerability was permitted. UFC was assessed on the penultimate day of each 2-week period. Patients were monitored until day 84. The primary endpoint was UFC $\leq$ ULN or a $\geq 50\%$ decrease from baseline at day 70 using the mean of 3 UFC samples collected during the week before day 70.
Results
Eleven patients (aged 25–55 years; four men) have been enrolled and nine have completed the study to date. Nine patients had prior surgery. Baseline UFC range was 1.6–17.0×ULN. UFC levels were normal on at least one assessment in 11 of 11 patients during the study. The primary endpoint was achieved by all nine patients who have completed the active treatment phase, eight of whom had normal UFC levels on day 70. After treatment discontinuation, UFC was $>$ ULN in six patients with measurements at day 84. The median dose of LCI699 associated with UFC normalization was between 5 and 10 mg bid. At day 70, mean SBP decreased by 13.1 mmHg from baseline. LCI699 was generally well tolerated; the most frequently reported adverse events were fatigue (5/11), nausea (4/11) and headache (3/11). Five patients experienced ACTH levels $>2\times$baseline. Four patients experienced study drug-related hypokalemia ($K^+ <3.5$ mmol/l; min 3.1 mmol/l). There were no serious AEs of suspected drug relationship.
Conclusion
LCI699 demonstrated efficacy with a satisfactory safety profile in this proof-of-concept study in patients with Cushing's disease.
Declaration of interest
The authors declare that there is a conflict of interest.
Funding
This work was supported, however funding details are unavailable.

### OC1.3

**Efficacy and safety of long-term treatment with pegvisomant in acromegaly: Italian pegvisomant observational study (ACROSTUDY)**

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ACROSTUDY is an international observational study to evaluate efficacy and safety of long-term treatment with pegvisomant (PEGA) in acromegaly. ACROSTUDY Italy was started in 2007 and we report data from the first 185 patients (93M, 92F, mean age, range, 49.9 y; 17–83.8 y) treated with PEGA (mean duration, range, 3.2 y, 0.1–8.5 y), from 24 centers, until 01/2010. Before and during PEGA, IGF1, GH-Ab, liver enzymes, metabolic parameters and pituitary MRI were assessed. Before PEGA, 70 patients had undergone surgery, 15 radiation therapy, 161 had received SSA ± DA and in 24 subjects no previous medical treatment of acromegaly was documented. At the start of treatment, 43.2% of patients received PEGA alone, 49.9% in association with SSA and 10% SSA + DA. During treatment, a progressive increase in the subjects receiving PEGA alone was observed. In 87% of cases PEGA was used in daily administration. The mean dose of PEGA received alone (mean starting dose 12.2 mg/day, at 5 y 18.5 mg/day) was not significantly different ($P=$NS) from that used in association with SSA ± DA (mean starting dose 12.4 mg/day, at 5 y 19.3 mg/day). In 74.4 and 75.5% of patients, IGF1 levels were normalized after 1 and after 5 y of therapy respectively. During PEGA, an increase in tumor lesion in 11 cases and a reduction in 16 cases was observed, but the centralized blinded reassessment confirmed the increase in three cases and a reduction in one case. In no case a significant and sustained transaminases elevation was reported. Discontinuation of treatment for serious adverse events was required in two cases. In conclusion, our data confirm that long-term PEGA therapy is highly effective in acromegalic patients resistant to other treatment and shows an excellent safety profile.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC1.4

**A multi-centre audit of the prevalence of cardiac valvulopathy in patients treated with dopamine agonists for hyperprolactinaemia**

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Bromocriptine (BC) and cabergoline (CAB) are ergot-derived dopamine agonists (DAs) used for the treatment of hyperprolactinaemia. Recently, concern has been raised about a possible association between long-term DA use and cardiac valvular abnormalities. These concerns are largely derived from studies in patients with Parkinson's disease receiving higher doses (typically CAB 3 mg/day vs 0.5–1 mg/week for hyperprolactinaemic patients). Studies in hyperprolactinaemic patients are generally reassuring but limited by their small size. We report the preliminary results of a UK-wide cross-sectional survey of echocardiographic findings in patients treated with BC/CAB for hyperprolactinaemia. The project was supported by the Clinical Endocrinology Trust and an unrestricted grant from Pfizer, UK. IRB permission was obtained at each centre. Anonymised data from 520 patients (163 male, median age 43, range 16–89) in 17 centres were collected. Studies were performed to British Society of Echocardiography standards. Patients were divided into two groups: completely normal ($n=192$); and those with any valvular abnormality of any severity (leaflet thickening, regurgitation, stenosis, reduced mobility or calcification, $n=328$). The two groups were then subdivided into BC- and CAB-treated. A two-tailed unpaired *t*-test was performed on the CAB-treated normal vs CAB-treated abnormal echocardiogram groups; and on the BC-treated normal vs BC-treated abnormal echocardiogram groups. There were no 'severe' and seven 'moderate' valve defects (two aortic valve thickening; two tricuspid regurgitation; one reduced aortic valve mobility; one aortic stenosis; one aortic regurgitation). Each patient's cumulative exposure to DA was calculated. There was no statistically significant difference in the cumulative dosage of either BC ($P=0.48$) or CAB ($P=0.13$) between those with normal vs abnormal echocardiograms. There was a trend towards a higher cumulative dose of DA in the groups with normal echocardiograms. This cross-sectional survey finds no evidence of an increased prevalence of valvulopathy in patients being treated with BC or CAB for hyperprolactinaemia.

Declaration of interest

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## OC1.5

**Initial hypothalamic involvement is the major risk factor for impaired prognosis and quality of life in childhood craniopharyngioma regardless of chosen treatment strategies: results of KRANIOPHARYNGEOM 2000**

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Hypothalamic obesity has major impact on prognosis and quality of life (QoL) in childhood craniopharyngioma (CP). The pathogenic relevance of initial hypothalamic involvement versus treatment-related hypothalamic lesions is a matter of controversy.

One hundred and twenty patients were recruited prospectively during 2001 and 2007 and evaluated after 3 years of follow-up. Body mass index (BMI) and QoL at diagnosis and 36 mo after diagnosis were analyzed based on reference assessment of tumour localization and post-surgical hypothalamic lesions using a standardized grading system (no, anterior, posterior involvement/lesion). Treatment was analyzed regarding neurosurgical strategy of 50 participating neurosurgical centres and the centre sizes. Based on patient load during the 6-year recruitment period, participating centres were categorized as small (1 pt/6 years), middle (2–5 pts/6 years) or large-sized centres (>5 pts/6 years).

BMI SDS at diagnosis was similar in patients with or without hypothalamic involvement. Surgical lesions of anterior and posterior hypothalamic areas were associated with higher increases in BMI SDS during 36 mo post-diagnosis compared to patients without or only anterior lesion ($+1.8$ BMISD, $P=0.033$, $+2.1$ BMISD; $P=0.011$), negatively impacting QoL in patients with posterior hypothalamic lesions. Surgical strategies varied between the 50 neurosurgical centres (3 large, 24 middle, 23 small centres). Patients treated in small centres presented with a higher rate of hypothalamic involvement compared to middle and large-sized centres. Treatment in large centres was less radical, the rates of complete resection and hypothalamic surgical lesions lower than those of middle-sized and small centres. However, a multivariable analysis showed that pre-operative hypothalamic involvement was the only independent risk factor for severe obesity ($P=0.002$).

Radical strategies leading to posterior hypothalamic lesions are not recommended due to potential to exacerbate hypothalamic obesity and impaired QoL. Because our results show that initial hypothalamic involvement has an *a priori* effect on the clinical course, our recommendations are based on recognizing CP as a chronic disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This work was supported, however funding details are unavailable.

## OC1.6

**Normal weight adult patients with Prader–Willi syndrome are not protected from insulin resistance during treatment with GH: results from a 12 month prospective study**

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Background

Diabetes mellitus is prevalent in adults with Prader–Willi syndrome (PWS), and GH therapy may deteriorate glucose balance.

Design

We prospectively investigated effects of 12 months of GH treatment on body composition and insulin resistance in relation to BMI in forty-two adults, mean($\pm$s.d.) age $28.5\pm6.7$ years with genetically verified PWS. Three patients with known diabetes were excluded. Data from baseline and 12 months of GH treatment in 35 patients, who completed the study are presented. The number of patients in each BMI-group (kg/cm$^2$) were 14(<25), 10(25–30) and 11(>30). Fat mass (FM) and lean mass (LM) were assessed by DEXA. Fasting blood samples and 2 h p-glucose after oral glucose tolerance test (OGTT) were made at baseline and after 12 months. Insulin resistance was estimated by homeostasis model assessment (HOMA-IR).

Results

With a mean final GH dose of $0.6\pm0.25$ mg we observed an increase in Insulin-like growth factor 1 (IGF1) from $115\pm35$ to $171\pm52$ µg/l, $P<0.001$, LM $39.9\pm8.1$ to $42.2\pm8.8$ kg, $P<0.001$, 2 h p-glucose $7.4\pm2.5$ to $8.1\pm2.5$, $P<0.05$, HOMA-IR $1.9\pm0.8$ to $2.3\pm0.7$, $P<0.01$ and a decrease in FM $29.4\pm12.5$ to $27.5\pm13.4$, $P<0.02$.

At baseline, IGF1 was lower with BMI (25–30) as compared to the other two groups, $95\pm27$ vs $124\pm33$ µg/l, $P<0.01$ and HOMA-IR was higher with BMI >30, $1.7\pm0.7$ vs $2.4\pm0.8$. Age and LM did not differ between the groups and variations in BMI were due to different FM. Final mean GH dose and IGF1 level did not differ between the groups. A reduction in FM was seen only with BMI (25–30), $29.1\pm3.0$ to $24.3\pm5.0$ kg, $P<0.01$ and HOMA-IR increased in the lowest BMI-group only, $1.6\pm0.6$ to $2.3\pm0.8$, $P<0.05$.

Conclusions

GH treatment in PWS patients results in the expected increase in insulin resistance irrespective of fat mass.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## Thyroid Clinical I

### OC2.1

**Overt hyperthyroidism is associated with increased mortality: a nationwide register-based study of disease discordant Danish twins**

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Introduction
Overt hyperthyroidism (OH) is associated with potentially lethal conditions such as atrial fibrillation, pulmonary embolism, stroke and coagulopathy. OH has also been connected with an increased mortality. However, this could be the result of common genetic and environmental factors affecting both OH and mortality.
Objective
To investigate if OH is associated with an increased mortality and to what degree the relation might be explained from genetic and/or environmental confounding.
Setting
Denmark.
Design
Register-based cohort study.
Participants
Twins ($n=96\,064$) and singletons from a 5% sample of the Danish population ($n=281\,549$) born from 1870 until 1990.
Methods
Unpaired and intrapair Cox regression analyses were compared. From the discordant twin pair design early environmental and genetic confounding was controlled by design. All participants were followed until December 31, 2008.
Results
Out of all participants, 4850 singletons and 1492 twins were identified as cases (OH). In the unpaired analyses, there was a significant higher mortality in individuals diagnosed with OH both in singletons (HR = 1.33, 95% CI 1.27–1.40) and in twins (HR = 1.31, 95% CI 1.18–1.46). In the intrapair analyses stratification for zygosity yield similar results for dizygotic twins (HR = 1.80, 95% CI 1.27–2.55), whereas the effect of OH attenuated in the monozygotic twins (HR = 0.95, 95% CI 0.60–1.50).
Conclusion
The findings of a raised mortality in singletons and within dizygotic twins pairs, but not within monozygotic twins, support a genuine relation between OH and mortality, which to some degree is influenced by shared genetic determinants.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC2.2

**Identification and functional analysis of DUOX2 variants: biallelic mutations are associated with permanent congenital hypothyroidism**

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Since the first identification of DUOX2 as an actor in the pathogenesis of congenital hypothyroidism (CH), several mutations have been associated with transient or permanent CH, with a high intra- and interfamilial phenotypic variability. In the present study, we report clinical and molecular studies of 7 unrelated children and 2 couple of siblings affected with CH and partial iodide organification defect (PIOD).
We identified nine novel and five previously reported DUOX2 mutations (seven missense, six stop codon – three nonsense and three frameshift – and one splice site mutations) using specific PCR primers able to distinguish the two highly homologous DUOX1 and DUOX2 sequences. The missense mutations involve conserved residues located in critical regions for protein function. Functional analysis of these variants indicated a significant impairment of $H_2O_2$ generation. Moreover, the nonsense mutants are shown to totally abolish the DUOX2 activity by different mechanisms: nonsense-mediated RNA decay, exon skipping and protein truncation.
Six out of eight cases with compound heterozygous variations had permanent hypothyroidism, but without evident correlations between the type of mutation and the phenotype in terms of TSH levels and discharge rates. However, two siblings showed biallelic mutations and transient CH. On the other hand, among the three patients harboring monoallelic mutations, one had transient and two permanent CH.
In conclusion, in our cohort most biallelic pathogenic DUOX2 variants were associated with permanent CH. The existence of other $H_2O_2$ generating systems and differences in age, ethnicity and iodine intake may account for the discrepant biallelic transient cases while the monoallelic cases with permanent CH could be explained by cryptic DUOX2 variations or alterations involving other thyroid mechanisms.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC2.3

**Thyroid hormone levels in euthyroid young men are associated with body composition and metabolic parameters**

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Introduction
Thyroid disorders affect metabolism and body composition; however, literature data have been conflicting on whether this is also the case for thyroid hormone levels within the euthyroid range. Therefore, we have investigated the relationship between indices of thyroid status and both body composition and metabolic parameters in a population of healthy euthyroid men.
Methods
Healthy male siblings ($n=941$, 25–45 years, median BMI 24.6) were recruited in a cross-sectional, population-based study; a history or treatment of thyroid disease and positive thyroid auto-immunity were exclusion criteria. Body composition and muscle cross-sectional area were assessed by dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). Total ($TT_3$, $TT_4$) and free thyroid hormones ($FT_3$, $FT_4$), TSH, Thyroxin Binding globulin (TBG) and reverse $T_3$ ($rT_3$) were determined using immunoassays.
Results
$FT_3$, $TT_3$ and TBG were positively associated with BMI, fat mass and serum leptin (all $P$ values between 0.02 and 0.0001), whereas $FT_4$ and $TT_4$ were solely associated with fat mass and serum leptin ((+); $P$ values $<0.005$). Inverse associations between lean mass ($P<0.005$) and other muscular parameters and thyroid hormones were observed. Higher levels of (F) $T_3$ and TBG were associated with lower insulin sensitivity, as assessed by HOMA-IR ($P=0.0001$). No associations between TSH and body composition or metabolic parameters were seen.
Conclusion
Thyroid hormone levels were positively associated with fat mass and leptin and inversely with indices of muscle mass and insulin sensitivity in this population of healthy young men with well characterized euthyroidism. Direction and

**Table 1**

| | TSH | $FT_3$ | $TT_3$ | $FT_4$ | $TT_4$ | $rT_3$ | TBG |
|---|---|---|---|---|---|---|---|
| Weight (kg) | −0.01±0.03 P=0.9 | 0.16±0.03 P<0.0001 | 0.14±0.03 P<0.0001 | −0.02±0 .03 P=0.6 | 0.04±0.03 P=0.2 | −0.04±0.03 P=0.08 | 0.09±0.03 P=0.004 |
| BMI (kg/m$^2$) | −0.01±0.03 P=0.9 | 0.18±0.03 P<0.0001 | 0.15±0.03 P<0.0001 | −0.03±0.03 P=0.4 | 0.03±0.03 P=0.3 | −0.02±0.02 P=0.2 | 0.09±0.03 P=0.004 |
| Whole body fat mass (kg) | 0.01±0.02 P=0.9 | 0.04±0.02 P=0.02 | 0.06±0.02 P=0.0005 | 0.06±0.02 P=0.0006 | 0.10±0.02 P<0.0001 | 0.06±0.02 P=0.0007 | 0.1±0.02 P<0.0001 |
| Whole body lean mass (kg) | −0.01±0.01 P=0.6 | −0.02±0.02 P=0.1 | −0.05±0.01 P=0.0003 | −0.04±0.01 P=0.004 | −0.09±0.01 P<0.0001 | −0.06±0.02 P<0.0001 | −0.1±0.01 P<0.0001 |
| HOMA-IR | 0.03±0.03 P=0.3 | 0.12±0.03 P=0.0001 | 0.12±0.03 P<0.0001 | −0.02±0.03 P=0.6 | 0.07±0.03 P=0.03 | −0.06±0.03 P=0.04 | 0.12±0.03 P<0.0001 |
| Leptin (μg/l) | 0.05±0.02 P=0.03 | 0.09±0.02 P=0.0001 | 0.08±0.02 P=0.0002 | 0.07±0.02 P=0.003 | 0.12±0.02 P<0.0001 | 0.07±0.02 P=0.003 | 0.11±0.02 P<0.0001 |

underlying mechanisms of these robust and coherent associations are presently not known.
Standardized estimates of mixed effects model describing the relationship between thyroid hormones (independent) and body composition and metabolic parameters (dependent)
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC2.4

**The thyroid hormone receptor-coactivator interface mediates negative feedback regulation of the human pituitary–thyroid axis**

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Corepressors and coactivators mediate thyroid hormone receptor-dependent repression and transactivation of positively-regulated target genes respectively, but their role in negative regulation is not understood.
A 4 years old boy was born at 31 weeks. He was jittery at birth, with neonatal respiratory distress. Childhood features included poor weight gain, heat intolerance, tachycardia and hyperactivity. Ongoing problems are low frequency hearing loss, poor sight, impaired coordination and learning disability. His circulating free thyroid hormones are consistently elevated ($FT_4$ 47.1 pmol/l (RR 9.8–23.1), $FT_3$ 13.7 pmol/l (RR 3.5–6.5) together with non-suppressed TSH (8.0 mU/l (RR 0.35–5.50)) levels; parental thyroid function is normal.
The proband is heterozygous for a novel mutation (E457Q) in the TRβ gene, involving a highly conserved residue located in its carboxyterminal transactivation domain; the mutation occurs *de novo*, with wild type parental TRβ sequence, consistent with their normal thyroid function. Although the mutant receptor binds T3 normally (WT $Ka = 1.29 \times 10^{10}\ M^{-1}$; E457Q $Ka = 1.26 \times 10^{10}\ M^{-1}$) hormone-dependent negative regulation (hTSHα) by E457Q TRβ is markedly impaired; in addition, when coexpressed, E457Q TRβ is a strong dominant negative inhibitor of WT receptor function. In protein-protein interaction assays, E457Q TRβ binds and dissociates from corepressors (NCoR, SMRT) normally; however, the mutant receptor fails to recruit coactivators (TRAP 220, SRC-1, RIPI40, LCoR). Crystallographic modelling indicates that substitution of glutamine for the negatively-charged glutamic acid at codon 457 abrogates electrostatic receptor-coactivator interactions.
Elevated thyroid hormones with non-suppressed TSH in the proband indicate impaired negative feedback in the pituitary–thyroid axis *in vivo*. consistent with impaired negative regulation of the TSHα promoter by E457Q TRβ seen *in vitro*. Together these observations suggest that proteins recruited to the thyroid receptor-coactivator interface mediate negative transcriptional regulation of target genes in the human pituitary–thyroid axis.
*Please note C Moran and M Agostini contributed equally to this work.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC2.5

**Thyroid spongiform nodules are the best candidates for percutaneous laser ablation. A 5 year follow-up study in 72 patients**

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Background
Percutaneous laser ablation (LA) is a therapeutic procedure used for the reduction of benign thyroid nodules. After LA a 50% nodule reduction was observed by most authors. However, eligibility criteria for LA based on long term efficacy have not been established.
Methods
Seventy-two patients (51 women and 21 men) with benign cold thyroid solitary nodules or a dominant nodule within a normo-functioning multinodular goiter underwent thermal Nd:YAG LA of thyroid nodular tissue by 1–4 optical fibers positioned into the tissue by 21-G needles under ultrasound real-time assistance. Mean ± SD nodule volume was 28.1 ± 29.3 ml. Energy delivered was 8842 ± 6086 Joules with an output power of 2–4 Watts.
According to internal content, nodules were classified as follows: a) compact: iso/hypoechoic, homogeneous, ≤10% of cystic content; b) mixed: solid, inhomogeneous, with a 20–50% cystic content; c) spongiform: aggregation of multiple microcystic components in more than 50% of the nodule. Cystic nodules (fluid content ≥50%) were excluded from this series.
Results
Five years after LA, nodule volume decreased to 14.5 ± 17.6 ml, with a reduction of −49.6% from pretreatment in the whole group of 72 patients. In 25 spongiform nodules, volume decreased from 24.8 ± 25.9 to 7.7 ± 7.5 ml, with a reduction of −58.7%. In 14 mixed nodules, volume decreased from 41.1 ± 47.3 to 16.1 ± 17.4 ml, with a reduction of −48.3%. In 33 solid nodules, volume decreased from 26.4 ± 24.5 ml to 17.9 ± 22.0 ml, with a reduction of −26.8%.
LA was more effective in patients with spongiform nodules as compared to mixed ($P \leq 0.01$) and compact ($P \leq 0.001$) nodules.
Conclusions
After 5 years, percutaneous LA achieved the expected 50% shrinkage in a variety of benign cold thyroid nodules. However, our data demonstrate that LA procedure was more successful and durable in patients with spongiform nodules. These patients are therefore the best candidates for percutaneous LA procedure.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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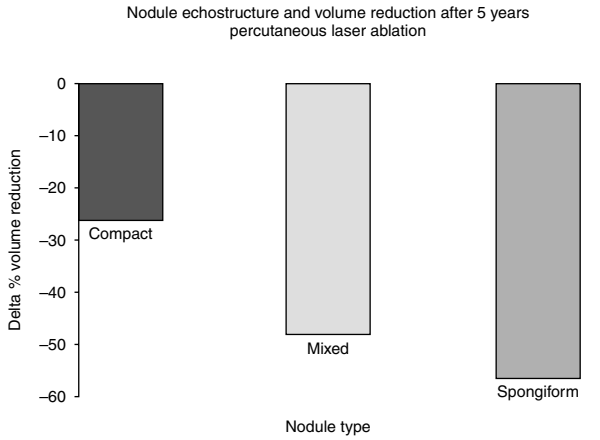


## OC2.6

**Concurrent presence of nonendocrine autoimmune diseases with Hashimoto's thyroiditis: a systematic assessment on more than 5000 consecutive patients**

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Systematic studies on the association of autoimmune thyroiditis (AIT), as pivotal disease and nonendocrine autoimmune diseases (NEAD) are scarce and mostly based only on the presence of circulating autoantibodies. In a large cohort of consecutive outpatients, AIT was diagnosed in 1541 (28.2%) and whenever additional autoimmune disorders were suspected, they underwent further screening. Diagnostic criteria were according with the Consensus Conferences for each suspected autoimmune disease. The association AIT + NEAD was detected in 250/1541 (16.2%) patients (227 W and 23 M; W/M 10:1; median age = 39 years). Of these, 64 patients (25.6%) had more than one autoimmune disease associated with AIT. The most frequent associated disease was chronic atrophic gastritis (CAG) (34.8%), followed by nonsegmental vitiligo (22.3%), celiac disease (11.0%), anti-phospholipids syndrome and multiple sclerosis (7.6%). Although most of patients with multiple autoimmune disorders were adult to old, 9.9% of them had a second autoimmune disease before the age of 30 years. In this group the most frequent diseases associated with thyroiditis were vitiligo (39%) and celiac disease (26%), while CAG was less observed (13%). Peculiar

characteristics of patients with AIT+NEAD were thyroxine malabsorption, chronic unexplained anemia and recurrent pregnancy loss (RPL). Thyroxine malabsorption was indeed higher than in patients with isolated thyroid diseases (31 vs 12.3%; $P<0.0001$; OR=3.60). RPL was observed in 6.9% of patients with AIT+NEAD as compared with 1.7% found in patients with isolated thyroid diseases ($P<0.0001$; OR=4.06). Unexplained anemia was found in 2.2% of patients with isolated thyroid diseases and in 18.3% of patients with AIT+NEAD ($P<0.0001$; OR=8.23). In conclusion: i) 1 patient out of 6 has NEAD associated with AIT and 1/10 of them is younger than 30 years; ii) atrophic gastritis, vitiligo and celiac diseases are the most frequent autoimmune disorders associated with AIT, being their prevalence age-related; iii) the presence of thyroxine malabsorption, chronic unexplained anemia and recurrent pregnancy loss in patients with AIT should induce to look for other autoimmune diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## Diabetes Clinical

### OC3.1

**Common variants of monogenic diabetes genes may affect β cell functional mass in patients with newly diagnosed type 2 diabetes**

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We tested the hypothesis that common genetic variability of β cell mass and/or function genes may influence β cell functional mass in type 2 diabetes (T2DM). We studied 590 drug-naive GAD-negative patients with newly diagnosed T2DM (age: median=60.0 yrs; I.Q. range: 52–66; BMI: 29.3 kg/m$^2$; 26.5–32.9). β cell functional mass was assessed by state-of-art mathematical modeling of glucose/C-peptide curves during a 240' frequently sampled OGTT, to provide the β cell responses to the rate of increase of glucose (derivative control: DC) and to glucose concentration (proportional control, PC). Insulin sensitivity, measured by the insulin clamp technique, acted as an internal control for the number of false positive hits. Forty-five SNPs, selected to cover over 90% of common genetic variability, were genotyped in 8 MODY (HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL) and 2 neonatal diabetes mellitus (NDM) (KCNJ11, ABCC8) genes. Allelic variants of 4 SNPs (rs1303722 and rs882019 of GCK, rs7310409 of HNF1A and rs5219 of KCNJ11, the latter being a known type 2 diabetes risk variant) were significantly associated to changes in DC of β cell function ($P=0.007-0.03$). Allelic variants of 5 other SNPs (rs2869084 and rs6031544 of HNF4A, rs10774580 of HNF1A, rs1801262 of NEUROD1, and rs7129639 of ABCC8) were found to influence significantly PC of β cell function ($P=0.001-0.04$). Only 1 (rs6721191 of KLF11) out of 45 SNPs was associated to insulin sensitivity ($P=0.047$). In multivariate regression models, combining GCK, HNF1A and KCNJ11 SNPs accounted for ~2.5% of DC of β-cell function, whereas combining HNF4A, HNF1A, NEUROD1 and ABCC8 SNPs accounted for ~3.6% of PC of β cell function. Thus, common variability of MODY and NDM genes is significantly associated to β cell functional mass in patients with type 2 diabetes, thereby potentially playing a role in the pathophysiology of the disease and in its metabolic prognosis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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### OC3.2

**Weight-loss independent metabolic benefits of gastric bypass surgery? Experiences from the Swedish obese subjects (SOS) study**

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Objective

Bariatric surgery has a marked effect on diabetes risk in the obese. The aim of this study was to evaluate weight-loss independent effects of gastric bypass on glucose and insulin levels over 10-years of follow-up.

Research design and methods

A total of 2010 obese individuals received bariatric surgery in the Swedish Obese Subjects (SOS) study (376 patients underwent non-adjustable or adjustable banding, 1369 underwent vertical banded gastroplasty (VBG), and 265 underwent gastric bypass (GBP)). Health examinations with anthropometric measurements and laboratory analyses were conducted at baseline and at 2- and 10-year follow-up. Follow-up rates were 92% and 74% at 2- and 10-years, respectively. Diabetes was defined by fasting venous whole blood glucose 6.1 mmol/litre or more, and/or self-reported diabetes medication. Weight loss was categorized in four groups: <30 kg; 30 to 25 kg; 25 to 20 kg; 20 to 15 kg. Changes in blood glucose and serum insulin levels were compared between the three surgical groups accounting for the degree of weight loss. Data was analysed separately for persons with and without diabetes at baseline.

Results

The average 10-year weight losses were 18 kg, 20 kg and 29 kg in the banding, VBG and GBP groups, respectively ($P<0.001$). Changes in fasting glucose and insulin were related to the degree of weight loss. There were no significant differences in changes in glucose and insulin between the three surgical groups, when patients within same weight loss categories were compared. This was observed for patients with and without diabetes at baseline. As expected, changes in glucose and insulin levels were more marked in patients with diabetes at baseline as compared to persons without.

Conclusions

Given same degree of weight loss over 10-years following bariatric surgery, there was no support for weight-loss independent benefits of GBP over banding and VBG on fasting glucose and insulin levels.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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### OC3.3

**Diabetic ketoacidosis at diagnosis influences complete remission after treatment of hematopoietic stem cell transplantation in adolescents with type 1 diabetes**

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**Abstract**

Objective

To determine if autologous nonmyeloablative hematopoietic stem cell transplantation (AHSCT) was worthwhile to do in type 1 diabetes adolescents with diabetic ketoacidosis at diagnosis.

Research Design and Methods

We enrolled 28 type 1 diabetes patients aged 14–30 years in a prospective AHSCT phase II clinical trial. Hematopoietic stem cells were harvested from the peripheral blood following a pretreatment consisting of a combination of cyclophosphamide and antithymocyte globulin. Changes of exogenous insulin requirement were observed and serum levels of hemoglobin A1c, C-peptide secretion during the oral glucose tolerance test (OGTT) and anti-glutamic acid decarboxylase antibody (GAD) were measured before and after AHSCT.

Results

After transplantation, complete remission (CR) defined as insulin independence was observed in 15 (53.6%, 15/28) patients for a mean period of 19.3 months over a follow-up ranging 4–42 months. The non-DKA patients achieved greater CR rate than the DKA ones (70.6%, 12/17 in non-DKA vs 27.3%, 3/11 in DKA, $P=0.051$). In non-DKA group, levels of fasting C-peptide, Cmax(peak value during OGTT) and AUCC(area under C-peptide release curve during OGTT) were enhanced significantly one month after transplantation and remained high during 24 months follow-up (all $P<0.05$). In DKA group, significant elevation of fasting C-peptide level and Cmax level were only observed at 18-month and 6-month respectively. There was no mortality.

Conclusions

We have performed AHSCT in 28 cases of type 1 diabetes. The data demonstrate AHSCT to be an effective long-term treatment of insulin dependence with greater efficacy achieved in patients without ketoacidosis at diagnosis.

Trial Registration clinicaltrials.gov Identifier: NCT00807651

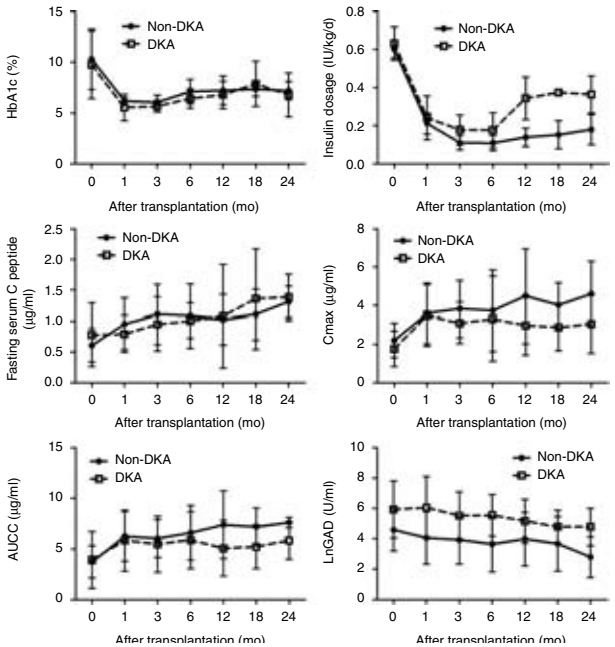


Fig1. Time course of HbA1c, Insulin dose, fasting C-peptide, Cmax, AUCC and LnGAD level in non-DKA group and DKA group respectively Note: Cmax, peak value of C-peptide during OGTT; AUCC (area under C-peptide release curve during OGTT); LnGAD, log form of GAD because of the uneven data distribution. Solid line, non-DKA group; Dotted line, DKA group.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC3.4

### Glucose lowering therapies and cancer specific mortality in adult insulin-treated diabetes

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**Abstract**
Aim
Following the general concern regarding the association between various diabetes treatments and cancer risk, the present study evaluated the cancer specific mortality in adult insulin-treated diabetes patients.
Materials and methods
All diabetes patients residing in a major urban area, aged over 40 years and receiving insulin treatment were included at the moment of their first diabetes outpatient visit from 01/01/2001 to 12/31/2008. A total of 11366 subjects (46.5% males) were followed-up for death of any cause until 12/31/2010 by crosslinking with the National Institute of Statistics mortality database, with a virtually 100% cover-up. Mortality data for the general population was obtained from the same source. Death related data was based on death certificate. Antidiabetic prescriptions were available from local pharmacies or prescribing institutions. Subjects with $<6$ months of follow-up were excluded (avoiding protopathic bias). Age and sex adjusted time-dependent Cox proportional hazard analysis (1 month update of treatment modalities) was performed.
Results
Mean age at baseline was $59.8 \pm 10.7$ years, mean follow-up period $6.75 \pm 2.98$ years (76697.9 person-years). Overall, unadjusted all-cause mortality was 49.7/1000 person-years (3811 deaths); cancer mortality was 6.9/1000 person-years (531 deaths). The major types of cancers were: pulmonary ($n=94$, 17.7%), pancreatic ($n=67$, 12.6%), liver ($n=64$, 12.1%), colorectal ($n=60$, 11.3%), breast ($n=55$, 10.4%) and stomach ($n=25$, 4.7%). Adjusted hazard ratios for cancer mortality (CI95%) were: biguanides 0.49 (0.35–0.68, $P<0.001$), sulfonylurea 0.9 (0.64–1.26, $P=0.54$), human rapid insulin 1.17 (0.96–1.41, $P=0.11$), rapid analogs 0.74 (0.46–1.22, $P=0.24$), human premixed insulin 0.85 (0.59–1.24, $P<0.4$), premixed analogs 0.91 (0.59–1.38, $P<0.64$), human intermediate insulin 0.82 (0.53–1.26, $P=0.36$), glargine 1.05 (0.64–1.74, $P=0.84$), and detemir 1.54 (0.75–3.15, $P=0.24$).
Conclusions
Biguanides are the only diabetes treatment choice associated with an independent, statistical significant (protective) effect on cancer mortality. Of interest, exposure to long acting analogues was not associated with increased cancer mortality in insulin treated subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC3.5

### Study of toll like receptor 2 and 4 expression in monocytes in type 1 diabetes mellitus with and without micro-vascular complications

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Background
Toll-like receptors (TLRS) are a class of proteins that play a key role in the innate immune system. They are single, membrane, non catalytic receptors which are expressed in multiple tissues predominantly cells of the innate immune system especially monocytes. So, they play a critical role in the regulation of immune function and inflammation.
Aim of our work
To study the expression of toll-like receptor 2 and 4 on monocytes in T1DM patients and its relation to microvascular complications.
Subjects and methods
This cross sectional study was conducted on 80 subjects above age of 18 years divided into Group I: 20 T1DM patients without diabetic complications. Group IIa: 9 T1DM patients with nonproliferative diabetic retinopathy (NPDR), Group IIb: 11 T1DM patients with proliferative diabetic retinopathy (PDR), Group IIIa: 7 T1DM patients with micro-albuminuria, GroupIIIb: 13 T1DM patients with macroalbuminuria, Group IV: 20 healthy subjects. They were subjected to full clinical history, thorough clinical examination, fundus examination, fasting plasma glucose level, (HbAIc), level albumin/creatinine ratio in random urine sample, measurement of TLR 2 and 4 using flowcytometry.
Results
There was highly significant difference between the studied groups as regards TLR2 and TLR4 percent ($P$ value $<0.01$) being higher in T1DM with microvascular complications than in T1DM without microvascular complications than in healthy control subjects. There was also highly statistical significant positive correlation ($P$-value $<0.01$) between TLR2 and age, duration of diabetes, SBP, DBP, FPG, HbA1c and albuminuria as well as highly statistical significant positive correlation ($P$ value $<0.01$) between TLR4 and age, duration of diabetes, SBP, DBP, FPG, HbA1c and albuminuria.
Conclusion
These results postulate that TLR2 and TLR4 may have a role in the microvascular complications of type 1 diabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## OC3.6

**Glucose disorders exert a detrimental effect on total brain volume in the elderly: a 2-year prospective MRI study**

K. Samaras[1,2], H. Lutgers[1], W. Wen[3,4], L. Campbell[1,2], B. Baune[5], H. Brodaty[3], J. Trollor[3] & P. Sachdev[3,4]
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Introduction
Long-standing type 2 diabetes (DM) is associated with brain atrophy. In this prospective study we examined the impact of glycaemic status on total brain volume in an elderly cohort.
Methods/Design
Two-year follow-up study of a population-derived cohort of non-demented community-dwelling adults aged 70–90 years (Sydney Memory and Aging Study). Prospective MRI and metabolic data were available in 312 of the 542 participants who had a MRI at baseline. At follow-up, participants were categorized in 4 groups: stable normoglycemia (NG at both time points, $n=102$), stable IFG (IFG at both time points, $n=120$), progression of glucose status (from NG to IFG/DM or from IFG to DM at follow-up, $n=57$), and, diagnosed DM at baseline ($n=33$). Total brain volume (TBV) was defined as the sum of grey and white matter ($cm^3$).
Results
$n=312$, mean age 78 ($\pm 4.4$) years; 54% Male, 41% had IFG and 13% had diagnosed DM at baseline. Compared to delta TBV in stable NG ($-18.4\ cm^3$), the decrease in TBV was 1.4-fold greater in stable IFG ($-26.6\ cm^3$, $P=0.2$), 2.3-fold greater compared to subjects with progression of glucose status ($-41.7\ cm^3$, $P<0.05$) and 2.3-fold greater in subjects with DM at baseline ($-42.3\ cm^3$, $P<0.05$). In a model that examined predictors of the decrease in TBV, glucose status at 2 years significantly predicted a decline, after adjusting for covariates (age, sex, TBV at baseline, history of hypertension, history of hyperlipidemia, use of lipid-lowering drugs), $\beta=-9.8$, $P<0.01$. Whilst 75% of the total variance in TBV at follow-up was explained by baseline TBV, 16% of the residual variance was explained by glucose status at follow-up.
Conclusion
This study in community-dwelling elderly people found that diagnosed DM as well as transition in glucose status to IFG or DM has an early and detrimental effect on brain volume assessed by MRI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details are unavailable.

# Pituitary basic

## OC4.1

**Obestatin plays an opposite role in the regulation of pituitary somatotrope and corticotrope function in primates and mice**

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Obestatin is a 23-amino acid amidated peptide that is encoded by the ghrelin gene and was therefore presumed to have regulatory effects on pituitary. However, the limited set of studies performed hitherto suggests that obestatin has no major effect on hormone secretion, *in vivo* or *in vitro*, from rodent pituitary, whereas no comparable data is still available on primates. Here, primary pituitary cell cultures from a non-human primate (baboon; Papio anubis) served to test the effects of obestatin (100 nM; 24 h) on the function of all pituitary cell types. Results revealed that obestatin did not alter expression or release of prolactin, LH, FSH or TSH. Conversely, obestatin treatment stimulated proopiomelanocortin (POMC) expression and ACTH release, while, surprisingly, it inhibited GH expression and release. Interestingly, these in vitro effects of obestatin on primate pituitary cultures were completely mimicked in mice treated in vivo with obestatin, suggesting that the effects of obestatin on pituitary cell function are conserved across species. Furthermore, obestatin treatment downregulated Pit-1 in both primate and mice pituitary cells, as well as mouse hypothalamic GHRH expression, which might contribute to the inhibitory effect of obestatin on GH expression and release observed in both species. Conversely, obestatin up-regulated expression of pituitary CRF-R1 in both models, and increased pituitary In2-ghrelin variant in mice, which may contribute to its stimulatory effects on POMC expression and ACTH release in both experimental models. Obestatin also down-regulated expression of somatostatin receptors 1 and 2 in both models, and also inhibited hypothalamic cortistatin in mice, thus reinforcing the evidence for its regulatory role on the GH and ACTH axes. Taken together, our results provide the first comprehensive experimental evidence to support a potential role of obestatin in the direct and opposite control of pituitary somatotrope and corticotrope function in mice and, most importantly, in a primate model.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC4.2

**Transcriptional regulation of prolactin by oestrogen *in vivo***

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Circulating levels of prolactin are subject to acute and long-term regulation by many factors including oestrogen and dopamine. We have studied the regulation of prolactin promoter activity in living pituitary cells using transgenic Fischer rats in which reporter gene expression is regulated by the human prolactin gene locus (hPRL-d2EGFP). We have previously identified pulsatile prolactin transcription patterns in living lactotroph cells in fetal tissue, that became stabilised during neonatal development. In order to assess how transcription patterns are affected during physiological and supraphysiological prolactin upregulation in the adult, we have evaluated the expression of the hPRL-d2eGFP transgene during the oestrous cycle and in males with long-term oestradiol releasing implants, respectively.
Rats, injected with LHRH to synchronise oestrous cycles, were culled and pituitary glands harvested at proestrus, oestrus and diestrus. Flow cytometry indicated a 1.8-fold increase in the number of cells expressing detectable levels of the d2EGFP reporter at oestrus ($n=7$) as opposed to diestrus ($n=5$). Mean fluorescence per cell increased by 10.6-fold. Validation by qPCR, confirmed a 4.1-fold increase in the expression of d2EGFP mRNA and a 3.7-fold increase in the endogenous rat PRL mRNA. Immunofluorescence confirmed induction of EGFP and prolactin protein expression in tissue sections. These data indicate a major increase in transcription rate in individual cells, which is likely to be necessary to sustain the high level secretory output during proestrus and oestrus. Supraphysiological oestrogen stimulation in males caused a 2.5-fold increase in pituitary weight and transgene expression was shown to be upregulated by flow cytometry and qPCR.
Using live cell imaging of tissue slice preparations from oestrous animals, fluctuating gene expression was detectable in a proportion of cells. The spatio-temporal nature of these transcription patterns is currently being subjected to mathematical analysis to determine how they are modified by relative cell positions in the tissue.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC4.3

**miR-26a targets PRKCD in ACTH pituitary adenoma**

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MicroRNAs (miRNAs) have several physiological functions, but have been also implicated in human neoplastic initiation and progression. We previously demonstrated that 30 miRNAs are differentially expressed in normal human pituitary as compared to pituitary adenomas. However, the most of miRNAs target genes remain unknown, hindering the understanding of the miRNAs contribute to pituitary tumorigenesis.

The aims of this study were to: (i) validate a murine ACTH-secreting pituitary adenoma cell line as a possible model to study pituitary miRNA deregulation; (ii) validate and investigate the role of potential targets of differentially expressed miRNAs. We analysed the murine AtT-20/D16v-F2 cell line, deriving from a murine ACTH-secreting pituitary adenoma, and normal mouse pituitary for the expression pattern of 11 miRNA, which expression was found to be different in human pituitary adenomas vs normal pituitary. Our results showed a partial agreement (50%) between expression trend of these miRNAs in human and mouse. In particular, we found that miR-26a has overlapping expression patterns in humans and mice, being up-regulated in adenomas vs. normal pituitary. Our results confirm that the 3′ untranslated region of PRKCD, a miR-26a putative target gene, is a functional target of this miRNA and provide evidence, by Real Time PCR, that this target is translationally suppressed. PRKCD, a member of the PKC subfamily, is dynamically involved in cell apoptosis in a specific stimulus manner. We observed that miR-26a inhibition led to a decrease in cell viability without increasing caspase 3/7 activity. These results indicate that miR-26a is overexpressed in human ACTH pituitary adenoma and can control cell viability in AtT-20/D16v-F2 cell line, by reducing the PRKCD expression, playing an important role in pituitary adenoma development. Our study provides new insights into potential contribution of these RNAs to pituitary neoplastic transformation and suggests that miR-26a might be a possible target for therapeutic strategies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC4.4

**Identification of coupling specificity between somatostatin receptor 5 (SST5) and G proteins by a bioluminescence resonance energy transfer (BRET) technique: the role of GoA protein**

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In this study we employed a novel bioluminescence resonance energy transfer (BRET) biosensor to study the coupling specificity of somatostatin receptor 5 (SST5) and its naturally occurring mutant R240W in living cells. Our previous data demonstrated that SST5 carrying the R240W mutation as well as other mutations in the third intracellular loop maintained the ability to inhibit intracellular cAMP levels similarly to the wild-type but failed to mediate the inhibition of intracellular calcium levels, GH release and cell proliferation, suggesting that different regions of SST5 are required for the activation of different signaling pathways. The BRET biosensor may monitor the activation of G proteins in response to SST5 activation by specific agonist BIM23206. The energy transfer occurs within two subunits of the heterotrimeric G protein complex, the energy donor (G alpha-Renilla Luciferase) and the acceptor (G gamma2-GFP10). G protein activation induces a structural rearrangement that increases the distance between the donor and the acceptor with a consequent decrease in the energy transfer. To detect specific G protein activation in living cells, we expressed in HEK293 cells wild-type or mutant SST5 together with different G protein (i1, i2, i3, oA, oB or q). We demonstrated that wild-type SST5 activated Gi (1, 2 and 3) and Go (A and B), whereas R240W SST5 maintained the ability to activate all Gi and GoB, but not GoA. As expected, neither the wild-type nor mutant receptors activated Gq. To investigate the role of GoA in SST5-mediated signal transduction in a pituitary cell model, we cotransfected cultured cells from GH-secreting adenomas with SST5 and a pertussis toxin (PTX)-resistant GoA protein. In PTX-treated cells, GoA restored the ability of BIM23206 to inhibit ERK1/2 phosphorylation and GH secretion. In conclusion, our data first demonstrated the coupling specificity of SST5 and revealed a crucial role for GoA in SST5 signaling in GH-secreting adenomas.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC4.5

**Targeting the IGF-IR system in pituitary tumors *in vitro*: antiproliferative action & pitfalls**

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IGF-I receptors (IGF-IR) and their aberrant signaling cascade contribute to the pathogenesis of several solid cancers. Pituitary adenomas express IGF-IR and present with overactivation of the IGF-IR pathway. The aim of the study was to identify the potential antiproliferative action of the small molecule IGF-IR inhibitor NVP-TAE226 on acromegalic ($n=11$) and nonfunctioning pituitary adenomas (NFPA; $n=15$) in primary cell culture. Changes in cell viability were determined by thymidine incorporation and a nonradioactive colorimetric assay. The compound at the 100 nM concentration suppressed cell viability by more than 20% in 9 out of 15 NFPA (mean growth suppression as % of vehicle control: $30\pm15$) and in 10 acromegalic tumors ($29\pm7$), while at 10 nM it was efficacious in 8 NFPA ($24\pm10$) and 4 acromegalic tumors ($28\pm14$). Inhibiting the PI3K pathway in the same tumors using BEZ235 suppressed cell viability at 10 nM concentration in all NFPA ($48\pm20$) and in two acromegalic tumors ($31\pm15$). In contrast, mTOR inhibition with everolimus was efficacious in 4 out of 15 NFPA ($25\pm10$) and in all but one acromegalic cases ($48\pm17$). Put together, these data indicate a tumor specific dependency to the different IGF-IR signaling branches, with NFPA being sensitive to PI3K inhibition and acromegalic tumors to the mTOR inhibition.

IGF-IR inhibition in acromegalic tumors *in vitro* was accompanied by increased GH secretion (GH normalized to cell viability assay counts; mean suppression as % of vehicle control: $46\pm10$). Similar GH increase was observed after mTOR but not in the same extend after PI3K inhibition ($42\pm31$ vs $20\pm14$; $P=0.043$), indicating a role for the mTOR pathway on GH synthesis.

Altogether, IGF-IR inhibitors have a potential as antiproliferative agents for the treatment of NFPA. In contrast their potent antiproliferative action in acromegalic tumors is compromised by the concomitant increase in GH secretion.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC4.6

**Cell cycle G2/M transition is modulated by microRNAs in pituitary adenomas**

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Background

Although pituitary adenomas are common endocrine neoplasms, the background of their pathogenesis has not clearly revealed. G1/S checkpoint alterations of the cell cycle have already been described in these tumours. MicroRNAs (miRs) which are small, non-coding RNA molecules, and posttranscriptionally regulate protein expression have also identified as potential pathogenic factors in certain cases. Our aim was to determine miR expression profile in pituitary adenomas, and validate targets of miRs that differentially expressed between normal and adenomatous pituitary.

Materials and methods
57 pituitary tissue samples were analyzed. MiR expression profiles were determined by TaqMan Low Density Arrays, selected miRs and genes were validated by real-time PCR. Multiple in silico target prediction algorithms were applied for identification of miR–mRNA interactions. Protein changes were detected using immunhistochemistry and Western blot. mRNA–miR interactions were proved using *in vitro* luciferase reporter system.
Results
162 differentially expressed miRs and those which were correlated with tumour size were determined in pituitary adenomas compared to normal tissues. Using target prediction we identified the downregulation of the Wee1 kinase protein by three overexpressed miRs targeting Wee1 proved by luciferase reporter gene experiment. 6 of 24 genes involved in G2/M transition were differentially expressed in adenoma tissues compared to normal tissue. Among CDC25 family that has opposite function to Wee1 Cdc25A and CDC25C were found to be overexpressed and its targeting miRs were underxpressed in pituitary adenomas.
Conclusion
Wee1 and CDC25 have opposite regulator effect on Cyclin B–CDK1 complex that controls G2/M transition of cell cycle. Our results show that in pituitary adenomas G2/M transition is complexly regulated by RNA interference through Wee1 and CDC25. Therapeutical approaches targeting miRs and members of G2/M transition are under development and based on our results these may have therapeutical significance in treatment of pituitary adenomas.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## Obesity basic

### OC5.1

**GH ameliorates impaired glucose tolerance in obese mice by modifying the visceral fat condition through adiponectin**

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Objectives
Secretion of GH, a pituitary and anabolic hormone that reduces visceral fat, is diminished in obese or elderly subjects. The reduced GH may induce visceral fat accumulation and subsequently accelerate metabolic syndrome in these population. In the present study, we sought the effect of chronic GH supplement on visceral fat and glucose metabolism in diet-induced obese (DIO) mice.
Methods and results
Male obese mice (C57BL/6, 12 weeks old) under high fat/high sucrose (HF/HS) diet were treated with daily injection of GH (5 μg/g body weight, $n=6$) or PBS (control, $n=6$) for 6 weeks. GH markedly reduced the mass of epididymal fat (Epi; PBS: $1.68\pm0.14$ vs GH: $1.13\pm0.04$ g, $P<0.0001$) and increased that of skeletal (gastrocnemius) muscle (PBS: $0.31\pm0.01$ vs GH: $0.36\pm0.02$ g, $P<0.0001$). Intra-peritoneal glucose tolerance test (IPGTT) demonstrated that GH dramatically ameliorated the impaired glucose tolerance (IGT) in the DIO mice. Real-time quantitative PCR analyses with Epi showed that GH significantly increased the expressions of adiponectin (40.8%, $P<0.05$), anti-inflammatory M2 macrophage markers (IL10: 66.4%, $P<0.05$; CD206: 41.4%, $P<0.01$), anti-oxidant enzyme (glutathione peroxidase: 36.6%, $P<0.05$) compared with control. Moreover, GH lowered the plasma levels of thiobarbituric acid reactive substance (TBARS), an oxidative stress marker, by 22.1% (PBS: $4.48\pm0.23$ vs GH: $3.49\pm0.17$ μM, $P<0.05$), in the DIO mice. However, in adipoectin-deficient mice under HF/HS diet, GH supplement neither changed the mass of Epi and skeletal muscle, nor modified IGT.
Conclusion
Chronic GH supplement mitigates IGT in DIO mice by modifying visceral fat mass, inflammation and oxidative stress likely through adiponectin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC5.2

**Production of galectin-1, -3, -9 and -12 in adipose tissue of lean and obese mice**

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Introduction
Obesity is characterized by an expanded and chronically inflamed adipose tissue (AT). The galectin (Gal) family of proteins exerts a variety of activities, including regulation of inflammation and adipogenesis.
Methods
We used RT-PCR, ELISA and flow cytometry to evaluate regulation of expression and production of 4 members of the Gal family – Gal1, Gal3, Gal9 and Gal12 – in visceral (VAT) and subcutaneous (SAT) adipose tissue of male C57BL6 mice fed a low (LFD) or high fat diet (HFD) for 5, 9, 13, 17 and 24 weeks.
Results
Feeding a HFD induced a progressive elevation in body weight and circulating leptin levels that was accompanied by a significant increase in serum levels of Gal1 and Gal3. Expression of each of the 4 Gal did not significantly change over time in either VAT or SAT of LFD mice. In mice fed a HFD there was a progressive and significant increase in expression of the anti-inflammatory Gal1 and Gal9 selectively in SAT, whereas expression of the pro-inflammatory Gal3 significantly increased in both VAT and SAT of HFD-fed mice. Expression of Gal12, which modulates adipogenesis, significantly declined over time in VAT, but not SAT, of HFD mice, following a trend comparable to adiponectin. Evaluation of cellular expression indicated that both adipocytes and the stromovascular fraction (SVF) produced Gal1 and Gal3, while Gal9 was predominantly produced by the SVF and Gal12 almost exclusively by adipocytes. Flow cytometry analysis demonstrated increased production of Gal3 and Gal9, but not Gal1, in both CD11c− and CD11c+ macrophages obtained from VAT, but not SAT, of HFD versus LFD mice.Conclusions: Our data indicate that feeding a HFD leads to differential modulation of Gal1, Gal3, Gal9 and Gal12 in macrophages and adipocytes in VAT and SAT, suggesting that Gal proteins may actively participate in regulating inflammation and adipogenesis in obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC5.3 The Young Investigator Winner

**Insulin action in brown adipose tissue is compromised during diet-induced obesity**

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Increasing evidences indicate that brown adipose tissue (BAT) functional activity is reduced during obesity. The nature of this deficit in BAT function, however, is still poorly understood. To analyze whether insulin function in the BAT is altered during obesity, we studied the in vivo metabolic activity of BAT by PET/CT imaging and euglycaemic hyperinsulinemic clamp in lean and diet-induced obese mice. Three groups of mice were analyzed after administration of different diet regimens leading to progressive obesity levels: standard chow diet (SD), high fat diet (HFD, 40% fat), super high fat diet (SHFD, 60% fat). Glucose uptake in the BAT was assessed analyzing 18F-FDG accumulation (a PET tracer glucose analogue) on PET/CT images. Insulin strongly increased PET signal in BAT of SD mice, whereas insulin induced 18F-FDG uptake was lower in HFD group, and completely absent in SHFD group. To analyze whether insulin exerts a role on BAT thermogenesis, we recorded BAT temperature during euglycaemic hyperinsulinemic clamp studies by the means of telemetric probes surgically implanted in the tissue. Insulin was able to reduce BAT thermogenesis in lean mice, whereas obese mice were not able to respond to insulin-induced modifications in BAT activity. To analyze the effect of insulin on glucose storage; glycogen accumulation into the BAT was studied analyzing glycogen content in the tissue at the end of the clamp procedure. Insulin-induced glycogen accumulation was impaired in BAT of obese mice. QT-PCR and Western blot analysis of the protein PTG (protein targeting to glycogen) confirmed the presence of a compromised glycogen metabolism in the BAT of obese mice.

These data show that during diet-induced obesity, BAT is not able to correctly respond to insulin with respect to glucose uptake, glycogen accumulation and thermogenesis modifications thus indicating that obesity leads to metabolic inflexibility in BAT.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC5.4

**Bile acid binding resin shows anti-obese effect through the modifications of intestinal microbiota**

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The growing epidemic of obesity has led health professionals to understand the pathophysiology of obesity and its complications. Gut microbiota are of special interest, since some kinds of microbiota such as Bacteroides are less in obese persons and some microbiota induce obesity in animals by effective fat storage through microbiota-mediated intestinal Fiaf (Fasting-induced adipose factor) suppressions. Recently, bile acid-binding resins (BARs), non-absorbable drug for dyslipidemia, were reported to prevent obesity in rodents and humans, but the precise mechanisms and the effects to microbiota are unclear. Here we report the impact of BAR to gut microbiota and environments.

Six-week-old male BALB-c mice were fed standard diet, high-fat diet or high-fat diet with 1.5% of BAR (colestimide). Body compositions and gene expression were analyzed after 12-week treatment. The compositions of gut microbiota were studied by 16S rDNA analysis of cecal contents. The same procedures were performed under the germ-free conditions.

The administration of BAR significantly reduced the body weight and intra-abdominal fat gain ($P<0.01$). The decrease of gut Bacteroides and the increase of Clostridium in high-fat group were observed compared with normal diet, and the administration of BAR induced the increase of Bacteroides and the decrease of Clostridium ($P<0.05$). The intestinal fiaf expression decreased significantly with high-fat diet and increased significantly by BAR. These anti-obese effects of BAR disappeared under germ free conditions and the germ-free mice conventionalized with standard flora showed reduced body weight gain as shown in normal BAR-treated mice.

In conclusion, BAR has anti-obese effects through the changes of gut microbiota and environments. The modulation of gut microbiota by changing intestinal bile acid flux would be the novel therapeutic target for obesity.

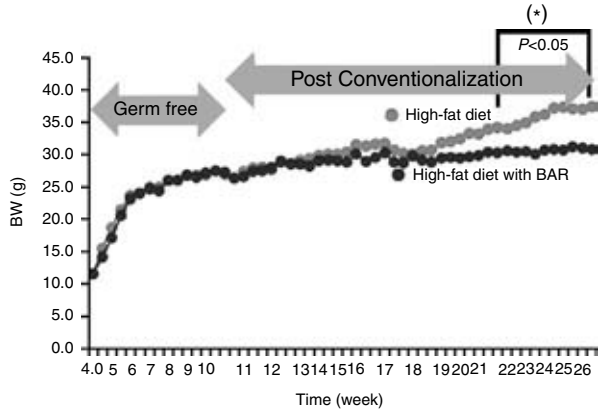


**Fig 1** BAR shows anti-obese effects through gut microbiota

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC5.5

**Development of leptin deficient *Lep$^{mkyo}$/Lep$^{mkyo}$* rat-evidence for its superiority over *Lep$^{ob}$/Lep$^{ob}$* mouse as a model for human obesity**

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Introduction

It is critical to consider species differences in translating the findings of obesity-model animals into human pathophysiology. Leptin plays an important role in regulating energy homeostasis. Although much has been learned from genetically obese leptin deficient *Lep$^{ob}$/Lep$^{ob}$* mice, some flaws were noted in mice as animal models for human diabetes.

Methods

To develop a rat leptin-deficient model, we use N-ethyl-N-nitrosourea (ENU) mutagenesis followed by high-efficient screening system. To compare the effect of thiazolidinediones (TZDs) on fatty liver between mouse and rat, we treated 12-week-old male leptin-deficient mice and rats with pioglitazone or rosiglitazone. Parameters of glucose and lipid metabolism, adiposity and PPARγ gene expression in the liver were examined.

Results

We found a leptin-deficient rat strain, Lepmkyo/Lepmkyo, which had a nonsense mutation (Q92X) within the leptin gene. *Lep$^{mkyo}$/Lep$^{mkyo}$* rats showed morbid obesity, glucose intolerance, hypertriglyceridemia and fatty liver, which are comparable to *Lep$^{ob}$/Lep$^{ob}$* mice. In a striking contrast, treatments with TZDs disclosed marked deterioration of steatohepatitis in obese *Lep$^{ob}$/Lep$^{ob}$* mice, while they improved steatohepatitis in obese *Lep$^{mkyo}$/Lep$^{mkyo}$* rats as in obese individual. TZDs differentially regulated hepatic peroxisome proliferator-activated receptor gamma (PPARγ) mRNA expression and body fat distribution between *Lep$^{ob}$/Lep$^{ob}$* mice and *Lep$^{mkyo}$/Lep$^{mkyo}$* rats. The present study demonstrates the superiority of *Lep$^{mkyo}$/Lep$^{mkyo}$* rats over *Lep$^{ob}$/Lep$^{ob}$* mice as animal models of obesity.

Conclusion

The present study provides an evidence of the superiority of *Lep$^{mkyo}$/Lep$^{mkyo}$* rats over *Lep$^{ob}$/Lep$^{ob}$* mice as a model for human obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC5.6

**Semaphorin 3C-a novel adipokine modulating human adipose tissue plasticity in weight change**

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Context

Alterations in white adipose tissue (WAT) function, including changes in extracellular matrix (ECM) composition, play an important role in conditions of both weight gain and loss.

Objective

We set out to identify novel genes regulated by obesity and cancer cachexia in human scWAT with potential roles for adipocyte function and/or tissue remodelling.

Design Candidate genes were selected by overlapping previously published global gene expression profiles in human WAT ($n=X$) with functional RNAi screens assessing lipid droplet morphology in Drosophila S2-cells. *In vitro* experiments were performed in primary cultures of human preadipocytes/adipocytes.

Results

33 genes regulated by obesity/cachexia had orthologues in Drosophila and of these two affected lipid droplet morphology in the RNAi screen. One of the two,

semaphorin 3C (SEMA3C), encodes a secreted factor not previously studied in human adipocytes. SEMA3C mRNA expression was increased in obesity and decreased in cancer cachexia. In addition, SEMA3C was predominantly expressed/secreted by mature adipocytes. Incubating human adipocytes with recombinant SEMA3C had no effect on differentiation, lipolysis, glucose transport or the expression of lipid oxidation genes which could be explained by the significant down-regulation of the SEMA3C receptors/co-receptors during adipocyte differentiation. In contrast, incubation of human pre-adipocytes with SEMA3C increased the production of the ECM-related factors fibronectin 1 and connective tissue growth factor in a concentration-dependent manner without any effects on differentiation.
Conclusions
SEMA3C is a novel adipokine altered by weight changes that regulates the expression of ECM genes in preadipocytes. SEMA3C may therefore play a role in tissue plasticity and remodelling.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## Female reproductioin basic

### OC6.1

**GnRH release failure in hyperprolactinemia is caused by a Kisspeptin deficiency**

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Hyperprolactinemia is the most common cause of hypogonadotropic anovulation in women. It is related to an alteration of pulsatile GnRH secretion. This gonadotropic deficiency has been proposed to result from direct suppression of prolactin (PRL) on GnRH release but its mechanism remains unknown. Because GnRH neurons do not express unequivocally the PRL receptor, and are stimulated by kisspeptin (Kp) neurons which do express PRL receptors, we hypothesized that GnRH deficiency in this condition could be due to a decrease in Kp secretion. To test this we developed and characterized a hyperprolactinemic female mouse model mimicking the human pathology and analyzed the ability of Kp10 i.p. administration to restore gonadotropin secretion and cyclicity
We demonstrated that 28 days administration of PRL by micropumps, significantly inhibited mouse ovarian cyclicity evaluated by vaginal smears, and decrease corpora lutea (CL) number, reflecting ovulation rate impairment. This anovulation was related to a significant downregulation of pituitary *LHβ* and *FSHβ* transcripts indicating a gonadotropin deficiency in this model. Hypothalamic *GnRH* expression was not altered while there was, a significant decrease of Kp mRNA and peptide (hypothalamic staining) suggesting a role of *Kp* decrease in GnRH release alteration in hyperprolactinemic female mice. We then demonstrated that Kp-10 administration restored cyclicity, ovulation and pituitary gonadotropin expression in hyperprolactinemic mice. Using hypothalamic explants, we also demonstrated that *in vitro* decrease of GnRH release induced by PRL was rescued by Kp-10 administration.
Together with the recent demonstration that Kp neurons express high levels of PRL receptor, our data suggest that PRL excess acts directly on Kp neurons to suppress Kp secretion and downstream GnRH secretion. Kp neurons appear, therefore, to be the missing link between hyperprolactinemia and GnRH deficiency in mammals.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC6.2

**Ghrelin modulates fertilization, early embryo development and implantation**

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Ghrelin (Ghr) acts as a link between energy balance and reproduction; hence, hyperghrelinemia reduces reproductive success. In addition, this hormone has an evident physiological role on reproduction, since Ghr and/or its receptor are synthesized by gametes/embryos and several reproductive tissues (including decidua/placenta); in addition, Ghr plasma concentration rises during gestation. The objectives of our study were to evaluate the effects of ghrelin administration (4 nmol/animal/day; sc) or endogenous ghrelin inhibition (by 6 nmol/animal/day of (D-Lys3)GHRP-6; sc) on mice fertilization, embryo development and implantation. We carried out three experiments treating female mice with Ghr and/or its antagonist: i) from one week previous to 12 hours after copula, sacrificing mice at Day 18 of pregnancy; ii) from ovulation induction to 80 hours after ovulation, when we retrieved embryos from uterus and iii) from Day 3 to Day 7 of pregnancy (peri-implantation period), sacrificing mice at Day 18.
Experiment 1: the antagonist increased the percentage of females with one/more atrophied fetuses (antagonist: 75.0% vs control: 11.1%; $n=8$–11 females/group; $P<0.0134$). Experiment 2: the antagonist significantly diminished induced ovulation and fertilization and both, Ghr and the antagonist, delayed embryo development (embryos in blastocyte stage: Ghr 40.8%, Ghr+antagonist 28.9% and antagonist 36.8% vs control 66.3%; $n=76$–136 embryos/treatment, $P<0.0001$). In experiment 3, Ghr and the antagonist significantly diminished fetuses weight and dams weight gain during gestation. Moreover, Ghr augmented the percentage of embryo loss (TM$\pm$SEM; Ghr: $17.3\pm6.58$ and Ghr+antagonist: $13.3\pm3.7$ vs control: $3.9\pm4.8$ and antagonist: $6.7\pm4.0$; $n=9$–12 females/treatment; $P=0.045$) and again, Ghr and the antagonist increased fetuses atrophy (Ghr: 71.4%, Ghr+antagonist: 44.4% and antagonist 62.5% vs control: 0%; $n=7$–10 females/group; $P<0.01$).
Our results suggest that hyperghrelinemia and/or endogenous ghrelin inhibition exerted immediate and long lasting effects on oocyte/embryo quality, implantation and embryo/fetal development, supporting the hypothesis that ghrelin has modulatory actions on these reproductive processes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC6.3

**LH and hCG produce different responses in human granulosa lutein cells *in vitro***

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Introduction
LH and hCG act on the same receptor (LHCGR), but it is not known whether they elicit the same cellular and molecular response. This study aims at comparing the activation of cell-signalling pathways and gene expression in response to LH and hCG.
Design
We evaluated the activation of cAMP, ERK and AKT-pathways and progesterone production by ELISA and Western blotting in human primary granulosa cells (hGLC) stimulated for up to 36 hours with equipotent doses of LH or hCG. The expression of target genes was measured by real-time PCR after 12-hours of stimulation with either gonadotropin, using specific ERK or AKT-pathways inhibitors (U0126 and LY294002) to differentiate their effects on gene expression.
Results
Continuous exposure of hGLC to equipotent doses of LH or hCG for 36 hours revealed that the intracellular cAMP production is pulsatile with a frequency of about 3–4 hours, with significantly higher stimulation by hCG vs LH (t-test; $P<0.05$; $n=3$), despite no differences in progesterone production, which increased progressively. Conversely, phospho-ERK and -AKT activation was more potent and sustained by LH vs hCG over 1 hour (t-test; $P<0.05$; $n=4$).

hCG was almost inactive on AKT. Finally, LH significantly increased the expression of NRG-1 and CYP19A1 genes after ERK inhibition, while hCG decreased the expression of AREG after AKT inhibition (t-test; $P<0.05$; $n=3$).
Conclusions
In terms of cAMP production, hGLC respond to equipotent, constant LH or hCG stimulation in a pulsatile fashion. Acutely, hCG is more potent on cAMP production, while LH is more potent on the ERK and AKT activation. The early inhibition of ERK and AKT results in the differential induction of expression of target genes depending on ligand, indicating that the LHCGR is able to differentiate the activity of LH and hCG.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC6.4

**Female fertility and activin-stimulated Fshb expression do not require SMAD2/3 in murine gonadotropes**

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In mammals, follicle-stimulating hormone (FSH), produced by pituitary gonadotropes, is required for proper gonadal function and female fertility. The TGFβ superfamily members, activins, are critical regulators of FSH synthesis. Activins signal through heteromeric type I/type II receptor complexes and the effector proteins SMADs 2 and 3 to regulate gene transcription. Numerous studies in gonadotrope-like cell lines suggest that activin-stimulated FSH β subunit (Fshb) transcription is mediated by SMAD2/3. To test the hypothesis that SMAD2/3 are required in gonadotropes for proper FSH synthesis and reproductive axis activity *in vivo*, we generated mice with gonadotrope-specific deletion of Smad2/3 (hereafter Smad2/3KO) by crossing Gnrhr-IRES-Cre (GRIC) mice with those carrying conditional (floxed) Smad2 and Smad3 alleles. Female Smad2/3KO mice had diminished estrous cycle frequency and spent more time in estrus than control females as assessed by daily inspection of vaginal cytology. However, in metestrus/diestrus, Smad2/3KO ovarian and uterine weights were comparable to those of control mice. Importantly, the frequency and size of litters produced over a six month period by Smad2/3KO females paired with wild-type males were indistinguishable from controls. Smad2/3KO males had slightly reduced testes weights but were fertile. Surprisingly, pituitary Fshb expression was normal in both male and female Smad2/3KO mice. In light of these results, we examined whether SMAD2/3 are required for activin A-regulated Fshb expression in gonadotropes ex vivo. Primary pituitary cells from mice carrying floxed Smad2/3 alleles were infected with control or Cre-expressing adenovirus. Despite >95% depletion of Smad2/3 mRNA in Cre infected cultures, inhibitory effects on basalFshb and activin A-induced Fshb expression were modest. Together, our data challenge the current model that FSH synthesis depends on SMAD2/3-mediated signaling.
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Declaration of interest
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## OC6.5

**Dihydrotestosterone treatment in mice induces a persistent polycystic ovary syndrome phenotype**

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Polycystic ovary syndrome (PCOS), the most common endocrine disorder in women in their reproductive years, is defined by two out of the following three criteria: hyperandrogenism, oligo/anovulation, and polycystic ovaries. Affected women are often obese and insulin-resistant. Recently, we developed a mouse PCOS-model through chronic exposure to dihydrotestosterone (DHT). Here, we studied whether the PCOS-phenotype remains after withdrawal of DHT treatment, which would more closely resemble PCOS in women.
Prepubertal mice were treated with a 90-days continuous release DHT or placebo pellet. After treatment (90 days) mice were sacrificed (chronic group, $n=17$) or allowed a 30-day wash-out period before sacrifice (sustained group, $n=17$). Before sacrifice mice vaginal smears were taken and an Intraperitoneal Glucose Tolerance Test (IPGTT) ($n=8$–9 per group) or an Insulin Tolerance Test (ITT) ($n=8$–9 per group) was performed.
After 90 days, DHT-treated mice were acyclic, their ovaries contained antral follicles with a cyst-like structure and an increased number of atretic follicles, bodyweights were higher, and chronically DHT-treated mice were glucose intolerant.
Mice of the sustained group remained anovulatory, their ovaries still contained cyst-like follicles and an increased number of atretic follicles. In contrast to the chronically DHT-treated mice, DHT-treated mice of the sustained group had an increased number of healthy follicles compared to placebo-treated mice. Bodyweight and weights of fat depots remained significantly increased in the sustained group and mice remained glucose intolerant. Insulin sensitivity did not appear to be affected in the DHT-treated mice, although the counter regulatory mechanisms after insulin administration were impaired in both the chronically and sustained DHT-treated mice.
We confirmed that chronic DHT-treatment induces a PCOS-like phenotype in mice. A 30-day wash-out period did not improve the ovarian and metabolic phenotypes. This suggests that in mice after a 90-day DHT treatment period, a sustained PCOS phenotype is induced.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC6.6

**Kit ligand-c-kit interaction regulates estradiol production by rat granulosa cells via oocyte factors**

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Kit ligand (KL)-c-kit interaction is critical for oogenesis and folliculogenesis in the ovary. However, the significance of KL-c-kit loop in ovarian steroidogenesis has yet to be elucidated. We here investigated the impact of KL-c-kit interaction in regulation of steroidogenesis using rat primary granulosa cells co-cultured with oocytes. Treatment with soluble KL suppressed FSH-induced estradiol production and aromatase mRNA expression without changing FSH-induced progesterone production. Blocking the KL-c-kit interaction by its neutralizing antibody increased FSH-induced estrogen production by granulosa cells, suggesting that endogenous KL is functionally involved in suppression of estrogen production by granulosa cells through the interaction between KL and its receptor c-kit on oocytes. The cAMP-PKA pathway activity was not involved in the KL and c-kit actions on estrogen regulation in granulosa cells. To explore the possible oocyte factors induced by KL-c-kit interaction, changes in the expression levels of major oocyte factors including BMP-15, GDF-9 and FGF-8 were investigated. It was of note that KL increased the expression levels of oocyte-derived FGF-8 and GDF-9, while it reduced the expression levels of oocyte-derived BMP-15 in the oocyte-granulosa co-culture. Based on the findings that FGF-8, but not BMP-15 or GDF-9, suppressed FSH-induced estrogen production by granulosa cells, oocyte-derived FGF-8 is possibly involved in suppression of FSH-induced estrogen production through the KL-c-kit interaction. Furthermore, the KL suppression of FSH-induced estrogen production in the co-culture was reversed by the FGFR inhibitor SU5402, which was additionally reversed by the combined treatment with extracellular-domain protein of BMPRII that antagonizes BMP-15 and GDF-9. These data suggest that endogenous oocyte factors including FGF-8 and BMP-15/GDF-9 are likely to be involved in the KL activity that inhibits FSH-induced estradiol production. Thus, KL-c-kit interaction plays a regulatory role in estrogen production through oocyte-granulosa communication.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## Adrenal Clinical

### OC7.1

**Progression-free survival without treatment of malignant pheochromocytoma and paraganglioma at one year**

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Pheochromocytoma and paraganglioma are defined as malignant by the occurence of metastasis in extra paraganglionic sites. The natural evolution of these tumors remains unknown.
The aim of our retrospective study was to define the progression-free survival (PFS) of untreated Malignant Pheochromocytoma and Paraganglioma (MPP) at 12 months (12 m-PFS) and to look for prognostic markers of 12 m-PFS. We analyzed clinical parameters of patients followed within 8 centers of the INCa-COMETE and GTE-RENATEN French networks. Patients were included whether they had a diagnosis of metastasis between 01/01/2001 and 01/01/2011. Progression was defined according to RECIST 1.1 for morphologic imaging and by the appearance of new lesions on scintigraphic imaging.
Ninety-three files were reviewed on site. Among them, 57 untreated patients for whom a follow-up strategy was first proposed, were included (34 males, 23 females), with a median age of 51 years (17–80). Median time since initial diagnosis was 17 months (0–341). Primary tumors were 47% pheochromocytomas ($n=27$), 40% abdominal paragangliomas ($n=23$) and 13% head and neck paragangliomas ($n=7$). A pheochromocytoma/paraganglioma genetic test was performed in 54 patients. None mutation was identified in 27 of the patients (50%) whereas 20 patients (37%) carried a SDHB mutation, one patient had neurofibromatosis type 1, one von Hippel-Lindau disease, two a SDHD- and two a SDHC-hereditary paraganglioma. Median follow-up was 2.4 years, (range = 0.4–5.7). 12 m-PFS without treatment was assessed to 46% (33–59). No significant prognostic markers of 12m-PFS were found. Untreated patients presented more frequent peritoneal metastases and less frequent bone metastases than directly-treated patients ($P<0.05$).
Forty-six percent of MPP have a stable disease without treatment at 1 year. If patients are asymptomatic, we recommend a first imaging work-up at 3 months, a wait-and-see policy and/or local treatments in the absence of progression on imaging.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC7.2

**Activation of the PKA pathway triggers formation of an illicit serotonergic regulatory loop in primary pigmented nodular adrenal disease (PPNAD) tissues associated with Cushing's syndrome**

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In the normal adrenal gland, serotonin (5-HT) stimulates cortisol secretion through activation of 5-HT4 receptors whereas, in some macronodular adrenal hyperplasia tissues, the corticotropic effect of 5-HT is mediated by ectopic 5-HT7 receptors. The aim of the present study was to investigate the role of 5-HT in the control of cortisol secretion in PPNAD tissues from 12 patients by using molecular, immunohistochemical and pharmacological approaches. RT-PCR studies revealed overexpression of the genes encoding tryptophan hydroxylase, the key enzyme of 5-HT synthesis, and the 5-HT4 and 5-HT7 receptors, in comparison with normal adrenals. Tryptophan hydroxylase was detected in hyperplastic nodules by immunohistochemistry. 5-HT dose-dependently increased cortisol production by PPNAD cells derived from 6 patients. The potency and efficacy of 5-HT to stimulate cortisol were higher in PPNAD than in normal adrenocortical cells. 5-HT was also able to increase expression of the CYP11B1 gene, which encodes the steroidogenic enzyme 11alpha-hydroxylase, in the PPNAD immortalized cell line LT2. Both the 5-HT4 receptor antagonist GR113808 and the 5-HT7 receptor antagonist SB269970 significantly inhibited the stimulatory effect of 5-HT on cortisol by reducing the potency and efficacy of the indolamine. These data indicate that, in PPNAD cells, the stimulatory action of 5-HT on cortisol release is mediated by both the eutopic 5-HT4 receptor and an ectopic 5-HT7 receptor. In most patients, PPNAD results from inactivating mutations of the PRKAR1A gene which cause enhancement of the PKA pathway. In the adrenocortical cell line H295R, inhibition of PRKAR1A gene expression markedly stimulated expression of tryptophan hydroxylase, 5-HT4 and 5-HT7 receptor mRNAs. Taken together, our results show that activation of the PKA pathway triggers formation of an illicit serotonergic regulatory loop in PPNAD tissues associated with Cushing's syndrome. This work was supported by INSERM U982, the Carney Complex network and ANR Genopat 2008.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC7.3

**Ribonucleotide reductase large subunit (RRM1) gene expression predicts efficacy of adjuvant mitotane in adrenocortical cancer**

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Purpose
Mitotane is the reference systemic therapy for adrenocortical carcinoma (ACC), but its mechanism of action and possible predictors of treatment response remain poorly defined. Our aim was to evaluate the gene expression of ribonucleotide reductase large subunit 1 (RRM1) and excision repair cross-complementation group 1 (ERCC1) in ACC as potential biomarkers for clinical outcome and response to mitotane.
Experimental design
Forty-five and 47 tissue samples from two cohorts (Orbassano, Italy; Wurzburg, Germany) of completely resected ACC were centrally analyzed using Real Time PCR for RRM1 and ERCC1 expression. Fifty-four patients received surgery alone and 38 received adjuvant mitotane after surgery. Clinical and pathological features were highly comparable in the two series. H295R and SW-13 ACC cell lines were also used for pharmacological tests.
Results
ERCC1 gene expression was not associated to clinical outcome. In contrast, high RRM1 gene expression was associated to shorter disease-free and overall survival at both univariate and multivariate analysis. In patients with low RRM1 gene expression adjuvant mitotane was associated with improved disease free survival, whereas this effect was lost in cases expressing high RRM1. *In vitro* mitotane induced strong up-regulation of RRM1 transcription (up to 25-fold increase) in mitotane-insensitive SW-13 cells but not in mitotane-sensitive H295R cells.
Conclusion
Our *in vitro* and *in vivo* data indicate that RRM1 gene expression is functionally associated to mitotane sensitivity and represents the first biomarker in ACC predicting response to adjuvant mitotane.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC7.4

**Differential diagnosis of primary aldosteronism by peripheral plasma levels of 18-oxo-cortisol: a noninvasive method with a high specificity**

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18-Oxocortisol (18-oxoF) is a derivative of cortisol (F) that is produced by aldosterone synthase (CYP11B2), and its clinical usefulness has not been clarified. We prospectively measured 18-oxoF, using highly sensitive liquid chromatography-tandem mass spectrometry (LC/MS/MS), in peripheral plasma of patients with primary aldosteronism (PA) who underwent adrenal venous sampling (AVS) to differentiate aldosterone producing adenoma (APA) from bilateral hyperplasia. The study was performed in 116 patients with surgically-proven APA and 121 patients with bilateral hyperplasia diagnosed by AVS which was successfully performed. The levels of 18-oxoF were significantly higher in the peripheral plasma from the patients with APA than that from the patients with bilateral hyperplasia (bilateral hyperaldosteronism (BHA) diagnosed by AVS and surgically-proven idiopathic hyperaldosteronism (IHA) were shown in Fig. 1). The cutoff value (4.7 ng/dl) of 18-oxoF levels was calculated by ROC analysis with sensitivity of 0.64 and specificity of 0.97. In the cases with CT-positive unilateral adrenal masses, the cutoff value (4.6 ng/dl) of 18-oxoF levels was calculated by ROC analysis with sensitivity of 0.73 and specificity of 0.97. In these cases, the 18-oxoF levels in 60 cases (80%) with APA were higher than 4.6 ng/dl, and while, those in 29 cases (97%) with bilateral hyperplasia and those in eight cases (89%) with contralateral CT-negative micro-APA were lower than 4.6 ng/dl. Thus, according to peripheral blood levels of 18-oxo-cortisol, 80% of APA patients with unilateral masses might be able to undergo ipsilateral adrenalectomy without AVS.18-oxoF levels can be a clinically useful biomarker for differentiating APA from bilateral hyperplasia.

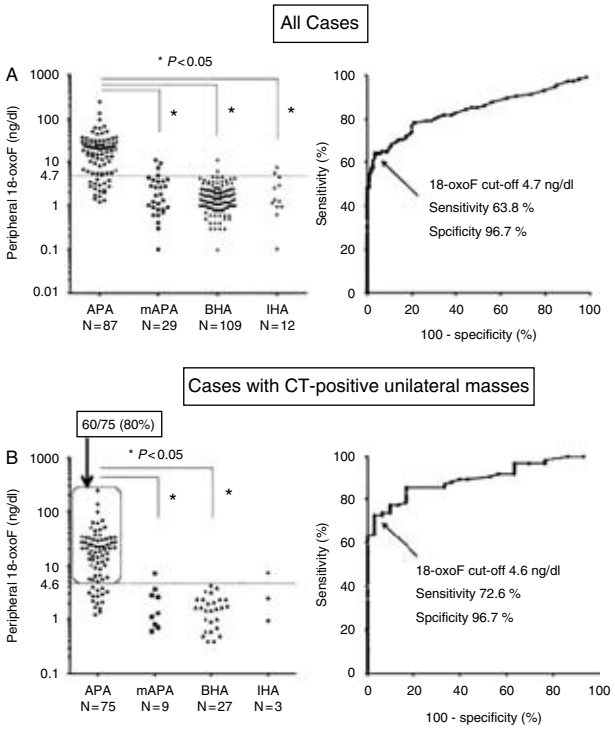


**Figure 1** ROC of 18oxoF plasma levels for the identification of APA versus bilateral hyperplasia in all cases (A) and cases with CT-positive unilateral adrenal masses (B): APA; cases with surgically-proven ipsilateral aldosterone producing CT-positive adenoma (A, B): mAPA (A); cases with CT-negative aldosterone producing adenoma: mAPA (B); cases with CT-positive non-functioning adenoma and contralateral CT-negative aldosterone producing adenoma: BHA; bilateral hyperaldosteronism diagnosed by AVS: IHA; surgically-proven idiopathic hyperaldosteronism.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC7.5

**Continuous subcutaneous hydrocortisone infusion (CSHI) as replacement therapy in Addison's disease (AD)**

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Background
Conventional glucocorticoid replacement therapy is unphysiological, and does not restore quality-of-life in AD. Here we evaluated the dosing regimens and glucocorticoid metabolism in 10 patients undergoing 24 h sampling during oral replacement therapy and CSHI.
Design, Subjects, Measurements
We set up a cross-over randomised multi-centre clinical trial to evaluate dosage and effects of CSHI in 40 Scandinavian AD patients, comparing 3 months of thrice daily oral hydrocortisone with 3 months on CSHI (NCT 01063569). Doses were adjusted according to serum cortisol 4 h after the morning dose (weight-adjusted oral treatment), and salivary cortisol (body-surface-area(BSA)-adjusted CSHI). Ten of the patients were admitted for 24 h blood sampling to determine whether circadian cortisol rhythm was restored, and to analyse the effects on ACTH levels.
Results
Oral treatment doses (median 0.24 mg/kg/day, range 0.2–0.5; median 10.4 mg/BSA/d, range 7.9–19.3) were slightly lower than CSHI doses (median 0.31 mg/kg/24 h, 0.26–0.5; median 13.1 mg/BSA/24 h, range 10.1–20.1).
Oral treatment yielded high post-dose serum cortisol peaks, whereas CSHI created a smooth circadian cortisol curve with median serum cortisol 472 nmol/L (interquartile range 467–589; 08 h), 336 nmol/L (291–415; 14 h), 141 nmol/L (126–212; 20 h) and 60 nmol/L (42.5–78.2; 02 h). The cortisone rhythm resembled the cortisol rhythm; the principal difference between the treatments was non-detectable levels during night-time on oral treatment, as opposed to a rise in the levels from 02 h on CSHI. Compared with oral treatment CSHI resulted in significantly lower ACTH levels from 06 h (median 49.1 pmol/L (interquartile range 9.0–271) vs 4.0 (1.0–28.6), $P=0.011$) to 12 h (21.5 pmol/L (2.1–91.6), vs 1.8 (1.0–5.9), $P=0.008$). Three patients on CSHI had morning ACTH values below the reference range.
Conclusion
CSHI safely established circadian cortisol rhythm in AD patients, which resulted in more normal ACTH levels. We suggest that with physiological replacement therapy ACTH is the appropriate effect parameter for individualisation of dose.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC7.6

**Progressively increased patterns of subclinical cortisol hypersecretion in adrenal incidentalomas differently predict major metabolic and cardiovascular outcomes: a large cross-sectional study**

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Background
Subclinical Cushing's syndrome (SCS) is defined as alterations in hypothalamic–pituitary–adrenal axis without classic signs or symptoms of glucocorticoid excess. It is still controversial whether SCS leads to metabolic and cardiovascular diseases, and if the degree of subclinical hypercortisolism may predict these clinical outcomes.
Aim
To evaluate the prevalence of hypertension, type-2 diabetes (T2D), coronary heart disease (CHD), ischemic stroke, osteoporosis, and osteoporotic fractures, and the relationship of these outcomes to increasing patterns of subclinical

hypercortisolism, in patients with adrenal adenomas classified as non-secreting (NSA) and SCS.
Methods
Using the 1-mg dexamethasone suppression test (DST) as primary diagnostic tool, 348 patients were classified as above: 203 were defined NSA and 19 SCS, using the most stringent cut-off values ($<50$ and $>138$ nmol/l respectively). Patients with cortisol post-DST between 50 and 138 nmol/l were considered intermediate phenotypes and classified as minor ($n=71$) and major ($n=55$) using plasma ACTH and/or urinary free cortisol as additional diagnostic tools.
Results
SCS patients showed higher prevalence of T2D, CHD, osteoporosis, and osteoporotic fractures, respect to NSA. Intermediate phenotypes showed also higher prevalence of CHD and T2D, respect to NSA. The prevalence of all clinical outcomes was not different between intermediate phenotype patients, which were therefore considered as a single group (IP) for multivariate logistic regression analysis. This analysis was performed to evaluate the relationships between potential risk factors (including the cortisol secreting pattern) and the adverse clinical outcomes: both IP and SCS secreting patterns showed a significant association with CHD (odds ratio – OR 4.09; 95% CI 1.47–11.38 and OR 6.10; 95% CI 1.41–26.49 respectively), independently of other potential risk factors. SCS was also independently associated with osteoporosis (OR 5.94; 95% CI 1.79–19.68).
Conclusion
Patterns of increasing subclinical hypercortisolism in adrenal adenomas are associated with increased prevalence of adverse metabolic and cardiovascular outcomes, independently of other potential risk factors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Bone

## OC8.1

### Hypophosphatasia: enzyme replacement therapy (ENB-0040) decreases TNSALP substrate accumulation and improves functional outcome in affected adolescents and adults

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Hypophosphatasia (HPP), a heritable metabolic bone disease, results from low alkaline phosphatase (TNSALP) activity. Inorganic pyrophosphate (PPi), an inhibitor of mineralization, and pyridoxal 5′-phosphate (PLP), are substrates that accumulate in HPP. There is no approved therapy. ENB-0040, a bone-targeted, recombinant, human TNSALP improves skeletal mineralization in affected infants and children with HPP.
Objective
Evaluate substrate levels and six-minute walk test (6MWT) following 24 weeks of treatment.
Methods
Six adolescents and 13 adults with HPP (mean age 42 years (14–68)) were randomized in an open-label, multicenter, concurrent control study of the safety and efficacy of ENB-0040. Patients received no treatment, 2.1 or 3.5 mg/kg per week via daily SUBCUTANEOUS injections of ENB-0040.
Results
Statistically significant decreases in PPi and PLP ($P=0.002$ and $P=0.009$, respectively) occurred in the treated groups (2.1 and 3.5 mg/kg per week) vs non-treated group. Serum PPi levels decreased from baseline levels of 5.5 to 3.5 μM at week 24 (2.1 mg/kg per week) and from 5 μM at baseline to 2.8 μM at week 24 (3.5 mg/kg per week). Serum PLP levels decreased from 324 ng/ml at baseline to 69 ng/ml at week 24 (2.1 mg/kg per week) and from 603 to 38 ng/ml at week 24 (3.5 mg/kg per week).
At baseline, patients averaged 349 m (6 – 620 m) during the 6MWT, with 10/19 requiring assistive devices during testing. At week 24, treated patients improved $+26$ m vs no improvement ($-14$ m change) for controls. Of 12 patients with functional impairment (BL 6MWT was 25%–75% of normal), nine improved (8/9 treated, 1/3 no treatment). The mean improvements for the 2.1 and 3.5 mg/kg per week groups were $+35.4$ m (n=5) and $+43.5$ m ($n=4$) respectively.
Injection site reactions occurred in seven patients, but did not cause discontinuation of treatment. Six serious adverse events (SAEs) were reported among 3 patients, two control (no treatment) patients had four of the SAEs. No SAEs were associated with ENB-0040.
Conclusion
ENB-0040 significantly decreases TNSALP substrates and improves function in adolescents and adults with HPP.
Declaration of interest
The authors declare that there is a conflict of interest.
Funding
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## OC8.2

### Comparison of high protein and normal protein weight loss diets on bone density in overweight post-menopausal women

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Introduction
The role of dietary protein in maintaining bone health is controversial. Traditionally the acidifying effect of high dietary protein (HP) has been thought to promote calcium loss from bone and hence hypercalciuria and so was considered harmful compared to a normal protein (NP) diet. However more recently the anabolic effect of increased dietary protein and its effect in increasing calcium absorption have been appreciated. There are no long term prospective clinical trials comparing high and normal protein diets in maintaining bone health.
Methods
We randomized 323 overweight (BMI $>$ 27 kg/m$^2$) post-menopausal women aged 40-70 to an isocaloric HP (approximately 100 g/day) or NP (approximately 67 g/day) weight loss diet for 2 years. We had excluded subjects with impaired bone health eg primary hyperparathyroidism, vitamin D insufficiency, previous low impact fracture, hip t score $<-2.0$ or using medications such as hormone replacements or steroids.
Results
Subjects were equally matched for age, weight, serum parathyroid hormone, vitamin D, cross-laps and osteocalcin at baseline. 69 of 164 HP subjects and 68 of 159 NP subjects completed the 2 year study. As expected there was matched weight loss across both groups with the HP group losing $9.6\pm1.6$ kg vs NP $10.3\pm1.9$ kg (NS). There was no significant difference in bone loss after 2 years at either hip, forearm or spine between groups (Table 1). ANCOVA analysis showed baseline bone density at any site and to a lesser extent baseline Vitamin D was the only significant predictors of bone density at that site at 2 years. Baseline weight, parathyroid hormone, cross-laps and 24 hour urea were not significant predictors.
Conclusion
The amount of dietary protein does not significantly affect bone health in post-menopausal women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details are unavailable.

**Table 1** Comparison of bone loss after 2 years of HP or NP diet at different sites

| Bone Loss at 24 months | L2-4 | Forearm | Hip |
|---|---|---|---|
| HP | $-0.7\pm0.6\%$ | $-1.2\pm0.4\%$ | $-1.2\pm0.3\%$ |
| NP | $-1.8\pm0.5\%$ | $-1.0\pm0.3\%$ | $-1.5\pm0.3\%$ |
| Significance | NS | NS | NS |

## OC8.3

### Profiling insulin like factor 3 (INSL3) signaling in human osteoblasts

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Insulin-like factor 3 (INSL3) is a testis-specific, Leydig cell derived hormone, which we recently demonstrated to play a role in bone metabolism. Young men with mutations in the gene for the INSL3 receptor (Relaxin family peptide 2, RXFP2) are at risk of reduced bone mass and osteoporosis. Consistent with the human phenotype, bone analyses of Rxfp2$^{-/-}$ mice showed decreased bone

volume, alterations of the trabecular bone, reduced mineralizing surface, bone formation, and osteoclast surface. The aim of this study was to elucidate the INSL3/RXFP2 signaling pathways and targets in human osteoblasts, by studying the effects of INSL3 on different molecular, cellular, and genetic mechanisms.
We analyzed the effects on alkaline phosphatase (ALP) production, protein phosphorylation, intracellular calcium, gene expression, and mineralization. INSL3 induced a significant increase in ALP production, and Western blot and ELISA analyses of multiple intracellular signaling pathway molecules and their phosphorylation status revealed that the MAPK was the major pathway influenced by INSL3, whereas it does not modify intracellular calcium concentration. Quantitative Real Time PCR and western blotting showed that INSL3 regulates the expression of different osteoblast markers. Alizarin red-S staining confirmed that INSL3-stimulated osteoblasts are fully differentiated and able to mineralize the extracellular matrix.
Together with previous findings, this study demonstrates that INSL3 is involved in bone metabolism by acting on the MAPK cascade and stimulating transcription of important genes of osteoblast maturation/differentiation, matrix deposition and osteoclastogenesis, and it stimulates mineralization.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC8.4

**Radiological vertebral fractures in hospitalized elderly patients with heart failure**

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Heart failure (HF) has been associated with bone loss and an increased risk of fragility fractures. Indeed, most of literature data on fractures were based on an historical and clinical approach focused on the identification of peripheral fractures, whereas the risk of vertebral fractures in this clinical setting is still unclear. In this study, we aimed at evaluating the prevalence of radiological vertebral fractures in 791 consecutive elderly and hospitalized patients (372 females and 419 males; median age: 75 years.; 190 patients with HF). The research focused on fractures of the thoracic spine, which were identified using chest X-ray routinely performed in the diagnostic work-up of HF. Exclusion criteria were: i) neoplastic diseases in progression; ii) systemic autoimmune diseases; iii) chronic therapy (>3 months) with oral and parenteral glucocorticoids; iv) chronic immobilization; v) trauma; vi) previous clinical history of HF without specific symptoms at the time of enrollment. Vertebral fractures were found in 170 patients (21.5%), the prevalence being significantly higher in patients with HF as compared to those without HF (38.9 vs 16.0%; $P<0.001$). The association between HF and vertebral fractures remained statistically significant (odds ratio 2.20, 95% C.I. 1.34–3.83) even after adjustment for age, sex, loop diuretic therapy, anticoagulant therapy, proton pump therapy, coexistent chronic obstructive pulmonary disease, diabetes mellitus and chronic liver diseases. In patients with HF, vertebral fractures were significantly correlated with duration of HF (odds ratio 1.09, 95% C.I. 1.06–1.10; $P<0.001$), female sex (odds ratio 5.85, 95% C.I. 1.90–17.4; $P=0.01$), ischemic heart disease (odds ratio 2.55, 95% C.I. 1.03–6.29; $P=0.04$) and treatment with anti-osteoporotic drugs (odds ratio 2.87, 95% C.I. 1.09–7.55; $P=0.03$).
Our study shows for the first time that patients suffering from HF are at higher risk of developing vertebral fractures as compared to age/sex matched subjects selected in the same clinical environment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## OC8.5 The Young Investigator Winner

**Pathogenetic chromosomal rearrangements in a large series of patients with pseudohypoparathyroidism type I**

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Pseudohypoparathyroidism (PHP) type I includes two major subtypes, Ia and Ib. About 70% of Ia patients, characterized by Albright hereditary osteodystrophy and multihormone resistance (PTH/TSH/GHRH/gonadotropins), carry point mutations in GNAS exons encoding Gsα. About 60% of Ib patients, with hormone resistance limited to PTH and TSH, have methylation defects within GNAS locus (sporadic or genetic-based). Recently, methylation defects were detected in pts with Ia phenotype, suggesting a molecular overlap between the two forms. Despite advances in the determination of molecular mechanisms underlying PHP, 30–40% of patients lack a molecular diagnosis. Moreover, it is unclear whether apparently sporadic imprinting defects are rather secondary to genetic defects. In order to simultaneously investigate for GNAS and STX16 deletions/duplications and for GNAS imprinting status (A/B-AS-NESP-XL DMRs), we performed methylation specific multiplex ligation-dependent probe amplification (MS-MLPA) in 96 patients (Ia=48/Ib=48), all negative for Gsα mutations. In 15 patients we detected rearrangements at GNAS and/or STX16 genes (known to be implicated in autosomal dominant Ib). In particular: i) of 8 PHP-Ia patients: two patients with apparent methylation defects carry a deletion of the entire GNAS locus, 1 had A/B loss of methylation (LoM) caused by STX16 deletions, five had deletions encompassing Gsα exon 1; ii) of 7 PHP-Ib patients: five had A/B LoM associated with STX16 deletions and two showed both extensive methylation defects and deletions within the AS region. In conclusion: a) MLPA proved to be a reliable method to detect genetic abnormalities associated with PHP-I, both known and novel to the literature; b) PHP-Ia and Ib may be caused by GNAS submicroscopic structural mutations; iii) All PHP-I patients negative for Gsα gene mutations should be considered for further molecular investigations to optimize genetic counselling. Ongoing studies are aimed to characterize the newly detected deletions and to investigate the function of the involved regions.

| Patologia | Ctrl | AD–Ib | Ia | | | Ib | | |
|---|---|---|---|---|---|---|---|---|
| ID pz | wt | 44 | 16 | 25 | 31 | 45 | 55 | 67 |
| STX16 Exon 01 | 0.96 | 0.97 | 0.99 | 0.98 | 0.95 | 0.96 | 1.17 | 1.12 |
| STX16 Exon 03 | 1.01 | 1.01 | 1.04 | 0.68 | 0.95 | 0.54 | 0.98 | 1.29 |
| STX16 Exon 05 | 0.99 | 0.52 | 1.03 | 0.61 | 1.17 | 0.48 | 0.89 | 1 |
| STX16 Exon 06 | 1 | 0.44 | 1.03 | 0.54 | 1.1 | 0.48 | 0.87 | 1.06 |
| STX16 Exon 08 | 1.02 | 1.11 | 1.04 | 0.55 | 1.11 | 0.55 | 0.95 | 1.15 |
| NESP Exon 01A | 0.96 | 0.98 | 1.06 | 0.58 | 1.01 | 1.01 | 0.89 | 1.01 |
| NESP Exon 01B | 1.04 | 1.16 | 0.7 | 0.67 | 1.01 | 1.05 | 1 | 0.78 |
| NESP Exon 01C | 1.05 | 0.86 | 0.7 | 0.5 | 1.11 | 0.91 | 0.87 | 0.84 |
| NESPAS Exon 01A | 0.94 | 0.98 | 1.04 | 0.57 | 1.01 | 1 | 1.1 | 1.23 |
| NESPAS Exon 01B | 1.08 | 0.86 | 0.76 | 0.6 | 1.19 | 1.09 | 0.57 | 0.43 |
| NESPAS Intron 01 | 0.99 | 1.07 | 0.97 | 0.52 | 0.97 | 1.03 | 1.29 | 1.09 |
| GNASXL Exon 01A | 0.95 | 1.11 | 1.06 | 0.58 | 1.43 | 1.03 | 0.96 | 0.82 |
| GNASXL Exon 01B | 0.98 | 0.95 | 1 | 0.6 | 1.08 | 1.19 | 1.03 | 0.87 |
| GNASXL Exon 01C | 1 | 1.03 | 0.99 | 0.65 | 0.96 | 1.28 | 0.99 | 0.75 |
| GNASXL Exon 01D | 1.02 | 0.95 | 0.95 | 0.6 | 1.4 | 0.94 | 1 | 1.1 |
| GNAS Exon 01A | 1.04 | 1.14 | 0.58 | 0.52 | 0.91 | 1.01 | 1.3 | 0.78 |
| GNAS Exon 01A | 0.98 | 1.11 | 0.62 | 0.55 | 1.06 | 1.07 | 1.11 | 0.89 |
| GNAS Exon 01A | 0.96 | 0.95 | 0.47 | 0.52 | 0.79 | 1.03 | 1.44 | 0.77 |
| GNAS Exon 01B | 0.98 | 1.09 | 0.53 | 0.5 | 0.87 | 0.98 | 1.17 | 0.98 |
| GNAS Exon 02 | 0.99 | 0.99 | 1 | 0.6 | 1.13 | 1 | 1.06 | 1.2 |
| GNAS Exon 03 | 1.05 | 1.06 | 0.97 | 0.46 | 0.88 | 0.98 | 0.95 | 0.96 |
| GNAS Exon 04 | 1.04 | 0.95 | 1 | 0.5 | 1.14 | 0.95 | 1.03 | 1.13 |
| GNAS Exon 06 | 1.05 | 1.02 | 0.99 | 0.54 | 0.87 | 1.02 | 1.03 | 1.17 |
| GNAS Exon 07 | 1.01 | 0.93 | 1.03 | 0.47 | 1.15 | 1.07 | 0.79 | 0.88 |
| GNAS Exon 09 | 1.01 | 1.02 | 1.02 | 0.48 | 1.24 | 0.91 | 1.01 | 1.19 |
| GNAS Exon 11 | 1.03 | 0.97 | 0.98 | 0.56 | 1.15 | 1 | 1.06 | 1.01 |
| GNAS Exon 13 | 1.05 | 1.27 | 1.03 | 0.57 | 1 | 0.98 | 0.86 | 1.04 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC8.6

**Simultaneous gain and loss of methylation at imprinted loci in a subset of patients with pseudohypoparathyroidism type 1b and GNAS epimutations**

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The majority of patients affected with pseudohypoparathyroidism type 1b (PHP-1b) display loss of imprinting (LOI) encompassing the GNAS locus responsible for decreased Gsa expression in target tissues and PTH resistance. In other imprinting disorders like Silver-Russell, Wiedemann-Beckwith syndromes or transient neonatal diabetes mellitus due to LOI of the imprinted 11p15 and 6q24 regions respectively, we and others have shown that the LOI may spread over to other imprinted loci in some patients. These findings suggested that multilocus imprinted disorders may result from dysfunction(s) of transacting factors.

Therefore, we hypothesized that similar mechanisms may underlie PHP-1b.

We have investigated, in 63 patients affected with PHP-1b, the methylation pattern of six human imprinted loci –in addition to GNAS- ZAC1, PEG1/MEST, ICR1 and ICR2 at 11p15, SNRPN and DLK1/GTL2 IG-DMR.

We found for the first time multilocus imprinting defects in five patients affected with PHP-1b and broad epigenetic changes at the GNAS locus. We observed both gain of methylation at the maternally PEG1/MEST ($n=3$) and at the paternally DLK1/GTL2 IG-DMR ($n=1$) methylated loci and loss of methylation at the PEG1/MEST locus ($n=1$). We propose that the mechanism causing the multilocus imprinting defects in PHP-1b differs from that of other imprinting disorders harboring exclusively loss of methylation in patients with multilocus defects.

Thereby, the epidominance assumption claiming that phenotypes are ruled by the most severely affected imprinted locus seems confirmed by our study, as we did not find clinical differences between patients affected with PHP-1b with or without multilocus defects.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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# Endocrine tumours & translation

## OC9.1 The Young Investigator Winner

**Temozolomide therapy for progressive metastatic paraganglioma/ pheochromocytoma: SDHB mutation as a prognosis biomarker for efficacy**

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Malignant pheochromocytoma and paraganglioma (PPGLs) are rare diseases with a heterogeneous behaviour. 131I-MIBG therapy and the cyclophosphamide-dacarbazine-vincristine chemotherapy regimen (CVD) constitute the most popular options in the metastatic setting. We have investigated the antitumor effect of temozolomide (TMZ), in patients with metastatic PPGLs. Efficacy was primary endpoint, safety and identification for prognosis factors of response were secondary endpoints.

Fifteen consecutive patients with progressive ($n=14$) or symptomatic ($n=1$) metastatic PPGLs received TMZ therapy. There were 12 men (80%); median age was 43 years. Germline mutations were screened: 10 patients harbored SDH B mutation and 5 patients had no mutation. Median number of cycle was 7, mean dose intensity for each cycle was 171 mg/m$^2$ per day (95% CI 160.7–181) for five days of a 28-days cycle.

The most frequent all grade toxicities included asthenia, nausea and anemia. Grade three toxicities were lymphopenia ($n=2$) and hypertension ($n=1$). According to RECIST 1.1 criteria or F18-FDG-PET evaluation, there was 5 (33%) partial responses, 8 (54%) stable diseases and 2 (13%) progressive diseases. With a median follow up of 29 months (range 6.4–37.9), median overall survival was not reached and median progression free survival (PFS) was 9.6 months.

Partial responses were observed in 4 out of 10 SDHB mutated patients but in no sporadic case. Patients with SDH B mutations or with no mutation had a median PFS of 16.7 and 2.6 months, respectively (log-rank, $P=0.013$); Patients with SDH B mutations or with no mutation had a 12-month PFS of 60 or 0% respectively. Methyl guanine methyl transferase (MGMT) expression was analyzed in four patients, including 3 responders and, was found negative.

This study demonstrates for the first time the antitumor efficacy of TMZ in a large series of metastatic PPGLs as well as the potential role for SDH B mutation as a prognosis biomarker of response.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC9.2

**GH protects breast cancer cells from chemotherapy by blocking cytotoxic-induced apoptosis in estrogen receptor negative breast cancer cells**

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Context

GH and insulin-like growth factor (IGF1) play important roles in breast cancer (BC) development. IGF1 has been shown to importantly influence estrogen effects on BC, suggesting that estrogen receptors (ER) may mediate also GH effects. We previously demonstrated that GH and IGF1 protect the ER positive BC-derived MCF7 cell line towards the cytotoxic effects of doxorubicin (D), independently of IGF1.

Aim of the study

Evaluate whether this holds true also in the ER negative BC-derived MDA-MB-231 cell line and in the normal MCF-12A breast cell line. In addition, we investigated the possible mechanisms implicated in the protective action of GH towards the cytotoxic effects of D.

Results

GH protects MDA-MB-231 cells from the cytotoxic effects of D but does not influence MCF-12A cell viability. The GH receptor antagonist Pegvisomant (Peg) reduces GH-induced DNA synthesis also in MDA-MB-231 cells. In addition, in both MDA-MB-231 and MCF7 cells GH does not revert D-induced G2/M accumulation, but significantly reduces basal and D-induced apoptosis, an effect blocked by Peg. GST activity is not implicated in the protective effects of GH, while D-induced apoptosis depends on JNK activation and GH reduces both basal and D-stimulated JNK transcriptional activity and phosphorylation.

Conclusions

In human BC cell lines GH directly promotes resistance to apoptosis induced by chemotherapic drugs independently of ER expression by modulating JNK, further supporting the hypothesis that GH excess might hamper cytotoxic BC treatment and that GH receptor antagonism may represent a new therapeutic pathway also in ER negative BC.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC9.3

**The central role of estrogen receptor α in IGF2 dependent adrenocortical carcinoma (ACC) cell proliferation suggests the use of selective estrogen receptor modulators (SERMs) for the treatment of ACC**

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Adrenocortical Cancer (ACC) is characterized by an increased production of insulin-like growth factor 2 (IGF2) and by estrogen receptor (ER) α

up-regulation. Aim of this study was to define IGF2 and estrogen signaling interactions in ACC, in order to give new indications for a better therapy. To this aim we used H295R cells and human ACC tissues which display common features: IGF2 activation of downstream effector pathways and over-expression of estrogen-related genes including ERα and aromatase, the enzyme required for estrogen production. We demonstrated that IGF2 controls expression of steroidogenic factor 1 (SF-1), that in turn increases aromatase transcription. 17β-estradiol ($E_2$) bound to ERα up-regulated IGF1R expression as a consequence of increased pCREB binding to IGF1R promoter. On the other hand, $E_2$ and PPT (a selective ERα agonist) stimulated IGF1R phosphorylation and caused ERK1/2 and AKT activation. In the $E_2$-dependent IGF1R transactivation we found the involvement of the scaffold protein PELP1/MNAR which acted as an adaptor protein for connecting ERα-IGF1R-Src-ERK1/2-AKT. These data suggest the ability for estrogens to activate IGF1R-downstream pathways even in the absence of IGF2. Furthermore, ERα regulated $E_2$- and IGF2-induced cyclin D1 expression, a gene controlling cell cycle progression overexpressed in ACC. Silencing ERα significantly blocked the ability of $E_2$ and IGF2 to induce cell proliferation more effectively than an anti-IGF1R monoclonal antibody, as used in phase I clinical trials. We also utilized H295R cells to generate xenografts in athymic nude mice to demonstrate the ability of SERMs such as tamoxifen to control ACC growth in vivo. Tamoxifen therapy significantly inhibited tumor growth further indicating that ERα contributes to the pathogenesis of ACC. These findings establish a critical role for ERα in IGF2-dependent ACC proliferation and provide rationale for targeting ERα (or PELP1) to control the proliferation of adrenocortical carcinoma.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC9.4

**Functional characterization of mutations in the multiple endocrine neoplasia type 1 (MEN1) gene suggest therapeutic strategies**

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Menin is the product of the multiple endocrine neoplasia type 1 (MEN1) gene which when inactivated causes an autosomal dominant disorder characterized by tumors of the parathyroids, endocrine pancreas and anterior pituitary.

We identified an MEN1 splice-site mutation leading to a menin Δ(184–218) mutant having an in-frame deletion of 35 amino acids, but otherwise of wild-type sequence. The transfected mutant was well expressed, and like wild-type menin, interacted directly with the transcriptional regulators JunD and NF-κB and inhibited their activities. However, the mutant had lost the normal interaction with Smad3 and was defective in mediating TGF-β-stimulated Smad3 transcriptional activity, stimulation of the cyclin dependent kinase inhibitors (CDKIs), p15 and p21, and cytostatic activity. Thus the mutant was stable, had selective loss of TGF-β signaling and growth inhibition and, importantly, identified the menin/Smad3 interacting region on a homology model of the human menin structure. These studies suggest the menin/Smad3 interface as a potential therapeutic target.

We functionally characterized a panel of 16 menin missense mutants, including W423R and S443Y identified in new MEN1 families and that are poorly expressed. Proteasome inhibitors, MG132 and PS-341, and inhibition of the chaperone, heat shock protein 70 (Hsp70), or the ubiquitin ligase, COOH-terminus of Hsp70-interacting protein (CHIP), by specific siRNAs, restored the levels of the mutants whereas that of wild-type menin was unaffected. Inhibition of CHIP restored the ability of mutants to mediate normal functions of menin – TGF-β upregulation of p15 and p21, as well as TGF-β inhibition of cell proliferation. Potentially, targeting specific components of the proteasome chaperone pathway could be beneficial in treating a subset of MEN1 cases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## OC9.5

**Glucagon-like peptide 1 receptor (GLP-1R) imaging for the preoperative localization of benign insulinomas in 30 patients**

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Background

Although biochemical diagnosis of endogenous hyperinsulinemic hypoglycemia is straightforward, surgical removal of an insulinoma is hampered by difficulties to localize it using conventional radiological procedures (endosonography, MRI, CT-imaging techniques).

*In vitro* data suggest that human insulinoma cells exhibit a high density of GLP-1R. 111In-exendin-4 is a 111In labeled GLP-1R agonist that binds with high affinity to GLP-1R and may be helpful in localizing benign insulinomas.

Aim

To localize benign insulinomas using 111In-exendin-4 in patients with proven endogenous hyperinsulinemic hypoglycemia but no or only one suspicious lesion on conventional imaging.

Material and methods

111In-exendin-4 was administered i.v. at a dose of (≈90 MBq; 30 μg peptide) over 5 min to 30 patients (18 females, 12 males, age ± 52.6 ± 14.8 years, mean ± S.D). Whole body planar images and SPECT/CT images (single photon emission computed tomography) of the abdomen were performed at 0.5 h, 4 h, 23 h, 96 h up to 168 h post injection. Diagnosis was confirmed by histology after surgical removal.

Results

Conventional imaging (MRI, CT, endosonography) was positive in 17 patients. $^{111}$In-exendin-4 SPECT/CT detected 23 true positive benign insulinomas and five additional positive lesions (one malignant insulinoma; two islets hyperplasia; two uncharacterized lesions). True negative tests were detected in two patients (one malignant insulinoma; one islets hyperplasia). There was no false negative result. The positive predictive value was 85% the negative predictive value 100%.

Conclusion

These data suggest that *in vivo* GLP-1R imaging defines a new non-invasive diagnostic approach to successfully localize small insulinomas.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This work was supported, however funding details are unavailable.

## OC9.6

**First *in vitro* study of human gastroenteropancreatic-neuroendocrine tumors: comparative effect of octreotide and pasireotide**

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Somatostatin analogs (SSAs) such as octreotide (OCT) are currently effective in controlling most hypersecretion associated symptoms of Gastoenteropancreatic-neuroendocrinetumors (GEP-NETs). The results of the phase IIIb PROMID trial showed that OCT doubled time to progression for patients with metastatic neuroendocrine midgut tumors compared with placebo. SSAs act on different intracellular pathways through different somatostatin receptor (Sst) subtypes. While OCT is mainly an Sst2 agonist, Pasireotide (PAS) is a new universal ligand, binding to all Sst (except Sst4). Our aim was to compare on primary culture of human GEP-NETs the effect of OCT and PAS on cell viability, hormonal secretion and transduction pathways.

Methodology

This study was performed on 12 human GEP-NETs *in vitro*. The expression of SST subtypes mRNAs was evaluated and the trafficking of SST2 in presence of SSAs was followed by confocal microscopy.

Results

1 Sst2 > Sst1 > Sst5 are expressed in all analysed GEP-NETs; 2/ Oct and PAS induced different Sst2 internalization patterns; 3/ OCT and PAS suppress cell

viability at similar levels in all analysed tumors (range 20–90%) through caspase dependant apoptosis process. 5/ Chromogranin A secretion is suppressed by both PAS and OCT in a dose dependant manner, close to the suppression observed in viability experiments. Conclusion: it is the first *in vitro* study on human GEP-NETs. PAS has a similar effect to OCT on cell viability and secretion of human GEP-NETs *in vitro*, despite a clear difference in Sst2 trafficking.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details are unavailable.

## Pituitary clinical 2

### OC10.1

**Predictors of morbidity and mortality in acromegaly: an Italian survey on behalf of the Italian study group of acromegaly**

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This study presents epidemiological data of 1512 Italian acromegalic patients who had been diagnosed from 1980 to 2002 and followed-up for more than 10 years, retrospectively collected by 24 tertiary referral centers. Data on co-morbidities and mortality were compared to those of the general Italian population obtained by the Italian National Institute of Statistic. At diagnosis median age of patients (41% M, 59% F) was 46 years, GH (mean ± S.D.) 31 ± 37 mcg/l, IGF1 744 ± 318 ng/ml (median SDS 8.53, IQR 5.82–12.34). Diabetes mellitus was reported in 16% of cases, hypertension in 33%. Older age and higher IGF1 but not GH levels at diagnosis were significant predictors of diabetes and hypertension. The patients were treated by surgery (80%), pharmacotherapy (75%), radiotherapy (18%), radiosurgery (6%) alone or in combination. The prevalence of neoplastic diseases (all causes) was significantly higher than in the general population. Older patients who had a greater delay of diagnosis and previous radiotherapy were also at higher risk to develop a neoplasm. Diabetes was a significant risk factor for neoplasm in the univariate analysis, only. At last follow-up 65% of patients had a controlled disease, with 55% off medical treatment. Multivariate analysis showed that male gender, extrasellar adenoma and high GH at diagnosis were predictors of disease persistence. Observed deaths were 61, with a standardized mortality ratio (SMR) of 1.13 (95% CI: 0.87–1.46). Mortality was significantly higher in the patients with persistently active disease (SMR 1.93; 95% CI: 1.34–2.70). Main causes of deaths were vascular diseases and malignancies with similar prevalence. Older age, higher GH at last follow-up, higher IGF1 levels at diagnosis, malignancies and radiotherapy were independent predictors of mortality.
In conclusion: basal IGF1 levels are important predictors of morbidity and mortality in acromegaly. The delay of diagnosis increases the risk to develop a neoplasm. The full hormonal control of the disease, nowadays reached in the majority of the patients, reverses the increased mortality.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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### OC10.2

**Food intake regulating hormones in adult craniopharyngioma patients**

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Introduction
Patients with craniopharyngeoma often have disturbances of the hypothalamic–pituitary axis and serious comorbidities as obesity. We hypothesized, that the pattern of hormones regulating the nutritional status is worsened in adult patients with craniopharyngioma (CP) compared to adult patients with non-functioning pituitary adenoma (NFPA).
Methods
We included 33 patients with CP ($m=16$, $f=17$, median age: 48 years (26–77)) and 33 patients with NFPA ($m=24$, $f=9$, median age: 66 years (44–80)) in the study. The prevalence of hormonal pituitary insufficiencies of adenohypophysis did not differ significantly between groups. We measured fasting glucose, insulin, leptin, serum total ghrelin, PYY and CCK. Furthermore, total body fat mass (= FM) was determined by dual-X-ray-absorptiometry.
Results
Significantly more patients with CP had diabetes mellitus ($n=7$ vs $n=1$, $P=0.027$). Patients with CP had significantly higher FM than patients with NFPA (38.48% (20.90–54.90) vs 32.48% (17.90–53.70), $P=0.017$). Moreover, glucose levels (82.5 mg/dl (68–120) vs 73.5 mg/dl (54–112), $P=0.004$) and leptin levels (15.9 µg/l (1.7–139.5) vs 9.3 µg/l (0.7–45.5), $P=0.007$) were significantly higher in CP than in NFPA. But insulin, ghrelin, PYY and CCK did not differ significantly between the two groups. When groups were divided by gender, ghrelin levels were significantly lower in female CP (132 ng/l (69–807) vs 287 ng/l (103–488), $P=0.003$).
Conclusion
Patients with craniopharyngioma have more metabolic complications including higher leptin levels and lower ghrelin levels in females than patients with NFPA. This might be caused by hypothalamic damage and not by pituitary insuffciency. Interestingly, the pattern of other food intake regulating hormones seems not be disturbed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This work was supported, however funding details unavailable.

### OC10.3

**Pathophysiology of renal calcium handling in acromegaly revisited**

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Background
Hypercalciuria is frequent in patients with acromegaly, but it is unclear how GH/insulin-like growth factor 1 (IGF1) regulate renal calcium handling. Elevated fasting plasma calcium levels despite increased glomerular filtration suggest enhanced renal calcium reabsorption.
Objective
To investigate the impact of acromegaly on phosphocalcium metabolism.
Design
Prospective sequential study (ClinicalTrials.gov Identifier: NCT00531908).
Setting
Tertiary referral medical center and clinical investigation center.
Intervention
16 consecutive patients (5F/11M) with acromegaly received a single IV infusion of 25 mg of furosemide to induce an acute increase in calcium and magnesium delivery to distal tubular segments during a high-sodium diet with stable dietary calcium, magnesium and phosphate intake.
Measurements
Baseline plasma and urine electrolytes, plasma calcitropic hormones, and furosemide-induced changes in the fractional excretion (FE) of Ca and Mg were measured before and 6 months (range: 1–12) after effective treatment of acromegaly.
Results
Serum IGF1 concentrations normalized in all the patients after acromegaly treatment. Compared with controlled acromegaly, active acromegaly was associated with significantly higher fasting plasma ($P=0.0002$) and urinary calcium ($P=0.0003$) levels, lower PTH levels ($P=0.0075$), higher calcitriol

levels ($P=0.0137$); higher phosphatemia ($P<0.0001$) and tubular phosphate reabsorption ($P=0.0002$); similar FGF23 concentrations; and a lower calciuric ($P=0.0327$) but not magnesiuric response to furosemide.
Conclusion
The IGF1-mediated and PTH-independent increase in calcitriol synthesis in acromegaly is responsible for both absorptive hypercalciuria and increased fasting plasma calcium linked to enhanced distal tubular calcium reabsorption, as shown by the selectively diminished calciuric response to furosemide, that may counteract the effect of increased glomerular filtration and prevent major urinary calcium losses. Physiologically, this calcium-retaining tubular effect of GH/IGF1 would limit calcium excretion during childhood, thus contributing to the positive calcium balance required for juvenile growth.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC10.4

**Higher glucocorticoid supplementation doses are associated with increased overall mortality in patients with non-functioning pituitary adenoma**

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Introduction
Current treatment guidelines for patients with insufficiency of the hypothalamic pituitary adrenal (HPA) axis recommend weight adjusted glucocorticoid supplementation doses to minimize risk of negative side effects (e.g. osteoporosis etc).
However, little is known on a potential dose-dependent effect of glucocorticoid supplementation on overall mortality in patients with pituitary disease. Non-functioning pituitary adenoma (NFPA) is one of the most frequent sellar pathologies. Therefore, the present study aimed at assessing whether higher glucocorticoid supplementation doses were associated with increased mortality in individuals with NFPA and HPA insufficiency.
Methods/Design
We included 105 patients (29 female, 76 male) referred to our tertiary Endocrine referral center after pituitary surgery due to NFPA and with glucocorticoid supplementation due to HPA insufficiency; 101 individuals with NFPA but without HPA insufficiency were included as comparators. Status (alive/death) and date of death were assessed per end of 2010. Mortality was assessed using Kaplan Meier methodology as well as Cox regression analysis with adjustments for age, body-weight and gender.
Results
Average age at inclusion was $57.6\pm15.0$ years, mean follow-up was $10.8\pm8.2$ years, mean hydrocortisone (HC) equivalent dose at follow-up was $22\pm13.1$ mg. Kaplan Meier survival probabilities differed significantly when comparing individuals with differing absolute HC dose (none, 5–19, 20–29, $\geq30$ mg, $P=0.011$) as well as using weight-adjusted doses ($P=0.047$). This result persisted when analyses were adjusted for age, weight, and gender with hazard ratios increasing from 1 (5–19 mg) to 1.36 (20–29 mg), and to 2.67 ($\geq30$ mg, $P$ for trend 0.036).
Conclusions
Higher glucocorticoid supplementation doses are associated with increased overall mortality in patients with NFPA and insufficiency of HPA axis. This further substantiates the importance of a balanced and adjusted glucocorticoid replacement therapy in these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## OC10.5

**Once-weekly, CTP-modified hGH (MOD-4023) is effective in growth hormone deficient adults: a phase II, dose and frequency finding study**

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Objective
GH replacement therapy currently requires daily injections, which may cause poor compliance and distress for patients. CTP-modified hGH (MOD-4023) is being developed for once-weekly administration in GH Deficient adults and children. The present study evaluated the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of MOD-4023 in GHD adults.
Design and methods
39 normalized GHDA patients currently treated with daily GH were randomized and switched to 3 dose levels of once-weekly MOD-4023 (30%, 45% or 100% of each patient's cumulative weekly hGH dose) to evaluate safety and PK/PD profile. The study was comprised of two stages. Stage I included an optimization period and 4 weeks of once-weekly subcutaneously administered MOD-4023. Stage II is an optional 16 week extension period of once weekly MOD-4023 administration to collect further safety information and confirm the results obtained in Stage I. Here we present the results of Stage I.
Results
MOD-4023 was well-tolerated and a dose dependent response of IGF1 concentration was demonstrated. In most patients, IGF1 levels were maintained within $\pm2$ SDS during the 4 weeks, without exceeding $+2$ SDS at peak levels. In two cohorts (45% and 100%) the mean IGF1 values were comparable to those obtained with daily hGH at steady state. The adverse effects reported were consistent with known hGH related side-effects, and were mostly mild. MOD-4023 was not immunogenic.
Conclusions
Once-weekly, repeated doses of long-acting MOD-4023 were safe and well tolerated in adult GHD patients. IGF1 levels were maintained within the normal range in most MOD-4023 treated patients for the entire 4 weeks of treatment.
Based on the positive results of Stage I, an estimated target dose range for a Phase III study has been established and the 16-week extension study was initiated to further confirm the estimated dose range and to provide additional safety information.
Declaration of interest
The authors declare that there is a conflict of interest.
Funding
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## OC10.6

**Second neoplasms in childhood cancer survivors with growth hormone deficiency treated or not treated with GH**

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Introduction
The relation between GH treatment and cancer is still matter of debate. Childhood cancer survivors (CCS) have a high risk to develop second benign and malignant neoplasms (SNs), mainly as a consequence of radiation therapy. Thus, in patients with GH deficiency it is difficult to discriminate the oncological risk due to GH treatment from that caused by anticancer therapy and by individual susceptibility.
Methods
We studied the prevalence of SNs in 49 CCS who developed GDH during childhood, after cancer therapy. 32 patient had a previous diagnosis of central nervous system tumor, 17 were cured for hematologic malignancies. 44 patients (90%) received radiation therapy involving the head, at doses ranging between 12 and 70 Gy. Patients were divided in two groups: GHD-treated patients ($n=25$, 18 males, 7 females) who received GH therapy during childhood for at least 12 month (mean $\pm$ S.D. $=3.9\pm1.9$ years), and GHD not-treated patients ($n=24$,

12 males, 12 females) who did not receive any GH replacement. Statistical analysis was performed using Chi-square and Student's t tests.
Results
Mean age at the time of the study was similar in the two groups (25.5±4.9; 28.3±6.7 years). Mean follow-up time was 16.7±7.0 years. In GHD-treated group, 8 patients (32%) developed 10 SNs (Table 1). In GHD not-treated group 6 patients (25%) developed 8 SNs (Table 1). None of the 5 GHD patients who did not received radiotherapy developed a second malignancy.
Conclusions
Data show that CCS with GHD are at high risk to develop SNs, mainly meningiomas. It is likely due to the very high percentage of GHD patients previously treated with radiation therapy, which is a risk factor for both GHD and SNs. Also if the difference between SNs in treated and not-trated patients is not statistically significant, these data should be considered when prescribing GH to CCS, especially in adulthood.

**Table 1** SNs in cancer survivors with GHD

| | GDH treated ($n$ = 25) | GDH not-treated ($n$ = 24) |
|---|---|---|
| Meningioma | 5 | 4 |
| Non Melanoma Skin Cancer | 3 | 3 |
| Thyroid Cancer | 1 | |
| Neurinoma | 1 | |
| Melanoma | | 1 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## Thyroid clinical 2

### OC11.1

**Growth retardation and severe constipation due to the first human, dominant negative thyroid hormone receptor alpha mutation**

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Introduction
Thyroid hormones act via receptors encoded by different genes (THRA and THRB) generating receptor subtypes (TRα1, TRβ1, TRβ2) with differing, tissue-specific expression. Resistance to Thyroid Hormone due to THRB defects is well recognised, but no THRA mutations have yet been reported. We describe the first case of human TRα-mediated thyroid hormone resistance due to a dominant negative THRA mutation.
Results
A 6-year-old female presented with lower segmental growth retardation (height < 10th centile), skeletal dysplasia (delayed bone age, femoral epiphyseal dysgenesis, delayed fusion of cranial sutures) and severe constipation. Thyroid function tests showed low/low-normal free T4 (fT4), high/high-normal free T3 (fT3), low reverse T3 (rT3) and normal TSH resulting in a markedly subnormal fT4/fT3 ratio. Heart rate, blood pressure (BP) and basal metabolic rate (BMR) were subnormal, but serum sex hormone binding globulin concentrations (SHBG), a hepatic marker of thyroid hormone action, were elevated.
Whole exome sequencing identified a heterozygous nonsense mutation (E403X) in THRA, generating a carboxyterminally truncated receptor protein which binds corepressors aberrantly and inhibits wild type receptor action in a dominant-negative manner. Thyroxine treatment suppressed TSH and normalised BMR with a further rise in SHBG; but heart rate, BP, growth retardation and intestinal dysmotility remained refractory.
Conclusions
This patient exhibits tissue-specific hypothyroidism paradoxically associated with only borderline abnormal thyroid hormone levels, synonymous with findings in TRα mutant mice. Some parameters (TSH, SHBG) responded to thyroxine treatment, but cardiac, gastrointestinal and skeletal tissues remained refractory. Such differential tissue sensitivity to thyroid hormone action, reflects preserved hormone responsiveness in TRβ-expressing tissues (e.g. hypothalamus, pituitary and liver) but resistance in TRα-expressing tissues (skeleton, gastrointestinal tract and myocardium). Recognition of hypothyroid features, but associated with a distinctive biochemical profile (subnormal fT4/fT3 ratio, low rT3), may enable future identification of additional cases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC11.2

**Subclinical thyroid disease and risk of new-onset atrial fibrillation**

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Background
It is still uncertain if subclinical thyroid disease or 'high-normal' thyroid function are risk-factors for atrial fibrillation (AF).
Objectives
To examine the risk of AF in relation to thyroid function.
Methods
Patients consulting their general practitioner from 2000–2009 in Copenhagen, Denmark, who underwent thyroid blood tests, were identified by individual-level linkage of nationwide registries. Patients with a history of thyroid disease, AF or related medication were excluded. Risk of AF was analyzed using cumulative incidence plots and Poisson regression models to gain Incidence Rate Ratios (IRR).
Results
Of 525,100 individuals in the study population (mean age 51.7 years [s.d. ±18.0]; 39.5% males) 504,113 (96.0%) were euthyroid, 1474 (0.3%) had clinical hypothyroidism, 10,679 (2.0%) subclinical hypothyroidism, 3421 (0.7%) clinical hyperthyroidism and 5414 (1.0%) subclinical hyperthyroidism. A 'dose-dependent' increased risk of AF was found in two levels of subclinical hyperthyroidism (TSH <0.1, 0.1–0.2 mU/l): IRR 1.8 [95% CI: 1.5–2.2], IRR 1.5 [1.2–2.0] and in 'high-normal' levels of euthyroidism (TSH 0.2–0.4 mU/l): IRR 1,3 [1.2–1.5]. Both clinical and subclinical hypothyroidism was associated with a lower risk of AF.
Conclusions
Subclinical hyperthyroidism and 'high-normal' thyroid function is a significant risk-factor for AF, whereas hypothyroidism is associated with decreased risk of AF.

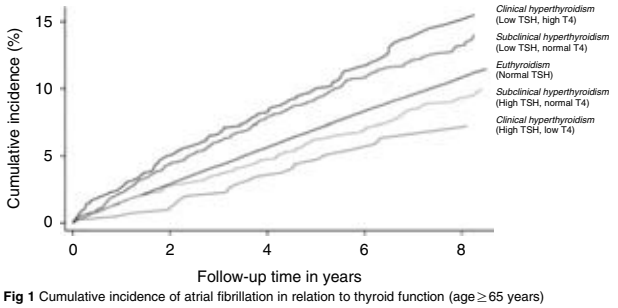

**Fig 1** Cumulative incidence of atrial fibrillation in relation to thyroid function (age≥65 years)

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC11.3

**Is overt hypothyroidism associated with increased mortality? A nationwide register-based study of disease discordant Danish twins**

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Background
Overt hypothyroidism (OH) has repeatedly been associated with a number of potential lethal conditions. However, it is still debated whether OH is linked with increased mortality. Moreover, a link between OH and mortality could be the result of common genetic and environmental factors affecting both OH and mortality. Investigating twin pairs discordant for OH can minimize this potential confounding. Our objective was to investigate, at a nationwide level, whether OH influence mortality.
Study populations
A random 5% sample of the Danish population ($n = 281\,549$) and all Danish twins ($n = 96\,064$) born between 1870 and 1990.
Methods
Unpaired and intrapair Cox regression analyses were compared. From the discordant twin pair design, early environmental and genetic confounding was controlled by design. The participants were followed until December 31, 2008.
Results
In the 5% sample of the Danish population, mortality was increased by 20% in subjects with OH (Hazard ratio, HR = 1.20, 95% CI, 1.14–1.27). The impact of OH on mortality remained significant after adjusting for the degree of comorbidity (HR = 1.16, 95% CI, 1.10–1.23). OH was not associated with an increased mortality in the analyses of twins discordant for OH (HR = 1.03, 95% CI, 0.75–1.42). Stratifying for zygosity yield essential similar results in monozygotic (HR = 1.00, 95% CI, 0.54–1.83) and dizygotic same sex pairs (HR = 1.04, 95% CI, 0.71–1.52).
Conclusion
Our findings of lack of association between OH and mortality within DZ and MZ twin pairs discordant for OH, implies, that the association found in the singletons may be due to genetic and environmental confounding.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details are unavailable.

## OC11.4

**Prevalence of TSH receptor mutation and clinical characteristics among 84 patients with hyperthyroidism with diffuse goiter and negative TBII**

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Introduction
Hyperthyroidism with diffuse goiter and negative TBII includes Graves' disease in the early stage or near remission and nonautoimmune hyperthyroidism (NAH) caused by constitutively activating germline mutations of TSH receptor. To verify the NAH in this situation, we examined TSH receptor mutation and followed-up the clinical course.
Patients and methods
From 2003 through 2011, 84 patients had hyperthyroidism with negative TBII and diffuse radioiodine uptake in the thyroid. Genomic DNA sequencing analysis of the TSH receptor gene was performed for these patients (11 men and 73 women; median, 36 years).
Results
Four families with novel heterozygous point mutations (L512Q,E575K,-D617Y,L267F) consisted of 11 members were detected. Subsequent *in vitro* functional assays except for L267F showed their constitutive activity. There was a family history of hyperthyroidism in 90.9% of the group with TSH receptor mutation and in 32.9% of the group without mutation. There were no significant difference of sex, age and thyroid volume at first visit between the two groups. Among 11 patients of the mutated group, 5 had consistently subclinical hyperthyroidism without therapy, 3 were treated with anti-thyroid drug or inorganic iodine (ATD/KI), and 3 underwent ablative therapy (surgery or radioiodine) during 1 to 8 years of follow-up. Meanwhile, among 57 patients of non-mutated group, 14 obtained remission, 15 had consistently subclinical hyperthyroidism without therapy, 8 were treated with ATD/KI, 3 underwent ablative therapy, 5 were suspicious for toxic multi-nodular goiter, and 12 had positive TBII during more than 1 year of follow-up.
Conclusion
The genetic analysis of TSH receptor shows the frequency of NAH family is about 5% among all subjects. Approximately half of non-mutated patients are considered as Graves' disease in the early stage or near remission.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC11.5

**Thyroid hormones and male sexual function**

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Objective
The role of thyroid hormones in the control of erectile functioning has been only marginally investigated. The aim of this study is to investigate the association between thyroid and erectile function in a general population sample (European Male Aging study, EMAS study) and in patients seeking medical care for sexual dysfunction (University of Florence Study, UNIFI study).
Participants
Two different cohorts of subjects were evaluated. The first one derives from EMAS study, a multicenter survey performed on a sample of 3370 community dwelling men aged 40–79 years (mean $60 \pm 11$ years). The second cohort is a consecutive series of 3203 heterosexual male patients (mean age $51.8 \pm 13.0$ years) attending our Andrology and Sexual Medicine Outpatient Clinic for sexual dysfunction at the University of Florence (UNIFI study). In the EMAS study all subjects were tested for thyroid-stimulating hormone (TSH) and free thyroxin

(FT4). Similarly, TSH levels were checked in all patients in the UNIFI study, while FT4 only when TSH resulted outside the reference range.
Results
Overt hyperthyroidism (reduced TSH and elevated FT4, according to the reference range) was found in 0.3 and 0.2% of EMAS and UNIFI study, respectively. In the EMAS and UNIFI samples, TSH levels were inversely related with erectile dysfunction (ED). Overt hyperthyroidism was associated with an increased risk of severe erectile dysfunction (ED, hazard ratio = 14 and 16 in the EMAS and UNIFI study, respectively; both $P<0.05$), after adjusting for confounding factors. These associations were conformed in nested case-control analyses, comparing subjects with overt hyperthyroidism to age, BMI, smoking status and testosterone-matched controls. Conversely, no association between hypothyroidism and ED was observed.
Conclusions
Erectile function should be evaluated in all individuals with hyperthyroidism. Conversely, assessment of thyroid function cannot be recommended as routine practice in all ED patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## OC11.6

### Is age a risk factor for hypothyroidism in pregnancy? An analysis of 5223 pregnant women

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Introduction
The guidelines of American Thyroid Association from 2011 include age over 30 as one of the risk factors for hypothyroidism in pregnancy. The aim of our study was to verify whether age increases the risk of autoimmune thyroid diseases (AITD) in pregnant women.
Methods
We performed a cross-sectional study in 5223 unselected consecutive pregnant women in the 9th–12th gestational weeks. In a single laboratory, we measured serum concentrations of thyroid-stimulating hormone (TSH) and antibodies against thyroperoxidase (TPOAb). Reference interval for TSH was 0.06–3.67 mU/l; the upper cut-off value for TPOAb was 143 kU/l.
Results
Overall, 857 (16.4%) women were positively screened. Of these, 294 (5.63%) had TSH elevation; 146 (2.79%) TSH suppression; 561 (10.74%) were TPOAb+ and 417 (7.98%) were euthyroid and TPOAb+. The average age was 31.1 years with 65.1% of women being $\geq$30 years of age. Of the 294 hypothyroid women, 189 (64.3%) were $\geq$30 years. Using logistic regression model, we did not find any significant association between age and neither serum TSH suppression, TSH elevation and TPOAb positivity ($P=0.553$. $P=0.680$ and $P=0.056$, respectively), nor between age and TSH elevation with TPOAb positivity combined ($P=0.967$). In a subgroup analysis of risk factors for hypothyroidism in 132 hypothyroid women, addition of age $\geq$30 increased the proportion of women identified in a case-finding screening according to ATA from 55.3 to 85.6%.
Conclusions
Prevalence of AITD does not increase with age in pregnant women; however, addition of age $\geq$30 to the case-finding screening strategy may substantially improve its efficacy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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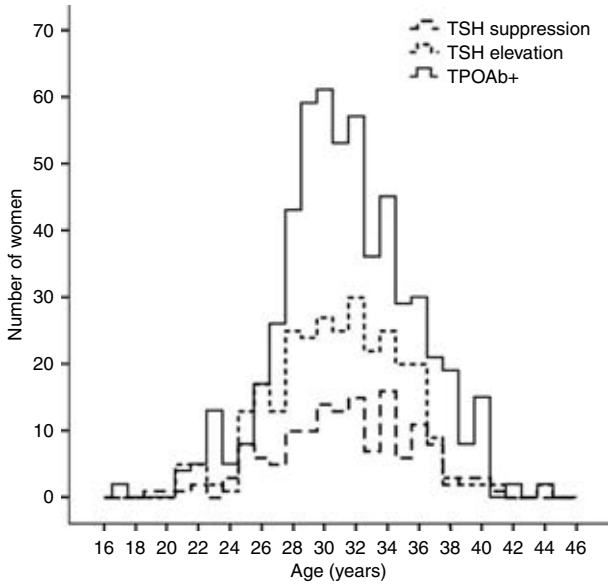

**Fig 1** Absolute frequencies of 857 pregnant women positively screened for AITD according to age

# Obesity clinical

## OC12.1

### Circulating endocannabinoids are associated to cardiometabolic alterations independently from adiposity

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Obesity is characterized by an increase of endocannabinoid circulating levels as anandamide (AEA) and 2-arachidonoyl-glycerol (2AG). Whether these lipids represent a biomarker of dysmetabolism or merely reflect the fat mass spill-over is still unclear. After generating rigorous, gender specific, plasma reference intervals by 2D-liquid chromatography-tandem mass spectrometry, we defined the 97.5thcentile of endocannabinoid distribution in the normal population as the cut-off above which they are considered pathological: 2AG = 4.23 and 3.12 pmol/ml; AEA = 1.66 and 1.59 pmol/ml, in males (M) and females (F), respectively. By using the cut-off we analyzed the endocannabinoid levels in a 348M and 439F population (106 and 160 normal weight (NW), BMI $\leq$ 24.9 kg/m$^2$; 242 and 279 overweight/obese subjects, BMI $\geq$ 25.0 kg/m$^2$) aged 18–90 y. 3.8%M ($n=4$) and 9.4%F ($n=15$) of NW and 8.7%M ($n=21$) and 17.2%F ($n=48$) of overweight/obese showed elevated 2AG, while 3.8%M ($n=4$) and 9.4%F ($n=15$) of NW and 9.5%M ($n=23$) and 29.4%F ($n=82$) of overweight/obese displayed increased AEA, respectively, indicating that obese/overweight F have more frequently pathologically plasmatic AEA levels. Within overweight/obese group, metabolic features were compared between subjects having normal or elevated 2AG and AEA levels. Males with elevated 2AG showed similar BMI but higher diastolic and systolic blood pressure (DBP and SBP, $P=0.018$ and $P=0.006$, respectively) and triglycerides ($P=0.036$) compared to those having 2AG in the normal range. Females with higher 2AG showed increased BMI ($P=0.012$), glycaemia ($P=0.020$) and triglycerides ($P<0.0001$), the latter maintaining the statistical significance after correction for BMI ($P=0.0005$). Both males and females with elevated AEA exhibited higher BMI and waist circumference compared to those with AEA in the normal range ($P=0.025$ and $P<0.0001$; $P=0.014$ and $P=0.001$, respectively). Females showing higher AEA had increased DBP, SBP and insulin even after correction for BMI ($P=0.006$, $P=0.016$ and $P=0.032$, respectively). In conclusion, circulating endocannabinoids are associated in a gender-specific fashion to cardiometabolic alterations independently from adiposity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC12.2

**Lipid-induced insulin resistance is associated with a reduction of IGF1 bioactivity independent of changes in IGFBP-1 and -2 in humans**

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Objectives
Insulin interacts with the GH – insulin-like growth factor (IGF) system by a reciprocal regulation of IGF-binding proteins (IGFBPs) and GH, which in turn interact to regulate insulin sensitivity (IS). We previously showed that IGFBP-2 is involved in the insulin-induced reduction in IGF1 bioactivity. We here address the modulation of IGF1 bioactivity during acute lipid-induced insulin resistance.
Methods
18 healthy men ($30.8 \pm 2.6$ years; BMI $23.6 \pm 0.5$ kg/m$^2$) were studied in a controlled, randomized crossover trial using lipid-heparin infusion at a dose inducing physiological elevations of free fatty acids (FFAs), vs saline-heparin infusion as control. IS was quantified by subsequent hyperinsulinemic euglycemic clamps. IGF1 bioactivity was estimated using an IGF1 kinase receptor activation assay (KIRA). Total IGF1, IGFBP-1 and IGFBP-2 were also measured under fasting conditions, during lipid or saline infusions and during the steady state of the subsequent clamps.
Results:
Lipid-infusion significantly reduced IGF1 bioactivity [$3.3 \pm 0.3$ µg/l (baseline) vs $2.5 \pm 0.2$ (180 min); $P<0.01$], total IGF1 [$135.2 \pm 10.8$ µg/l (baseline) vs $121.9 \pm 9.7$ (180 min); $P<0.01$] and IGFBP-1 [$27.1 \pm 4$ µg/l (baseline) vs $15.2 \pm 2.4$ (180 min); $P<0.01$]. This was associated with significant reduction in peripheral glucose-uptake [GIR.kg-1BW: $4.3 \pm 0.4$ (lipid) vs $6.1 \pm 0.5$ (saline); $P<0.01$]. No changes were detected after saline-infusion ($P>0.05$).
Subsequently, euglycemic hyperinsulinemia induced further reductions in IGF1 bioactivity and in IGFBP-1 levels ($P<0.01$) in both intervention arms, but did not change total IGF1 levels ($P>0.05$).
IGFBP-2 levels did not change neither after lipid- nor saline-infusions ($P>0.05$) but significantly increased during steady state ($P<0.05$).
Conclusions
We here show that lipid-induced reduction of insulin sensitivity was associated with reduction in IGF1 bioactivity. The impact of elevated free fatty acids/triglycerides on IGF1 bioactivity was not mediated by changes in IGFBPs, but may relate to the concomitant suppression of total IGF1.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC12.3 The Young Investigator Winner

**Transient increase of beta-cell first phase secretion after smoking cessation: a possible contributor to carbohydrate craving and body weight gain?**

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Despite the known health hazards of cigarette smoking, many smokers fail to quit smoking, mainly due to nicotine's addictiveness, but also because of fear from gaining weight. However, the mechanism of weight gain after smoking cessation is not fully elucidated yet.
Healthy long-term smokers ($28 \pm 2$ years, 10F/20M, $23.4 \pm 1.3$ kg/m$^2$) participating in a smoking cessation programme underwent 3-hour oral glucose tolerance tests (oGTT) and body composition measurements while still smoking (Visit A; $n=27$) and after a minimum of 3 (3 m; $n=14$) and 6 months (6 m; $n=8$) of non-smoking. Fasting (QUICKI) and dynamic insulin sensitivity (OGIS) were calculated. First phase beta-cell secretion was calculated as the ratio of C-peptide and glucose areas under the curve in the first 40 min of oGTT (IGI40). Appetite was quantified with a free-choice-buffet. Fasting plasma concentrations of neuropeptide-Y (NPY), peptide-YY (PYY), glucagon-like-peptide-1 (GLP-1), leptin, ghrelin and visfatin were measured.
Body weight and fat mass increased after 3 m (+4% and +22% respectively) and after 6 m non-smoking (+5% and +35% respectively, $P<0.05$ vs baseline).

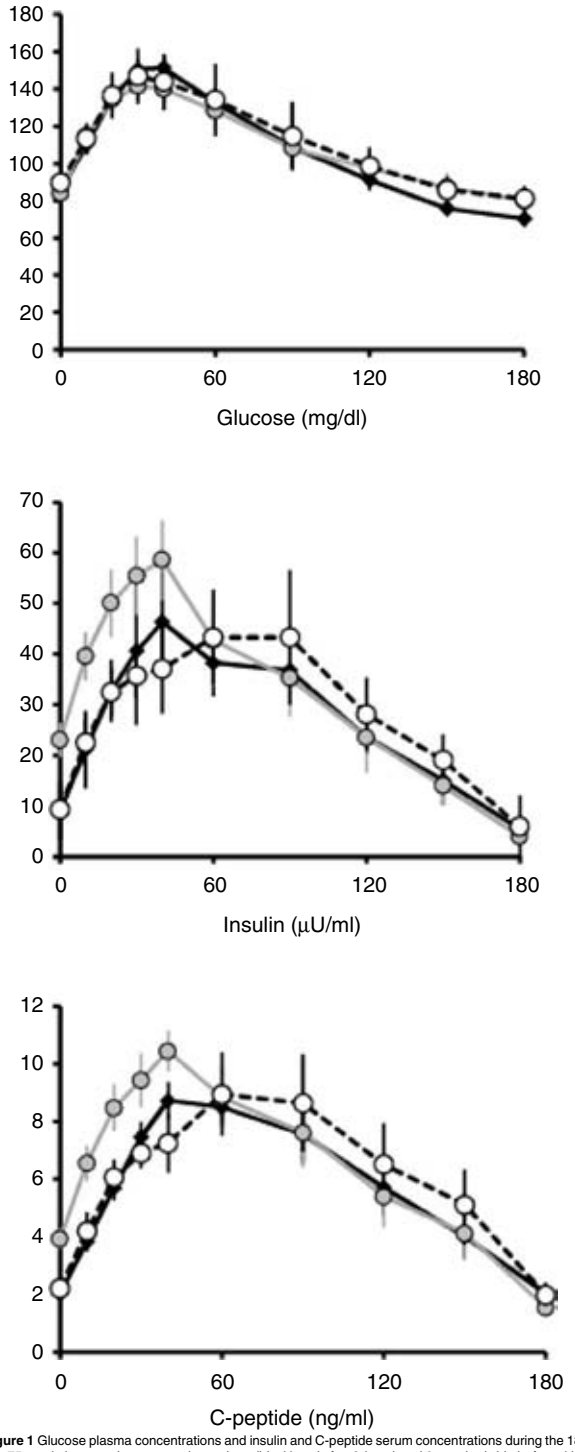


**Figure 1** Glucose plasma concentrations and insulin and C-peptide serum concentrations during the 180 min 75g oral glucose tolerance test in smokers (black) and after 3 (grey) and 6 months (white) of smoking cessation.

Participants showed significant fasting insulin resistance (QUICKI: 3 m:0.37 ± 0.02 vs baseline:0.41 ± 0.2; $P < 0.05$) at 3 m, but not at 6 m, while OGIS remained unchanged throughout. IGI40 increased by 31% after 3 m (baseline:0.299 ± 0.11 vs 3 m:0.391 ± 0.11, $P < 0.01$), but reversed to normal after 6 m (0.265 ± 5). Accordingly, carbohydrate ingestion was significantly increased after 3 m (3 m:500 ± 39 kcal vs baseline:396 ± 45 kcal; $P < 0.05$), but not after 6 m. Fasting NPY was increased at 3 m (3 m: 0.41 ± 0.03 ng/ml vs baseline:0.26 ± 0.04 ng/ml; $P < 0.05$), but not at 6 m. Interestingly, increased beta-cell-secretion at 3 m was less pronounced in participants finally succeeding to quit smoking for at least 6 m, as opposed to those relapsing after 3 m ($P < 0.05$).

Smoking cessation leads to a transient increase of first phase beta-cell secretion in response to glucose, fasting insulin resistance and NPY plasma levels. These alterations could contribute to increased carbohydrate craving and consequent body weight gain after smoking cessation (Fig. 1).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC12.4

### Genetic susceptibility, birth weight and obesity risk in young chinese

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Thus far, approximately 30 loci influencing body mass index (BMI) and the risk of obesity have been identified. In this study, we aimed to examine the individual and joint associations of 23 BMI-associated loci identified from recent genome-wide association studies in Caucasians with obesity risk in young Chinese. Birth weight reflects prenatal metabolic adaption and has been related to later-life obesity risk. We particularly assessed whether these genetic variants interacted with birth weight in relation to obesity risk. We recruited 540 young (14–30 years) and obese patients (BMI $\geq$ 30 kg/m$^2$), and 500 age- and sex-matched normal-weight healthy individuals (BMI $<$ 23 kg/m$^2$).We genotyped genetic variants in those 23 BMI-associated loci. Six loci, including SEC16B, GNPDA2, BDNF, FTO, MC4R and TMEM160, were significantly associated with obesity risk, with odds ratio from 1.314 to 1.701. These risk loci accounted for 4.84% of the genetic variance in obesity. We created a genetic risk score (GRS) by summing the risk alleles of these associated genetic variants. Prediction of obesity was significantly improved ($P <$ 0.001) when the GRS was added to a model with age and gender, with improvement of discrimination for obesity by 2.7%. In addition, we found that the GRS interacted with birth weight in relation to obesity ($P$ interaction $<$ 0.001). The genetic effect appeared to be more pronounced in individuals with normal range of birth weight (25–75%) than those with either low ($<$25%) or high ($>$75%) birth weight. In conclusion, we showed that the combined genetic risk of variants identified from European populations might significantly improve the identification of high-risk group of obesity in young Chinese. For the first time, we demonstrated birth weight might interact with genetic susceptibility in relation to obesity risk in later life, which deserves consideration in future efforts to prevent obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC12.5

### Brown adipose tissue activation is inversely related with central obesity and metabolic parameters in adult human

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Background

Recent studies have shown that adult human possess active brown adipose tissue (BAT), which might be important in affecting obesity and related metabolic risk. However, the supporting evidence in large population based studies is sparse.

Methods

We studied 4011 (2688 males and 1323 females) tumor-free Chinese adults aged 18–89 for BAT activities, visceral/subcutaneous fat areas and metabolic parameters. *In vivo* 18F-fluorodeoxyglucose (18F-FDG) uptake into adipose tissue and abdominal fat distribution were measured by whole body FDG-positron-emission tomography and computed tomography (PET/CT) and CT scans at umbilicus level.

Results

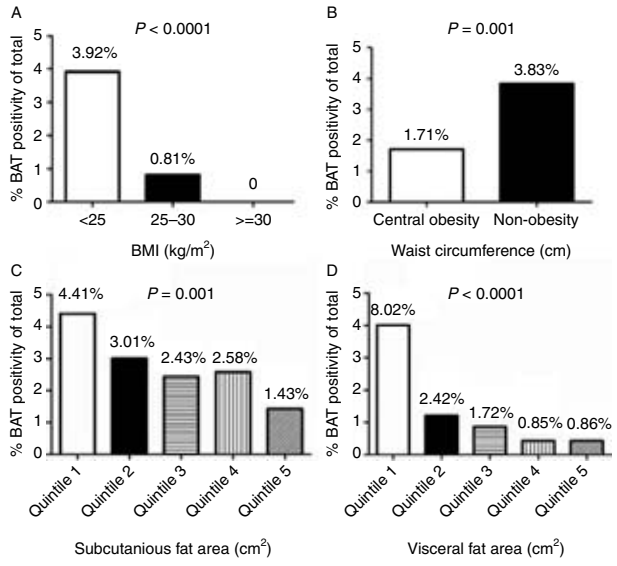
We found that the prevalence of BAT was around 2.7% in our study participants, with a significant sexual difference (5.5% in the females vs 1.3% in the males; $P < 0.0001$). BAT detection was increased in low temperature and declined in elderly subjects. The BAT positive subjects had lower BMI ($P < 0.0001$), less subcutaneous fat areas ($P < 0.01$), visceral fat areas ($P < 0.0001$), waist circumferences ($P < 0.0001$), lower fasting glucose and triglyceride levels (both $P < 0.01$) and increased HDL cholesterol concentrations ($P < 0.0001$), compared with the BAT negative subjects. Robust logistic regression revealed that after adjustment for covariates (including age, sex, BMI, visceral and subcutaneous fat areas and waist circumferences), age and BMI in the males (OR 0.92 and 0.84, both $P < 0.008$) while age and visceral fat areas in the females (OR 0.87 and 0.98, respectively, $P < 0.05$) were independently associated with detectable BAT.

Conclusion

We found the amount of active BAT is inversely related with central adiposity and metabolic parameters in adult humans, suggesting a potential role of BAT in the control of body weight and metabolic status.

**Table 1** The unadjusted and adjusted ORs and 95% CI from the logistic regressions predicting the likelihood of positive BAT

| | Male subjects | | | | Female subjects | | | |
|---|---|---|---|---|---|---|---|---|
| | unadjusted | | adjusted | | unadjusted | | adjusted | |
| | OR (95% CI) | *P* | OR (95% CI) | *P* | OR (95% CI) | *P* | OR (95% CI) | *P* |
| Age (years) | 0.90 (0.86–0.94) | <0.0001 | 0.92 (0.88–0.96) | 0.0002 | 0.87 (0.83–0.89) | <0.0001 | 0.87 (0.83–0.91) | <0.0001 |
| BMI (kg/m$^2$) | 0.80 (0.71–0.90) | 0.0003 | 0.84 (0.75–0.96) | 0.008 | 0.75 (0.67–0.83) | <0.0001 | – | – |
| Waist circumferences (cm) | 0.93 (0.90–0.97) | 0.0001 | – | – | 0.93 (0.90–0.97) | <0.0001 | – | – |
| Subcutaneous fat areas (cm$^2$) | 0.993 (0.985–1.001) | 0.08 | – | – | 0.989 (0.984–0.994) | <0.0001 | – | – |
| Visceral fat areas (cm$^2$) | 0.993 (0.985–1.001) | 0.10 | – | – | 0.96 (0.95–0.98) | <0.0001 | 0.98 (0.97–0.99) | 0.02 |




Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## OC12.6

**Eating speed and the risk of type 2 diabetes mellitus: a case-control study**

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Introduction
Diabetes mellitus is one of the main public health issues. It is becoming a world pandemic. Type 2 diabetes appears to involve interaction between susceptible genetic backgrounds and environmental factors. It's important to identify modifiable risk factors that may help reduce the risk of type 2 diabetes. For the meantime no data in scientific literature or eating speed could influence on the risk of developing type 2 diabetes mellitus. Therefore the aim of the study was to assess the relationship between eating speed and the risk of type 2 diabetes mellitus.
Subjects and methods
A case-control study included 234 cases with newly confirmed diagnoses of type 2 diabetes mellitus and 468 controls those who were free of the disease. Cases and controls (ratio 1:2) were matched by gender and age (+5 years). A specifically designed questionnaire was used to collect information on possible risk factors of type 2 diabetes mellitus. Anthropometrical measurements were made according to WHO recommendations. The odds ratios (OR), and 95% confidence intervals (CI) for type 2 diabetes mellitus were calculated by a conditional logistic regression.
Results
The cases had higher body mass index and significantly lower education level, compared to the controls.
Variables such as a family history on diabetes, education, morning exercise, body mass index, waist circumference, cigarette smoking and plasma triglycerides level were retained in multivariate logistic regression models as confounders because their inclusion changed the value of the OR by more than 5% in any exposure category. After adjustment for possible confounders more than two-fold increased risk of type 2 diabetes mellitus was determined for subjects eating faster (OR = 2.52; 95% CI 1.56–4.06) vs subjects eating slower.
Conclusions
Our data support a possible relationship between faster eating speed and the increased risk of type 2 diabetes mellitus.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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# Adrenal basic

## OC13.1

**Targeting multiple signalling pathways showed high anti-tumour potential in two mouse phaeochromocytoma cell lines**

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Introduction
Since there is no completely effective therapy available for malignant phaeochromocytomas (PCCs) and paragangliomas, we have been investigating novel targeted therapies utilising one more benign (MPC) and one more malignant (MTT) PCC cell line. We have previously shown that the IGF1-receptor-inhibitor NVP-AEW541 led to compensatory ERK and mTORC1 up-regulation at suboptimal doses and that the dual PI3K/mTORC1-inhibitor NVP-BEZ235 also resulted in compensatory ERK activation at low doses. Therefore we speculated that the efficacy of NVP-BEZ235 may be enhanced by combination with the established agent lovastatin which inhibits ERK-signalling.
Methods/Design
Both cell lines, generated from heterozygous Neurofibromin-1 knock-out-mice, were treated with varying concentrations of lovastatin, NVP-BEZ235, or the combination of both drugs, for different time periods. Metabolic viability was assessed by cell proliferation assay (MTS). Protein levels of phospho-(p) and total AKT, ERK and p70S6K were investigated by Western blotting in MPC cells. Apoptosis was measured by a caspase assay (Caspase-Glo®3/7) in MPC and MTT cells.
Results
Lovastatin significantly dose- and time-dependently decreased MPC and MTT cell viability and diminished pAKT and pERK, but increased pp70S6K. Pre-treatment for 24 h with 10 uM lovastatin followed by addition of 50nM NVP-BEZ235 for 48 h showed a marked additive effect in both cell lines. Combination treatment decreased both pAKT and pp70S6K without ERK up-regulation. Single treatment with NVP-BEZ235 did not induce apoptosis. However, lovastatin significantly enhanced apoptosis compared to the vehicle. Combination treatment increased apoptosis compared to single NVP-BEZ235 treatment but decreased apoptosis compared to single lovastatin treatment.
Conclusions
Targeting PI3K/AKT-, mTORC1/p70S6K- and ERK-signalling simultaneously suggests a novel therapeutic approach for malignant PCCs. Targeting only one or two of these pathways results in compensatory activation of the remaining ones. ERK inhibition appears to specifically increase apoptosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC13.2

**VSNL1 is upregulated in aldosterone producing adenomas with KCNJ5 mutations and protects from calcium-induced apoptosis**

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Visinin-like 1 (VSNL1) is upregulated in aldosterone-producing adenomas (APA) compared to normal adrenals. We demonstrate that VSNL1 over-expression in adrenocortical carcinoma cells (NCI H295R) upregulates basal and angiotensin II (Ang II)-stimulated CYP11B2 gene expression 3.2- and 1.5-fold, respectively. Conversely, silencing VSNL1 by RNA interference decreases Ang II-stimulated CYP11B2 expression and aldosterone secretion by 41 and 34.5%, respectively. Mutations in the potassium channel KCNJ5 have been identified in APA that result in sodium influx, membrane depolarization and are postulated to result in calcium influx in adrenal glomerulosa cells. VSNL1 and CYP11B2 are 8.1- and 6.0-fold more highly expressed, respectively, in APA harbouring KCNJ5 mutations compared to those without, and the upregulation of VSNL1 in these APA accounts for the overexpression of VSNL1 in the total APA sample set compared to normal adrenals. Silencing VSNL1 in H295R cells renders them sensitive to ionomycin-induced apoptosis indicating that VSNL1 protects these cells against calcium-induced cell death. Concomitant expression of mutated KCNJ5 (G151R) and silencing VSNL1 results in apoptosis of H295R cells, an effect that is blocked by nifedipine and is absent using a control siRNA or when wild-type KCNJ5 is expressed and VSNL1 is silenced. These data demonstrate that VSNL1 plays a dual function in vitro in the regulation of CYP11B2 gene expression and in the inhibition of calcium-induced apoptosis. Additionally, VSNL1 may play a role in the pathophysiology of APA harbouring mutations in the potassium channel KCNJ5 via its anti-apoptotic function in response to calcium cytotoxicity and its effect on aldosterone production.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC13.3

**Cortisol secretion is dependent on intraadrenal production of ACTH in macronodular bilateral adrenal hyperplasia causing Cushing's syndrome**

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Illicit expression of membrane receptors for circulating regulatory factors, such as gastric inhibitory polypeptide (GIP), luteinizing hormone (LH) and serotonin (5-HT) receptors, has been well documented in ACTH-independent macronodular adrenal hyperplasias (AIMAHs) causing Cushing's syndrome. In addition, we have observed an abnormal expression of ACTH in some steroidogenic cells in two AIMAH tissues. The aim of the present study was to investigate the role of local production of ACTH in the control of steroidogenesis in a series of 30 AIMAH tissues. Expression of pro-opiomelanocortin (POMC) mRNA and ACTH-like immunostaining were detected in all tissues studied. ACTH co-localized with 17-hydroxylase, the HDL-cholesterol receptor SR-B1, prohormone convertase 1 and secretogranin II immunoreactivities in clusters of cells disseminated throughout hyperplasia tissues. Perifusion experiments demonstrated that adrenal slices spontaneously released detectable amounts of ACTH in a pulsatile fashion. ACTH secretion was significantly increased *in vitro* by GIP, hCG and 5-HT in tissues previously sensitive *in vivo* to the stimulatory action of food intake, hCG and 5-HT4 receptor agonists. In addition, measurement of ACTH concentrations in plasma obtained from two AIMAH patients during adrenal vein sampling showed a significant ACTH gradient versus periphery indicating that AIMAH tissues actually secrete ACTH *in vivo*. The ACTH receptor antagonists corticostatin and ACTH(7–38) reduced basal as well as GIP-induced cortisol production from perifused hyperplasia tissues. These data indicate that, in AIMAH tissues, ACTH released by a subpopulation of steroidogenic cells exerts an intraadrenal stimulatory tone on cortisol secretion. They also suggest that macronodular bilateral adrenal hyperplasia may be regarded as a cause of ACTH-dependent Cushing's syndrome due to ectopic expression of corticotropin within the adrenal cortex. This work was supported by grants from INSERM, University of Rouen, Assistance Publique des Hôpitaux de Paris, the COMETE network, the Société Française d'Endocrinologie and Novo Nordisk Laboratory.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC13.4

**A genome-wide methylation study of adrenocortical tumors shows specific alterations linked to gene expression, revealing new aspects of the molecular classification of adrenal carcinomas**

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Introduction

DNA methylation is a mechanism for gene expression dysregulation in cancer. Little is known about methylation in adrenocortical tumors (ACT). Previous transcriptome studies proposed an original classification of ACT, first discriminating carcinomas from adenomas, then separating the carcinomas in two groups with different prognosis (C1A and C1B), the poor outcome group (C1A) being divided in three subgroups: p53 inactivation sub-group (C1Ap53), β-catenin activation sub-group (C1Aβcatenin), and a third with no known molecular alteration (C1Ax). Here we studied the genes promoter methylation, and the relation between methylation and gene expression in ACT, especially with the transcriptome classification of ACT.

Patients and methods

Genome-wide methylation patterns of 84 adenomas and 51 carcinomas were studied by the Infinium HumanMethylation27 Beadchip (Illumina). Gene expression data were available for 87 tumors, including 34 carcinomas (HG-U133Plus2.0 AffymetrixGeneChip, Affymetrix).

Results

Overall, the carcinomas are more methylated than the adenomas (Wilcoxon-test, $P = 7e\text{-}07$). Methylation varies among transcriptome-based subgroups of carcinomas: two subgroups of poor outcome harbor a high level of methylation (C1Ap53 and C1Ax), one of poor outcome a low level of methylation (C1Aβcatenin), and one of better outcome an intermediate level of methylation (C1B).

A few genes are recurrently hypermethylated in carcinomas whatever the group (ex: H19), other genes having subgroup-specific methylation. The transcriptome/methylation correlation shows 1741 genes (out of 12 250) negatively correlated; among the top genes are the expected H19 and several tumor suppressors (PLAGL-1, G0S2, NDRG2).

Methylation and expression also show regional patterns, among which are the regions 1q22, 8p21.3, 11p15 and 17p13.1.

Conclusions

This first ACT genome-wide methylation study shows that specific methylation profiles characterize the transcriptome-identified subgroups of carcinomas. It identifies tumor suppressor genes with recurrent methylation aberrations, related to gene expression deregulation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC13.5

**KCNJ5 mutations in Japanese patients with aldosterone-producing adenomas**

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Background

Primary aldosteronism (PA) has been reported to affect about 10% of patients with essential hypertension. In Western countries, about one third of them have aldosterone-producing adenomas (APA), and mutations of the KCNJ5 gene have recently been identified in approximately 30% of APAs. In contrast, most Japanese (~80%) with PA have APA. Therefore, Japanese PA may have different characteristics from patients in Western countries, and those with mutations of the KCNJ5 gene may have different features from those without.

Design

We sequenced KCNJ5 cDNAs in 25 tumors from patients with APA operated on at Gunma University Hospital and compared their clinicopathological features between patients with and without mutations. In addition, we investigated levels of KCNJ5 mRNA in the two groups and compared to those in cortisol-producing adenomas (Cushing's syndrome) and pheochromocytomas.

Results

Of the 25 patients with APA, 16 (64%) had two recurrent somatic mutations of the KCNJ5 gene: 12 cases of p.G151R (8 with c.451G > A, and 4 with c.451G > C) and 4 cases of p.L168R (c.503T > G). Levels of KCNJ5 mRNA were significantly higher in the APAs with mutations than those without. Immunohistochemistry also showed a stronger staining of KCNJ5 on the cell membrane in the tumor with a mutation. The level of KCNJ5 mRNA in cortisol-producing adenomas was approximately 30% of that in APAs, and almost no expression was observed in pheochromocytomas. In addition, the patients with mutations were statistically younger and showed higher plasma aldosterone concentrations and lower serum potassium levels.

Conclusions

i) We identified a high prevalence of mutations of the KCNJ5 gene in Japanese patients with APA, ii) KCNJ5 mRNA levels were higher in the APAs with KCNJ5 mutations, and iii) the expression of KCNJ5 mRNA was significantly higher in APAs than cortisol-producing adenomas and pheochromocytomas, and iv) patients with APAs associated with somatic mutations of the KCNJ5 gene may exhibit early onset and high serum aldosterone level.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC13.6

**GATA-4 overexpression induces adrenocortical tumorigenesis and ectopic expression of luteinizing hormone receptor in C57Bl/6J mice**

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We have earlier shown a reciprocal feed-forward amplification link between expression of transcription factor GATA-4 and luteinizing hormone receptor (LHR) during adrenocortical tumorigenesis, and also that chronically elevated LH levels may induce LHR in mice, but not GATA-4. Hereby, our goal was to analyze the consequences of ectopically expressed GATA-4 on murine adrenal cortex in the presence and absence of gonadectomy (GDX) induced elevated LH levels. For this purpose, we established a transgenic (TG) murine model overexpressing ectopically GATA-4 under the adrenal specific 21-hydroxylase (21-OH) promoter (21-OH-GATA-4) in C57Bl/6J genetic background. In intact 21-OH-GATA-4 females, but not in males, a gradual age-dependent increase of adrenal Gata-4 expression was followed by slowly progressing hyperplasia of non-steroidogenic spindle-shaped cells (A cells) in the subcapsular cortex. This phenotype was markedly enhanced by GDX in both sexes. Additionally, adrenocortical hyperplastic areas of GDX 21-OH-GATA-4 mice, besides A cells, were also composed with large lipid-laden cells (B cells). Long exposure on elevated LH levels resulted with adrenocortical adenoma in 21-OH-GATA-4 females. Intact and GDX 21-OH-GATA-4 adrenals displayed high Fog-2 but downregulated Gata-6 expression. In contrast to WT adrenal cortex and neoplastic B cells, areas with spindle-shaped A cells in intact and GDX 21-OH-GATA-4 mice were SF-1, DAX-1 and CYP11A1a negative. Both normal and neoplastic adrenocortical cells were StAR positive. 21-OH-GATA-4 mice of both sexes expressed ectopic LHR already by the age of 2 mo. LHR was localized in morphologically normal adrenocortical cells and large lipid-laden B cells of intact and GDX 21-OH-GATA-4 adrenals. Our findings provide the molecular pathways into the induction of adrenocortical tumorigenesis and expression of LHR as a consequence of ectopic adrenocortical expression of GATA-4.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This work was supported, however funding details are unavailable.

## Male reproduction

### OC14.1

**X chromosome-linked copy number variations in male infertility**

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The role of CNVs in male infertility is poorly defined, and only those linked to the Y chromosome have been the object of extensive research. Although it has been predicted that the X chromosome is also enriched in spermatogenesis genes, no clinically relevant gene mutations have been identified so far. In order to advance our understanding of the role of X-linked genetic factors in male infertility, we applied high resolution X chromosome specific array-CGH in 199 men with different sperm count followed by the analysis of selected, patient-specific CNVs in large groups of cases and controls. We identified 73 CNVs, among which 48 are novel, providing the largest collection of X-linked CNVs in relation to spermatogenesis. We found 21 patient-specific CNVs with potential clinical implication. Cancer Testis Antigen gene family members were the most frequently affected genes, and represent new genetic targets in relationship with altered spermatogenesis. The most relevant finding of our study is the significantly higher global burden of CNVs in patients than controls due to an excessive rate of deletions/person and to a higher mean sequence loss/person. Our observation adds 'CNV burden' to the list of those genetic anomalies which may link spermatogenic failure with genomic instability. The implications of this finding may not be restricted to infertility and should be taken into consideration in the era of assisted reproductive techniques, which allow infertile men to conceive their own biological children.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC14.2

**Deficient expression of genes involved in the endogenous defense system against transposons in cryptorchid boys with impaired mini-puberty**

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Mini-puberty is the period between 30 and 80 days after birth when testosterone and gonadotropin surges occur in male infants to induce the transformation of gonocytes into Ad (adult/dark) spermatogonia. Cryptorchid boys with impaired mini-puberty develop infertility despite timely and successful surgical treatment. The decreased germ cell count found in this group of boys could be the result of uncontrolled transposon activity inducing genomic instability and germ cell death. A genome-wide analysis of 18 cryptorchid and 4 control testes was performed with Affymetrix chips. We found that 6 of 8 genes that are important for transposon silencing were not expressed in the high azoospermia risk (HAZR) group of cryptorchid boys but were expressed in the low azoospermia risk (LAZR) and control groups. Two genes, CBX3 and DNMT1, were equally expressed in all 3 groups. Impaired expression of the DDX4, MAEL, MOV10L1, PIWIL2, PIWIL4, and TDRD9 genes in the group of cryptorchid boys at high risk of infertility indicates that gene instability induced by impaired expression of transposon silencing genes contribute to the development of azoospermia. Intact mini-puberty appears to be essential for the development of the endogenous defense system mediated by transposon silencing.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC14.3

**Comparison of serum testosterone and estradiol levels in 3174 European men as measured by a platform immunoassay and mass spectrometry; relevance for the diagnostics in aging men**

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Testosterone (T) and estradiol (E2) are the two most important sex steroids in men and women, respectively, and their accurate determination in serum is of crucial importance in assessing gonadal function both in clinical management and research. The limitations of serum T and E2 measurements using non-extraction platform immunoassays (IA) are widely recognized. Switching to more specific mass spectrometry (MS)-based methods has been advocated but directly comparative data on the two methods are scarce. We compared here serum T and E2 measurements in a large sample of middle-aged/elderly men using a common platform IA and a gas chromatography (GC)-MS method, in order to assess their limitations and advantages, and to diagnose male hypogonadism, in subjects of the European Male Aging Study ($n=3174$; age 40–79 years). Peripheral serum T and E2 were analyzed using established commerical platform IAs (Roche Diagnostics E170) and in-house GC-MS methods. Over a broad concentration range, serum T levels measured by IA and MS showed high correlation ($R=0.93$, $P<0.001$), which was weaker in the hypogonadal range ($<11$ nmol/l; $R=0.72$, $P<0.001$). The IA/MS correlation was less robust for E2 levels ($R=0.32$, $P<0.001$, at E2 $<40.8$ pmol/L, and $R=0.74$, $P<0.001$, at E2 $>$ 40.8 pmol/L). Using MS as 'gold standard', IA ascertained low T compatible with hypogonadism ($<11$ nmol/L) with 75% sensitivity and 96.3% specificity. The same parameters with IA for detection of low E2 ($<40.7$ pmol/L) were 13.3%

and 99.3%, and for high E2 (>120 pmol/L) 88.4% and 88.6%. In conclusion, validated platform IA is sufficient to detect subnormal T levels in the diagnosis of male hypogonadism. The IA used for E2 measurements showed poor correlation with MS and may only be suitable for the detection of high E2 in men.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC14.4

**The prevalence of hypogonadism in men on methadone maintenance**

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Opioid analgesia impairs gonadal function in healthy men and women. Methadone, a synthetic opioid is used in opioid addicts to prevent relapse due its longer-lasting effects and is known to cause hypogonadism. Little data exists on the prevalence of this and the mechanism through which this occurs.
Men ($n=147$), mean age 36.2 years (S.D 7.2) attending a drug-treatment rehabilitation centre for methadone maintenance therapy had early morning gonadotrophins, prolactin, thyroid hormones and sex-hormone binding-globulin (SHBG) levels measured.
35.4% ($n=52$) of men had total testosterone (TT) below the reference range (8.6–29 nmol/L). The mean TT in the hypogonadal group was 5.18 nmol/L (S.D. 2.28) compared to 18.1 nmol/L (S.D. 7.65) in the eugonadal group ($P<0.001$). SHBG concentrations were increased in the whole group, 62.5 U/l (S.D. 28.9) compared to the normal reference range (14.5–48.4 U/l). SHBG concentrations were significantly lower in the hypogonadal group compared to the eugonadal group, 49.7 U/l (S.D. 22.0) versus 69.3 U/l (S.D. 29.9) respectively ($P<0.001$).
There was no significant difference in methadone dose, antidepressant, benzodiazepine or anti-psychotic use between groups. Prolactin levels were normal and not significantly different in the low TT compared to the normal TT group, 223.5 mU/l and 250.6 mU/l respectively.
LH levels were significantly lower in the hypogonadal group, 4.10 U/l (S.D. 3.46) compared to the eugonadal men, 5.87 U/l (S.D. 2.97) ($P<0.005$). There was no difference in FSH levels. TSH was significantly greater in the hypogonadal group 5.82 mU/l (S.D. 14.92) compared to the eugonadal group 1.96 mU/l (S.D. 1.51) ($P<0.05$), with normal free T4 levels in both groups.
In summary, there is a high prevalence of hypogonadism in male methadone users. The whole group have high SHBG levels. However, compared to eugonadal users, hypogonadal users have significantly lower SHBG and LH levels and higher TSH levels. The clinical implications of this are not known.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC14.5

**The muenster EXAKT project: X-inactivation in klinefelter patients**

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Introduction
Klinefelter Syndrome (KS; 47,XXY) is the most common sex chromosome disorder in men, characterized by hypergonadotropic hypogonadism. EXAKT (Epigenetics, X-chromosomal features and clinical Applications in Klinefelter syndrome Trial) is a Muenster-based prospective project involving Klinefelter patients and their parents assessing a wide area of cardiovascular, inflammatory and metabolic factors as well as a broad range of genetic and epigenetic investigations especially regarding X-inactivation that could be disturbed due to the aberrant sex chromosomal constitution. Therefore, we have examined the methylation profile of XIST, which drives X-inactivation, in KS blood samples taking into account the X-chromosomal origin.
Material & methods
Klinefelter blood samples ($n=130$), male ($n=50$) and female ($n=50$) controls; Pyrosequencing; Microsatellite analysis.
Results
X-origin was determined in 80 KS patients: 56% of patients had a paternal origin of the supernumerary X-chromosome, whereas 24% showed a maternal MII origin and 20% MI origin of the extra X. In men, XIST methylation levels reached nearly 100% while in women on average 65% were found, indicating the correct silencing of one of the two X-chromosomes. XIST methylation level in 130 KS patients was 75% on average. The XIST methylation pattern of KS patients with paternal origin of the X-chromosome (74%) resembled that of KS patients with MI origin (73%) and both significantly differed from the methylation pattern of patients with MII X-origin (80%).
Conclusion
In contrast to previous studies, we detected hypermethylation of XIST in KS. The role of the altered XIST methylation pattern remains to be further studied. The higher XIST methylation in KS patients with MII origin points to a disturbed X-inactivation depending on the parental inheritance.
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Declaration of interest
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## OC14.6

**Erectile dysfunction after liver transplantation**

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Despite it's believed that sexual function may improve after liver transplantation (LT), many patients complain about unsatisfactory sexual life. There are few longitudinal data on the endocrine changes which occur after liver transplantation.
Aim
To evaluate erectile sexual dysfunction (ESD) prevalence and factors associated with its development, especially with relation to the status of sex steroids. Patients and Methods: 54 post-LT male patients were invited to answer a five-item International Index of Erectile Function (IIEF-5) and Hamilton Depression Rating Scale (HS). ESD was defined as a IIEF-5 $\leq 21$ points and depression as a HS $\geq 8$ points. Patients were tested for serum levels of testosterone (T), bioavailable T (bioT), androstane-3alpha,17beta-diol (3-DIOL-G) (glucuronide metabolite of dihydrotestosterone), SHBG, glucose and insulin.
Results
Main aetiologies of cirrhosis were HCV, HBV, alcohol, cryptogenic and NASH, showing a prevalence of 30, 22, 17, 11, and 11% respectively; median age was 60 (20–78) years old and follow up was 6 to 168 months. 85% were on calcineurin inhibitors as immunossupressors. 49 patients answered the questionnaires: 5 patients (10.2%) had more than 21 points in IIFE-5 (normal erectile function) while 21 (42,9%) had $<8$ points (no depression), $P<0.003$. No correlation was found between age/ESD – age/HS – ESD/HS. An inverse correlation between age and T ($P<0,02$) and bioT ($P<0,003$), and a direct correlation between IIFE-5 and T ($P<0,04$), bioT ($P<0,03$) and 3-diol ($P<0,03$) were established.
Conclusions
i) A surprisingly high prevalence of ESD was found in our series, and it seems not to be related to psychological status, ii) Hypogonadism does not seem to improve after LT, perhaps because of pre-existing gonadal alterations (toxic-metabolic damage) and immunosuppressive pharmacological side effects, iii) Further studies are needed to find out other reasons for the relationship between hypogonadism and LT outcome, and to explore the possibility of post transplant androgen therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## Thyroid basic

### OC15.1

***PATZ1* is a new candidate tumour-suppressor gene in thyroid cancer**

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Thyroid carcinoma arising from the thyroid follicular epithelium represents the most frequent endocrine malignancy and is mainly associated with gene rearrangements generating RET/PTC and TRK oncogenes, as well as BRAFV600E and RASV12 activating point mutations. Except for p53, which appears to be involved only in poorly differentiated and aggressive histotypes, a role of tumor-suppressor genes in the pathogenesis of thyroid cancer is still poorly known.
We found that the POZ/AT-hook/kruppel Zinc finger 1 (PATZ1) gene is down-regulated, with an inverse correlation to the degree of malignancy and differentiation, in the vast majority of thyroid tumours compared to normal thyroid, suggesting a tumour-suppressor role mainly involved in the late stages of thyroid carcinogenesis.
To explore this possibility, we performed functional studies in the papillary thyroid cancer (PTC)-derived TPC1 and the anaplastic thyroid cancer (ATC)-derived FRO cell lines, in which the expression of *PATZ1* is strongly down-regulated with respect to normal thyroid cells. Restoration of *PATZ1* expression, by stable DNA transfection, had no effect on the growth rate of both cell lines. Conversely, it led FRO cells to death and highly reduced migratory and invasive capabilities of both cell lines. Finally, we examined the transformed phenotype of the FRO cell transfectants expressing *PATZ1*, by analyzing their ability to grow in soft agar and to induce tumours in athymic mice. Differently from the parental cell line expressing the empty vector, these transfectants did not form colonies in soft agar and had reduced transforming ability *in vivo* compared to their controls. These data indicate that the loss of *PATZ1* expression exerts a functional role in the pathogenesis of thyroid cancer, and they are consistent with a specific role of PATZ1 in the signaling pathways involved in cell survival and metastatic progression.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC15.2

**Ligand bound-thyroid hormone receptor contributes to reprogramming of pancreatic exocrine cells to insulin-producing cells via induction of Ngn3 and MafA**

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Introduction
One goal of diabetic regenerative medicine is to instructively convert mature pancreatic exocrine cells into insulin-producing cells. We recently reported that liganded thyroid hormone receptor α (TRα) plays a critical role in expansion of the β-cell mass during postnatal development.
Materials and Methods
AdTRα is a recombinant adenoviral vector that expresses human TRα1 under the control of the cytomegalovirus promoter. To analyze whether TRα gene transfer induces reprogramming of pancreatic exocrine cells to insulin-producing cells, AdTRα were injected into the pancreas of immunodeficient mice. Rat pancreatic AR42J cells that possess exocrine and neuroendocrine properties were infected with AdTRα. The expression of transcription factors that are involved in the differentiation of pancreatic endocrine cells was then analyzed by quantitative RT-PCR, western blot or immunocytochemistry. To explore whether liganded-TRα-induced reprogramming of pancreatic exocrine cells is direct or indirect effect, AdTRα-infected AR42J cells were concomitantly transfected with siRNA of Ngn3 or MafA.
Results
Small scattered clusters of insulin-producing cells, which also expressed lipase, were observed in AdTRα-infected mice. T3-treatment of AR42J cells that were infected with AdTRα and pretreated with activin A increased the mRNA and protein expression levels of Ngn3 and MafA, compared to no T3-treatment. Overexpression of TRα together with T3-treatment also induced insulin expression in activin A-treated AR42J cells. The siRNA-induced inhibition of expression of Ngn3 or MafA significantly inhibited AdTRα-induced reprogramming of AR42J cells into insulin-producing cells.
Conclusions
These results suggested that combination of liganded-TRα and activin A leads to reprogramming pancreatic exocrine cells to insulin-producing cells via induction of Ngn3 and MafA. Our findings also support the hypothesis that liganded-TRα plays a critical role in β-cell regeneration during postnatal development.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### OC15.3

**Radioiodine therapy of non-thyroidal cancer following systemic sodium iodide symporter (NIS) gene transfer using Transferrin-receptor (Tfr) targeted non-viral gene delivery vectors**

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We have recently demonstrated the high potential of non-viral polyplexes for tumor-specific delivery of the sodium iodide symporter (NIS) after systemic application. In the current study we used novel polymers based on linear polyethylenimine (LPEI), shielded by polyethylene glycol (PEG), and coupled with the synthetic peptide B6 (LPEI-PEG-B6) as a transferrin (Tf)-receptor specific ligand to achieve active tumor targeting to human hepatocellular cancer (HuH7) cells after systemic delivery of NIS DNA.
We complexed LPEI-PEG-B6 with a NIS-expressing plasmid and analyzed levels of functional NIS expression after transfection of HuH7 (high Tf-receptor expression level) as compared to control cancer cells with low Tf-receptor expression level (colon cancer, RKO) *in vitro* and *in vivo*.
*In vitro* incubation of HuH7 cells with LPEI-PEG-B6/NIS resulted in a 9-fold increase in iodide uptake activity as compared to RKO cells. After establishment of subcutaneous HuH7 and RKO tumors in nude mice, NIS-conjugated nanoparticles or control vectors were injected i.v. followed by analysis of radioiodine biodistribution using I-123 scintigraphy. After injection of LPEI-PEG-B6/NIS, a significant perchlorate-sensitive iodide accumulation (8.5–10.9% ID/g I-123; eff. half-life of 5 h) was observed in HuH7 tumors resulting in a tumor absorbed dose of 50 mGy/MBq I-131 after systemic NIS gene transfer using LPEI-PEG-B6/NIS polyplexes. Tumoral iodide uptake activity and NIS mRNA expression were significantly lower in RKO cells confirming the specificity of Tf-receptor targeted nanoparticle vectors. After four cycles of polymer application followed by therapeutic application of I-131 (55.5 MBq), tumor growth was significantly reduced as compared to control groups.
These results clearly demonstrate that systemic *in vivo* NIS gene transfer using nanoparticle vectors coupled with a Tf-receptor targeting ligand is capable of inducing tumor-specific radioiodide uptake, which represents a promising innovative strategy for the NIS gene therapy approach in metastatic cancer.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details are unavailable.

### OC15.4

**The DNA methylation as a predisposition factor in the pathogenesis of congenital hypothyroidism in premature infants**

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Introduction
Epidemiological data indicate that children born prematurely have a risk 3–5 fold higher of congenital hypothyroidism (CH). In addition premature infants born small for gestational age (SGA) have a risk of 12% higher to develop IC compared to prematures with appropriate development (AGA). The mechanisms that justify the increased risk of IC are still unknown. Some studies report a

pattern of aberrant methylation associated with prematurity, intrauterine fetal development and the onset of some diseases. This project is focused on the study of DNA methylation, as predisposing factor of thyroid diseases.
Methods
Using the Illumina Infinium-HumanMethylation27 technology we analyzed the global DNA methylation patterns (AVGβ) and selected the differentially methylated genes (DMGs) between 31 CH-cases born premature, AGA or SGA, and 31 term or preterm controls. The following groups were selected according to the gestational age at birth: 12 CH-with severe prematurity (CH-SP < 32 weeks) and 19 CH-premature infants (CH-P 32–37 weeks); Controls: 9-CPS, 6-CP, 12-term birth (CT > 37 weeks). The same subjects were then analyzed according to intrauterine growth (20 CH-SGA, 11 CH-AGA than 6 C-SGA and 20 C-AGA) or the degree of CH: 19 with overt CH (CH-O, TSH > 10 μU/l) and 12 with mild CH (CHM, TSH < 10 μU/l) than 16 CP and 12 CT.
Results and Conclusions
The global and gene-specific methylation analysis showed that infants born prematurely and SGA have a significant hypomethylation than term-controls. The 95% of the DMGs are hypomethylated and the 70% are represented by CpG sites located in DNA non-coding regions. The gene ontology analysis revealed that DMGs involved in fetal growth and thyroid hormone metabolism were deregulated. In conclusion, these data suggests that genomic instability caused by global hypomethylation maybe related to premature birth and fetal growth delay. Thyroid defects could be caused by an increased expression of predisposing-genes, rather than a reduced expression of protective-genes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## OC15.5 The Young Investigator Winner

**Lymphatic endothelial markers podoplanin and Prox1 in differentiated thyroid tumors**

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It is believed that differentiated thyroid tumors (DTC) metastasize via different pathways: papillary carcinoma (PTC) usually via lymphatic spread whereas follicular cancers (FTC) mainly hematogenously. However, the mechanism/s contributing to these tumors invasion is not fully understood.
We examined the expression of lymphatic endothelial markers: Podoplanin (type-1 transmembrane mucin-like glycoprotein) and Prox1 (Prospero homeobox protein 1) in the PTC and FTC derived cell lines and series of DTC using quantitative Real-Time PCR (Q-RT-PCR), Western blot and immunochemical (IHC, IF) methods.
Podoplanin (PDPN) was highly expressed on both transcript and protein levels in papillary cell lines BcPAP and TPC1 whereas follicular cell lines: FTC-133, CGTH-W-1 and ML-1 were negative. Papillary tumor cells showed PDPN mostly restricted to and distributed in the cytoplasm. Cell lines with podoplanin expression showed low or undetectable Prox1 transcript level. By contrast PDPN negative FTC cell lines highly expressed Prox1 mRNA. There was no correlation between Prox1 protein and transcript. In all examined cell lines Prox1 protein was present at similar level exhibiting diffuse nucleocytoplasmic pattern.
The majority (72/120) of PTC and all FTC tissues were PDPN negative. However, in 48/120 (40%) of PTC cases podoplanin was expressed in cytoplasm of tumor cells with heterogonous intensity. This cytoplasmic neoexpression was correlated with patients age. Normal thyroid (NT) and peritumoral tissue (PT) cells were totally negative. In these tissues antibody labeled solely lymphatic vessels used as internal positive control. Prox1 protein was strongly expressed in the cytoplasm, weakly in some nuclei of PDPN positive PTC and clearly in the nuclei of NT.
Our study is the first to demonstrate the difference in the expression of lymphatic specific markers podoplanin and nuclear transcription factor Prox1 in DTCs. This might suggest different spreading pathways of PTC and FTC. Further on-going studies will define this mechanism/s and its potential implications.
This work was supported by CMKP 501–1–25–01–11.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC15.6

**Thyroglobulin induces thyroid cell growth through suppression of miR-16 and miR-195**

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Introduction
Thyroglobulin (Tg) is stored in the follicular lumen of the thyroid and serves as a substrate of thyroid hormone biosynthesis. However, it was shown that Tg significantly induces thyrocyte cell growth and suppresses expression of thyroid-functional genes. Despite having such functions, molecular mechanisms of Tg action are largely unclear. We have recently demonstrated that TSH-induced thyroid cell growth is partly mediated by a downregulation of microRNAs (miRNA) and induction of their target genes required for cell growth. In this study, we examined possible role of such miRNAs on Tg-induced thyroid cell growth.
Methods
Rat thyroid FRTL-5 cells maintained without TSH, insulin, and with 0.2% rather than 5% serum were treated with physiological concentrations of Tg. Total RNA was extracted and expression of miRNAs was determined using miRNA microarray and real-time PCR. The effect of miRNAs on cell growth and the expression of their target genes were assessed by bromodeoxyuridine (BrdU) incorporation, cell count, and real-time PCR using specific miRNA agonists.
Results
We have identified 21 miRNAs whose expression was downregulated by Tg. Those included miR-16 and miR-195, which were previously shown as important mediators of TSH action. Overexpression of miR-16 and miR-195 resulted in suppression of Tg-induced BrdU incorporation and cell growth. In that condition, induction of their target genes essential for cell growth, Mapk8, Ccne1 and Cdc6, were attenuated.
Conclusion
Our results indicate that Tg induces thyroid cell growth through suppression of miR-16 and miR-195 and induction of their target genes, which is similar to the mechanism of TSH action. miR-16 and miR-195 might be essential miRNAs for the regulation of thyroid cell growth.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Female reproduction clinical

## OC16.1

**The association between birth weight and PCOS in adult life. A register-based study on 523, 757 Danish women born 1973–1991**

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Introduction
PCOS is characterized by insulin resistance and an increased risk of type 2 diabetes.
Objective
To study the association between birth weight and risk of polycystic ovary syndrome (PCOS) in adult life in Danish women born 1973–1991.
Methods
Female offspring born in Denmark during 1973–1991 of Danish mothers ($n = 523,757$) were included and followed for a total of 4, 739, 547 person-years at risk. Information on birth weight was extracted from the Danish Medical Birth Register. The cohort was followed-up for the diagnoses hirsutism or PCOS from age 15 years until the end of 2006 in the Danish National Patient Register (NPR). Information on maternal diabetes diagnoses was also extracted from the NPR. Incidence Rate Ratios (IRR) were estimated from Poisson regression models with

95% confidence intervals (CI). All analyses were adjusted for attained age and period.
Results
The risk of PCOS was significantly increased in women with birth weight > 4500 grams (IRR =1.57 (95% CI 1.21–2.03)) compared to women with birth weight 3000–3499 grams. The risk of PCOS was independent of size for gestational age. Offspring of women with diabetes diagnoses had an increased risk of PCOS. In this population low birth weight was associated with increased risk of PCOS.
Conclusions
Our data suggest that the risk of PCOS was increased in girls with birth weight >4,500 grams. Girls born of diabetic mothers had increased risk of PCOS, but in this population low birth weight was associated with increased risk of PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC16.2

**The first missense mutation of BMP15 mature domain identified in a Chinese family with primary ovarian insufficiency causes defective production of the bioactive protein**

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Primary Ovarian Insufficiency (POI) is an ovarian defect characterized by the premature depletion of ovarian follicles before 40 years and represents one major cause of female infertility. POI is a heterogeneous disease but, despite its idiopathic origin in most of patients, there is a strong genetic evidence, in particular for X chromosome-linked defects. BMP15 gene maps to Xp11.2 within a Turner locus critical for ovarian function and mutations in this gene have been found in the heterozygous state in association with both primary and secondary amenorrhea in several POI cohorts. All the variations are located in the proregion of the protein, except for the R329C substitution, identified very recently in the Chinese population, co-segragating with POI in mother and daughter, and is the first reported located in the mature peptide. Involving an Arg to Cys change, the mutation was predicted in silico to be possibly damaging by impairing the correct folding of the protein. To verify the in silico prediction, we further in vitro studied the mutation. By western blot analysis, we observed a reduced production of both precursor and mature peptide, moreover the mutant form showed a significant reduction of the BMP signalling pathway activity compared to the wild-type by a luciferase reporter assay in COV434 granulosa cells. We demontrated that the first mutation discovered in the BMP15 mature domain is responsible for an impairment of either the maturation process or the precursor stability, resulting in a defective secretion of the bioactive protein. This results suggest that the BMP15 haploinsufficiency could have caused the onset of POI in the Chinese family harbouring the R329C mutation. In conclusion, our analysis confirm the importance of the screening of BMP15 for the prediction of POI risk and the importance to evaluate functionally possible disease-linked mutations to be distinguished from rare polymorphisms.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC16.3

**Anti-müllerian hormone as a predictor of ovarian response to weight loss in overweight women with polycystic ovary syndrome**

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Introduction
Weight loss is a key initial treatment strategy in obese women with polycistic ovary syndrome (PCOS) and improves insulin sensitivity and metabolic risk factors. In some women with PCOS it may reduce hyperandrogenism and restore menstrual function, ovulation and fertility. The aim of this study was to reveal clinical predictors of menstrual response to weight loss in overweight women with PCOS.
Design
overweight unovulatory women with PCOS (age 25.7±5.9 yr, body mass index (BMI) 32.3+5.3 kg/m$^2$) followed a 6-month weight loss program. We estimated ovulation induction (by ultrasound scanning), change in menstrual cycle, anthropometric measurements, endocrine parameters and insulin sensitivity.
Results
Mean reductions in weight by 9.0±5.9 kg ($P<0.001$) and BMI by 3.3±2.1 kg/m$^2$ ($P<0,001$) occurred for the subjects over the study duration. That was followed by reduction in fasting insulin (from 20.8±17.4 to 11.1±7.8 mU/liter, $P=0.002$), glucose (from 5.7±0.6 to 5.3±0.5 mmol/L, $P=0.01$) and the homeostasis model assessment of insulin sensitivity (HOMA) (from 5.2±4.2 to 2.7±1.9, $P=0.06$). Of 30 subjects, 15 (50%) responded to the intervention with improvements in menstrual cyclicity (responders). Compared to nonresponders, responders had lower baseline anti-mullerian hormone (AMH) levels (6.3±3.3 vs 9.8±4.2 ng/ml; $P=0.015$) and higher BMI (34.8±5.6 vs 30.4±4.2 kg/m$^2$, $P=0.026$). Using ROC-curves we calculated that the value of AMH less than 6.5 ng/ml can predict improvement of menses (sensitivity 73%, specificity 67%) by weight loss in overweight women with PCOS.
Conclusions
AMH measurement can be useful in the pretreatment identification of women with PCOS who will benefit from lifestyle intervention by menstrual improvements.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC16.4

**Epidemiological survey on the prevalence of hyperandrogenic states in adolescent and young women**

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The aim of this epidemiologic study, performed in a wide population of high-school students in Northern Italy, was to obtain an unbiased estimate of the prevalence of hyperandrogenic states in adolescents and young females.
2052 female students, aged 15–19 years, were consecutively contacted. 939/2052 (45.8%) refused to participate in the study, but 431 of the 939 non-participants (45.9%) agreed to fill out a brief questionnaire aimed to broadly assess clinical hyperandrogenism. 1113/2052 (54.2%) participated in the study (group A) that included a medical visit and a nutritional interview. Hirsutism was scored through the modified Ferriman-Galley score and androgenic alopecia through the Ludwig scale. Number of menses in the previous year was recorded and used to define menstrual dysfunction. 570/1113 participants (group B) also agreed to give a blood sample for the measurement of testosterone by isotopic dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS); the reference interval was established in-house using a subgroup of 149 healthy, normal-weight and untreated students. 203 students were subsequently excluded from group A because of oral contraceptive pills (OCPs); 105 students were subsequently excluded from group B because of OCPs and 20 students because of thyroid dysfunction or hyperprolactinemia. Within group A we found: 109 students (12.0%) with isolated menstrual dysfunction; 134 students (14.7%) with isolated clinical hyperandrogenism (hirsutism and/or androgenic alopecia); 32 students (3.5%) with the association between menstrual dysfunction and clinical hyperandrogenism. Within group B we found: 40 students (9.0%) with isolated menstrual dysfunction; 66 (14.8%) with isolated clinical hyperandrogenism; 17 (3.8%) with Polycystic Ovary Syndrome (menstrual dysfunction, clinical hyperandrogenism and high testosterone levels); 26 (5.8%) with isolated hypertestosteronemia; 5 (1.1%) with the association of clinical hyperandogenism and high testosterone levels, but with regular menses. These data show that hyperandrogenic states are common in adolescent and young women, thereby supporting the need for specific diagnostic criteria.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC16.5

**Urinary glucocorticoid metabolite excretion is associated with insulin resistance independent of body mass index (BMI) in patients with polycystic ovary syndrome**

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Polycystic ovary syndrome (PCOS) is a triad of insulin resistance, hyperandrogenism and anovulation. PCOS is associated with increased adrenocortical drive, which may have adverse metabolic consequences. Here we analysed the relationship of urinary androgen and glucocorticoid metabolite excretion with insulin resistance in a large PCOS cohort.

We compared results from 127 PCOS patients (Rotterdam criteria) with 100 BMI-matched controls. All subjects underwent anthropometric assessment including BMI, and metabolic testing with oral glucose tolerance test (OGTT) and homeostatic model assessment of insulin resistance (HOMA-IR). 24-h urine samples from patients and controls were analysed by gas chromatography/mass spectrometry for total androgen (androsterone + etiocholanolone) and total glucocorticoid metabolite excretion (μg/24 h). HOMA-IR values were log-transformed to normalise their distribution. Linear regression was used to measure the impact of urinary steroid metabolite excretion on HOMA-IR.

OGTT results were abnormal in 26 PCOS patients (21%). Dysglycaemic PCOS women did not differ by age from normoglycaemic patients but had a significantly higher BMI (35.6 ± 6.2 vs 30.5 ± 6.7 kg/m$^2$, $P=0.001$). Of those PCOS patients with a BMI > 30, 33% (22/67) had dysglycaemia on OGTT, compared to 7% (4/53) of those with BMI < 30. Total glucocorticoid excretion was significantly higher in PCOS patients compared to controls (9612 ± 4194 vs 8067 ± 4165, $P=0.013$). After adjustment for age and BMI, total glucocorticoid excretion was highly predictive of HOMA-IR levels, with HOMA-IR values increasing by 7% (95% CI, 2–11%, $P=0.003$) for each increase of 1000 μg/24 h in glucocorticoid metabolites (Table 1). Total urinary androgen metabolites were not predictive of HOMA-IR, $P=0.068$.

In PCOS, total glucocorticoid metabolite excretion is strongly correlated with markers of insulin resistance. Increased adrenocortical drive in PCOS may have adverse metabolic consequences.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1**

| FACTOR | COEFF. (95%CI) | SIG.* |
|---|---|---|
| Age | −0.8% (−2.3 to 0.7)** | 0.275 |
| BMI | +6.2% (4.0 to 8.6)** | <0.001 |
| Total glucocorticoid metabolites | +6.7% (2.3 to 11.2)*** | 0.003 |

*$P<0.05$. **% change in HOMA-IR for each unit increase in factor. ***% change in HOMA-IR for each 1000 μg/24 h increase.

## OC16.6

**Kisspeptin-10 stimulation of gonadotropin secretion in women is modulated by sex steroid feedback**

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Background

Sex-steroid feedback regulates gonadotropin (LH and FSH) secretion. Kisspeptin, a novel hypothalamic neuropeptide, stimulates gonadotropin secretion by stimulating GnRH secretion, and has been shown in animal models to play a central role in mediating sex steroid feedback.

Hypothesis

As estrogen feedback occurs at both the hypothalamus and the pituitary in women, we hypothesized that the stimulatory effect of kisspeptin-10 would be dependent on prevailing sex-steroid milieu.

Methods

Responses to intravenous kisspeptin-10 (0.3 μg/kg) in women in the follicular phase ($n=10$) were compared with a low sex steroid/high gonadotropin condition (post-menopause, $n=6$) and with a high sex steroid/low gonadotropin condition (combined contraceptive pill, $n=4$; progestogen contraceptive implants, $n=4$). 60-min AUC of gonadotropin secretion before and after kisspeptin-10 were compared using paired *t*-tests. By deducting 60-min mean baseline value from observed gonadotropin concentrations, inter-individual variability in baseline secretion was controlled for.

Results

Kisspeptin-10 stimulated LH secretion in follicular phase (ΔAUC 2.3 ± 0.8 IU/l × h, $P=0.009$), post-menopausal (5.3 ± 0.9 IU/l × h, $P=0.002$) and progestogen implant (2.6 ± 0.8 IU/l × h, $P=0.05$) groups but not in women taking the combined pill (0.9 ± 0.4 IU/l × h, $P=0.13$).

FSH secretion was significantly increased only in post-menopausal women (ΔAUC 2.6 ± 0.8 IU/l × h, $P=0.03$) with changes of <0.5 IU/l × h observed in the other three groups.

Gonadotropin responses in post-menopausal women were significantly larger than the other groups (one-way ANOVA analysis of ΔAUC; LH ($P=0.012$) and FSH ($P=0.001$)) without significant differences between the other groups.

Conclusions

Gonadotropin responses to kisspeptin-10 in women are enhanced in sex-steroid deficient post-menopausal women and suppressed in women taking pharmacological doses of exogenous estrogen and progestogen. Moreover, in keeping with other studies of kisspeptins, kisspeptin-10 preferentially stimulates LH over FSH.

These results are consistent with the notion that the pituitary gonadotrope is a functionally important locus of estrogen feedback in women and also inform potential translational applications of kisspeptin in reproductive endocrine disorders.

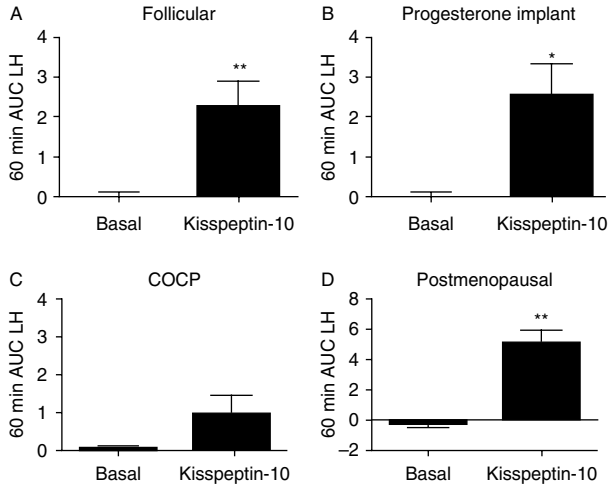


**Figure 1** 60-min AUC of LH before and after i.v. administration of 0.3 μg/kg kisspeptin-10 to healthy women. A, women in follicular phase ($n=10$); B, women using progesterone implants for contraception ($n=4$); C, women taking combined oral contraceptives ($n=4$); D, postmenopausal women ($n=6$).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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# Diabetes basic

## OC17.1

**Pdk1/Foxo1 pathway regulates macrophage migration, polarization, and insulin sensitivity**

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Chronic inflammation in adipose tissue contributes to obesity-related insulin resistance. The 3-Phosphoinositide-dependent Protein Kinase 1 (Pdk1)/forkhead transcription factor (Foxo1) pathway is important in regulating glucose and energy homeostasis, but little is known about this pathway in adipose tissue macrophages (ATMs). To investigate this, we generated transgenic mice that

carried macrophage/granulocyte-specific mutations, including a Pdk1 knockout (LysMPdk1-/-), a Pdk1 knockout with transactivation-defective Foxo1 (Δ256LysMPdk1-/-), a constitutively active nuclear (CN) Foxo1 (CNFoxo1-LysM), or a transactivation-defective Foxo1 (Δ256Foxo1LysM). The LysMPdk1-/- mice exhibited elevated M1 macrophages in adipose tissue and insulin resistance. Insulin-stimulated IRS1 or IRS2 and Akt phosphorylation were significantly decreased in epididymal fat and liver of LysMPdk1-/- compared to control mice. F4/80+ Kupffer cells in liver were significantly decreased. Furthermore, expression levels of Il4 and Il10 were significantly decreased and bone marrow derived Cd11b+ cells were significantly increased in liver of LysMPdk1-/-. LysMPdk1-/- exhibit increased Ccr2 expression in stromal vascular fraction (SVF) and peritoneal macrophage. Transwell migration assays revealed that Pdk1-deficient bone marrow derived macrophages (BMDM) exhibited significantly increased capacity of migration compared to BMDM from control. Overexpression of transactivation-defective Foxo1 rescued these phenotypes. CNFoxo1LysM promoted transcription of the C-C motif chemokine receptor 2 (Ccr2) in ATMs and increased M1 macrophages in adipose tissue. On a high fat diet, CNFoxo1LysM mice exhibited insulin resistance. Furthermore, Pdk1 deletion or Foxo1 activation in bone marrow-derived macrophages abolished insulin- and interleukin-4-induction of genes involved in alternative macrophage activation. These data suggested that Pdk1-Foxo1 pathway regulates migration and polarization of macrophage and plays an important role in the regulation of insulin sensitivity *in vivo*.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC17.2

### Placental restriction co-ordinately alters hepatic expression of micro-RNAs and targets related to non alcoholic fatty liver disease, in adult male sheep

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Placental restriction (PR) of fetal growth impairs insulin action and metabolic control in postnatal life. MicroRNAs (miRNAs) are important post-transcriptional regulators, down regulating mRNA abundance or translation. We therefore determined if PR in the sheep alters miR expression in liver, skeletal muscle and adipose tissue of adult offspring and if this associates with altered abundance of their predicted molecular targets.

PR was induced by pre-pregnancy removal of most uterine implantation sites. Control (8 male, 8 female) and PR (6 male, 8 female) sheep had insulin sensitivity and metabolism measured at 12 months, then tissues collected for RNA extraction. MiRNA expression was measured by Exiqon miRarray (v8.1) (R, with adjustment for multiple comparisons) and RTPCR, as was expression of predicted targets (identified by algorithms, then networks and pathways by IPA). Effects of PR and sex were assessed by ANOVA, associations by Pearson's correlation, with statistical significance at $P<0.05$.

PR mostly increased microRNA expression in insulin sensitive tissues of adult sheep. In males, PR increased hepatic expression of eight miRNAs by ~1.5-3.5-fold, with differential expression of four independently confirmed (miR 1, 21, 142-3p and 144), each predicted to target molecules involved in insulin signalling, metabolism and hepatic disease. The latter include p85α, Pparα, Igf1, Foxo3 and Acox1, all exhibiting reduced hepatic expression (~ 2.3-4.0 fold) following PR in males, with the abundance of miR-1, 142-3p and -144 correlating negatively with Acox1 expression.

These findings show for the first time that PR co-ordinately alters hepatic expression of miRs and predicted targets related to non-alcoholic fatty liver disease (NAFLD), in adult male offspring. Reduced hepatic expression of Pparα (regulates lipid catabolism), and Acox1 (peroxisomal fatty acid β-oxidation) characterise or promote development of NAFLD, increasingly common following fetal growth restriction in humans, and microRNAs may partly mediate this prenatal programming of NAFLD.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This work was supported, however funding details are unavailable.

## OC17.3

### Caerulin precursor fragment (CPF-AM1): a novel insulinotropic peptide from the skin secretion of the clawed frog, Xenopus amieti

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We reported the isolation and structural characterization of CPF-AM1 and other peptides from the skin secretion of Xenopus amieti. This study investigated the insulin-releasing activities of synthetic CPF-AM1 using clonal pancreatic cell line, BRIN-BD11 and Swiss TO mice with diet-induced insulin resistance.

Acute insulin-release studies were performed in Krebs Ringer bicarbonate buffer supplemented with 5.6 mM or 16.7 mM glucose, purified synthetic peptide (0–3 mM) and known modulators of insulin secretion. Insulin-release was measured by radioimmunoassay. Membrane potential and intracellular calcium ([Ca2+]i) were measured by flourometric assay using FLEXstationTM. Degradation of CPF-AM1 by plasma enzymes was investigated using reversed-phase HPLC and MALDI-TOF spectrometer.

At 5.6 mM glucose, CPF-AM1 significantly stimulated insulin-release over concentration range of 30 nM (1.4-fold, $P<0.05$) to 3 μM (3.4-fold, $P<0.001$) without beta-cell cytotoxicity. Without extracellular calcium, the stimulation was reduced by 25.3%. CPF-AM1(1 μM)-induced stimulatory effects were inhibited by co-incubation with 50 μM verapamil (57.4%, $P<0.001$) and 300 μM diazoxide (51.8%, $P<0.001$). Insulin-secretion increased by 3.3-fold with KCl (30 mM, 16.7 mM glucose) and CPF-AM1 (1 μM). At 5.6 mM glucose and 1 μM CPF-AM1, co-incubation with 200 μM IBMX and 200 μM tolbutamide caused 1.2-fold and 1.1-fold increase in insulin-release respectively. The peptide induced membrane depolarization (2.6-fold) and increased [Ca2+]i (2.0-fold) at 5.6 mM glucose. *In vivo*, intraperitoneal administration of CPF-AM1 (75 nmol/kg bw) with 18 mmol/ kg glucose significantly enhanced insulin-release (1.5-fold) and improved glucose tolerance by 28% ($n=6$, $P<0.05$). CPF-AM1 is resistant to degradation by plasma proteolytic enzymes up to 8 hr.

The study showed that CPF-AM1 is a novel peptide with potential for development into a new antidiabetic drug.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## OC17.4

### Syntaxin-3 regulates recruitment and exocytosis of newcomer insulin granules and granule-granule fusion during biphasic glucose-stimulated insulin secretion in pancreatic β-cells

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Introduction

The molecular basis of exocytosis of secretory insulin-containing granules (SGs) during biphasic glucose-stimulated insulin secretion (GSIS) of pancreatic β-cells remains unclear. Syntaxins (Syns) Syn-1A and Syn-4 are t-SNARE proteins situated on the plasma membrane that have been well studied to mediate insulin exocytosis. However, Syn-3, peculiarly more abundant in insulin SGs, has unclear function in GSIS.

Methods/Designs

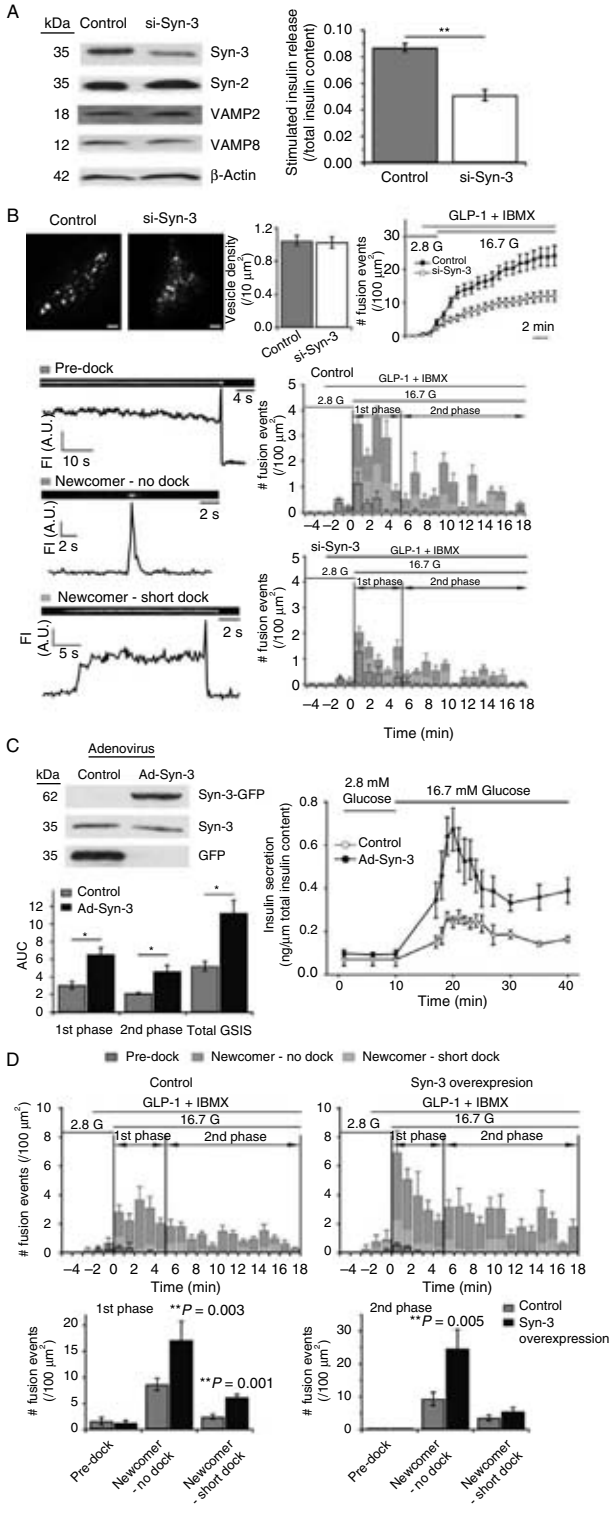
Confocal microscopy confirmed the localization of Syn-3 to insulin SGs in insulin secretory cells. Syn-3 function was assessed by loss of endogenous function by siRNA downregulation of Syn-3 expression, and gain of function by Syn-3 overexpression in INS-1 cell line and rat islet β-cells. Readouts included electron microscopy (EM), insulin secretion by RIA, and visualizing single insulin SG behavior by total reflection fluorescence microscopy (TIRFM).

Results

Depletion of endogenous Syn-3 in INS-1 cells inhibited GSIS by 42%. Time-lapse TIRFM showed no change in the number and fusion competence of docked SGs, and instead, a marked reduction in the recruitment of newcomer SGs to plasma membrane and subsequent slowing of their exocytotic fusion kinetics per se in both fist and second phases encompassing biphasic GSIS. Conversely, overexpression of Syn-3 into mouse islets and INS-1 cells caused enhancement of first and second phases of GLP-1-potentiated GSIS by 2-fold, accounted for by an increase in newcomer SGs, and remarkably, also by increased SG-SG/compound fusion events, the latter confirmed by EM.

Conclusions

Syn-3 in insulin SGs functions to mediate SG-SG fusion and mobilization of newcomer SGs to the plasma membrane, contributing to both first and second phases of GSIS in pancreatic β-cells.




A) Reduction of endogenous Syntaxin-3 levels impairs glucose-stimulated insulin secretion (GSIS). B) Reduction of endogenous Syntaxin 3 levels inhibits recruitment and exocytosis of newcomer insulin granules but with no effect on docked granules. C) Overexpression of Syntaxin 3 enhances GSIS. D) Enhancement of biphasic GSIS by Syntaxin 3 overexpression is due to recruitment and fusion of newcomer insulin granules with plasma membrane.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## OC17.5

### Glucocorticoid receptor α expression is downregulated in gluteal subcutaneous adipose tissue of black South African women and associates with increased metabolic risk

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Introduction

Increased capacity for glucocorticoid regeneration in subcutaneous adipose tissue (SAT) by 11β-hydroxysteroid dehydrogenase-1 (11HSD1) is associated with obesity and associated risk factors. We hypothesised that down-regulation of SAT 11HSD1 and/or glucocorticoid receptor-α (GRα) may explain differences in body fat distribution and metabolic risk between black and white women. The study aimed to compare the expression of 11HSD1 and glucocorticoid receptor-α (GRα), and glucocorticoid-responsive genes in gluteal SAT depots, and determine their relationships with body composition and metabolic risk factors in South African women.

Methods

Body fatness (DXA) and distribution (computerized tomography), insulin sensitivity (SI, intravenous glucose tolerance test) and the expression of 11HSD1, GRα, PPARγ, adiponectin, CD68 and TNFα were measured in gluteal SAT of 56 normal-weight and obese black and white premenopausal South African women.

Results

11HSD1 expression was increased with obesity in both black and white women ($P<0.001$), but did not differ by ethnicity. In contrast, GRα mRNA levels were significantly lower in both normal weight and obese black compared to white women ($0.86\pm0.25$ vs $1.31\pm0.65$ AU and $0.52\pm0.21$ vs $0.91\pm0.26$ AU, respectively, $P<0.01$). Lower GRα expression in black women was associated with increased CD68 ($r=-0.64$, $P<0.001$) and TNFα ($r=-0.39$, $P<0.01$), reduced PPARγ ($r=0.84$, $P<0.001$) and adiponectin mRNA levels ($r=0.47$, $P<0.001$), as well as increased fat mass ($r=-0.61$, $P=0.001$) and serum triglycerides ($r=-0.43$, $P=0.022$), and reduced HDL-cholesterol ($r=0.48$, $P=0.010$) and SI ($r=0.47$, $P=0.016$).

Conclusions

Expression of GRα is downregulated in gluteal SAT of black South African women, and associates with reduced adipogenic capacity and increased metabolic risk factors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## OC17.6

### Meta-analysis of differentially expressed microRNAs in type 1, type 2 and gestational diabetes mellitus

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Introduction

MicroRNAs (miRNA) are noncoding RNAs that play a central role in governing a variety of physiological and pathological processes, and there are few studies on

miR involvement in diabetes mellitus. There are three principal types of diabetes, i.e., type 1 (T1DM), type 2 (T2DM) and gestational diabetes mellitus (GDM). In this study we performed a meta-analysis of the miRNA expression profiling of peripheral blood mononuclear cells (PBMC) from T1DM, T2DM and GDM patients by using the microarray technology.
Methods/Design
Agilent human miRNA microarray kits (v3) $8\times15$ K were used to perform hybridizations of samples from T1DM ($n=4$), T2DM ($n=4$) or GDM ($n=5$) patients that were quantified and analyzed using GeneSpring GX11 pack (Agilent). ANOVA ($P<0.05$) with Benjamini-Hochberg FDR multiple testing correction was performed. Finally, miRNAs exhibiting fold change $\geq2.0$ were considered for the study.
Results
Ten miRNA were differentially expressed in the 3 types of diabetes: hsa-miR-328, hsa-miR-181b, hsa-miR-1306, hsa-miR-181c, hsa-miR-1275, hsa-let-7b*, hsa-miR-939, hsa-miR-623, hsa-miR-595, and hsa-miR-1268. None of these miRNAs were previously described in association with any type of diabetes. Interestingly, two members of miR-181 family contribute to T cell tolerance, and this miRNA family has been involved in the inhibition of IL-2 expression.
Conclusion
Considering that autoimmunity is characteristic of T1DM, considering the similarities of mRNA expression profiles between T1DM, T2DM and GDM, and considering that miR-181 family modulates some cytokine expression, these results indicate that miRNA may be used as marker for diabetes. Financial support: FAPESP, CNPq.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## Paediatric endocrinology

### OC18.1

**Mutations in the NR5A1 gene in patients with 46,XY disorders of sex development (DSD): high frequency of familial multi-generational occurrence**

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The nuclear receptor SF1/NR5A1 regulates transcription of genes involved in reproduction, steroidogenesis and male sexual differentiation. Mutations in humans cause gonadal dysgenesis with or without adrenal failure in both 46,XY and 46,XX individuals. In a cohort of patients with familial 46,XY DSD, we identified 6 heterozygous NR5A1 mutations in 19 subjects from 5 unrelated families (F1-F5). Moreover, a de novo heterozygous mutation in one patient with 46,XY DSD and no affected relatives was also detected. Low ovarian reserve with preserved fertility was detected in females harboring NR5A1 mutations. Extreme within-family variability was found in 46,XY DSD affected patients, with phenotypes ranging from severe fetal undervirilization, prompting female sex of rearing, to spontaneous pubertal development and even preserved fertility. Analyses revealed a W279X heterozygous mutation and an intronic deletion (g3314-3317delTCTC (IVS 4+8) in F1, and a Y183X heterozygous mutation in F2. A novel R313H heterozygous variation was found in F3, and a novel S303R mutation in F4. A novel heterozygous R69H mutation was found in the only 46,XY DSD patient studied in F5, and a de novo G77E mutation in the sporadic case. All new mutations were predicted to affect protein function by prediction models (SIFT, Polyphen and MutationTaster). Mode of inheritance seems to be autosomal dominant with variable penetrance. We emphasize the extreme phenotypic variability, even in siblings with the same mutation. As previously reported, we found spontaneous puberty in 46,XY DSD individuals raised as males, and for the first time we report preserved fertility in one of these affected individuals. Subjects with heterozygous NR5A1 mutations and mild phenotypes, such as isolated hypospadias in 46,XY patients, compensated ovarian dysfunction and early menopause in 46,XX subjects, might easily go undetected. A careful family screening of 46,XY as well as 46,XX individuals is recommended whenever an index case is detected.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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### OC18.2

**Clinical profile, gender choice and long term follow up of subjects with 5 alpha-steroid reductase 2 deficiency**

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Introduction
5 alpha-steroid reductase deficiency (5α SRD) is a rare autosomal recessive disorder. Presented here is the clinical profile and long term outcomes of subjects with 5α SRD examined in our hospital during the last 30 yrs.
Methods
Records of patients registered in the endocrine clinic of our hospital and new patients with diagnosis of 5α SRD were compiled. Details of history,physical examination,chromosomal analysis, hormonal studies, psychological assessment, nature of surgery and follow up were recorded.
Electrochemiluminiscence method was used for the estimation of LH,FSH and T. DHT was done by Radioimmunoassay (RIA)[kit based method (Immunotech, Prague)]after Celite chromatography.
Results
There were 30 patients, age 2 to 28 yrs ($13.01\pm7.4$) (from 27 families) were diagnosed to have 5α SRD during the last 31 years. One family had 4 boys, all with 5α RD. The mean age at which medical attention was sought was 5.6 yrs. 23 patients were assigned female sex while 7 patients were assigned male sex at birth. Among the 23 who were assigned female sex,11 sought medical attention peripubertally,10 opted for male gender re-assignment while 1 opted to continue her female gender status. Eleven patients received medical attention during infancy to early childhood, 9 had male gender assignment and 2 had female gender assignment on medical advice. One of them,11 years, reared as female is under evaluation. The 2 patients who had feminizing genitoplasty during infancy presented with gender dysphoria at age 8 and 11 years. One of them had gender reassignment after 2 years of follow up. Gender dysphoria was not observed among any of the children initially assigned male gender. Two patients are married, wife of one of them was pregnant at the last follow up. The second patient had normal LH, FSH and Testosterone at the initial evaluation. Semen analysis at age 16 revealed sperm count of 16 million/ml. He married at age 24, semen analysis at age 26 revealed azoospermia, elevated LH and FSH and subnormal testosterone.
Almost all patients who grew up as females without medical attention had opted for male gender assignment peripubertally. No gender dysphoria was observed among those reared as boys.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### OC18.3

**Effects of vitamin D insufficiency and its correction on insulin sensitivity and serum osteocalcin concentration in obese children**

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Background
Vitamin D insufficiency (VDI) was reported to be associated with decreased insulin sensitivity (IS). Individuals with higher osteocalcin (OC) were shown to have better IS. Vitamin D regulates OC synthesis. Obese children carry an abnormal glucose homeostasis risk. Whether correction of VDI improves IS in obese children and mediates such effect via OC are unclear. We, therefore, studied glucose homeostasis, IS, vitamin D status and serum OC concentration in 230 obese children and examined vitamin D treatment effect on those parameters in 42 obese children with VDI.
Methods
Serum 25-hydroxyvitamin D (25-OHD) and OC levels were measured. An oral glucose tolerance test (OGTT) was performed. IS indices including HOMA-IR, Matsuda index and QUICKI and β-cell function, insulinogenic index (IGI) were calculated. Patients with VDI (25-OHD $<30$ ng/ml) were treated with 560 000 units of vitamin D2 and underwent the second OGTT after treatment completion.

Results
Of 230 patients, 58 (25%) had impaired glucose tolerance, impaired FPG and diabetes. One hundred and fifty-nine (69%) had VDI (25-OHD 22.3 (4.5) ng/ml), while the remaining 69 patients had adequate 25-OHD level of 34.6 (2.9) ng/ml. Comparing between patients with vitamin D sufficiency and insufficiency, there were no differences in BMI Z-score, OC, IS and β-cell function. However, 42 VDI patients who had 25-OHD increased to 55.5 (14.8) ng/ml after treatment completion had Matsuda index and QUICKI significantly increased from the pre-treatment values. Furthermore, the percentage of patients with abnormal OGTT was reduced from 33 to 19%. There were no changes in IGI and OC. OC was positively correlated with IGI ($r=0.218$, $P=0.001$) but not with IS.
Conclusion
Correction of VDI in obese children could enhance IS but not β-cell function. Critical level of 25-OHD may be needed to augment IS. IS enhancement seems not to be mediated by OC.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## OC18.4

### Mortality in GH-treated (Tx) patients (pts) enrolled in the global genetics and neuroendocrinology of short stature international study (GeNeSIS)

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Preliminary data from the French SAGhE study of 6928 pts with isolated idiopathic GH deficiency (IsIGHD), idiopathic short stature (ISS) or born small for gestational age (SGA) who started GH treatment during childhood (1985–1996) & were followed up in 2009, suggested increased mortality vs the French general population (pop; standardized mortality ratio (SMR): 1.3, 95% confidence interval (CI): 1.1–1.6; 116 403 person-years (PY))[1].
To assess mortality in a global pediatric observational study, deaths in GeNeSIS pts (all diagnoses, incl. organic & syndromic) were identified; for reference to SAGhE data, mortality rates were calculated for GH-Tx pts with IsIGHD, ISS & SGA. SMR/95% CI were calculated using age- & sex-specific pop rates from CDC (USA) & WHO (other countries).
Among 18 147 pts (all diagnoses) 33 deaths were reported (30/17 692 GH-Tx, 3/455 non-GH-Tx) during median (Q1, Q3) follow-up of 2.0 (0.9, 3.8) years. Deaths were due to acute illness (16), recurrence of pre-existing intracranial neoplasms (8, incl. the 3 non-GH-Tx pts), second cancers (2, both irradiated), accidents (3), underlying conditions (2) & unknown causes (2). 5 deaths were in pts with IsIGHD, ISS & SGA (Table).
The total death rate in GH-Tx pts with IsIGHD, ISS & SGA in GeNeSIS was similar to the 2007 US age adjusted mortality rate for children aged 5–14 years (rate: 15.3/100 000 PY)[2]. SMRs for each diagnosis individually & combined were not elevated.
There was no evidence of increased mortality in the GH-Tx IsIGHD, ISS or SGA pts in GeNeSIS. However, our analysis in pts during GH treatment is not directly comparable with SAGhE. Other limitations include comparison with general pop data (lacking untreated controls) & limited follow-up time.
References
[1] Carel J-C *et al*. 2011 *Endocr Rev* **32** LB-5.
[2] Xu J *et al*. 2010 *National Vital Statistics Reports* **58**(19).

**Table 1** GeNeSIS mortality rates & SMR in GH-Tx pts with IsIGHD, SGA & ISS

| Diagnosis | *n* | Deaths | PY[a] | Mortality rate (95% CI)[b] | Expected deaths[c] | SMR (95% CI) |
|---|---|---|---|---|---|---|
| IsIGHD | 7712 | 3 | 18 714 | 16.0 (3.3–46.9) | 12.0 | 0.3 (0.1–0.7) |
| ISS | 2538 | 1 | 5060 | 19.8 (0.5–110.1) | 2.2 | 0.5 (0.0–2.6) |
| SGA | 826 | 1 | 1860 | 53.8 (1.4–299.6) | 1.4 | 0.7 (0.0–4.0) |
| Total | 11 076 | 5 | 25 634 | 19.5 (6.3–45.5) | 15.6 | 0.3 (0.1–0.8) |

[a]Follow-up from study enrolment.
[b]Rate/100 000 PY.
[c]Based on: http://wonder.cdc.gov (US) & http://apps.who.int/ghodata (other countries).

Declaration of interest
The authors declare that there is a conflict of interest.
Funding
This work was supported, however funding details are unavailable.

## OC18.5

### Earlier reactivation of the hypothalamo-pituitary-gonadal axis and advancement of puberty in boys under labour stress

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The reawakening of the hypothalamo-pituitary-gonadal (HPG) leading to attainment of sexual maturation and reproductive capacity is influenced by several internal and external factors. Amongst external factors, adverse physical or psychological conditions may alter the timing of onset of puberty. The present study examined the effect of persistent and severe physical stress on timing of onset of puberty. The study included non-working school/college going and working boys of 10–20 years of age ($n=594$). Blood samples were obtained, plasma was separated and concentrations of (LH), (FSH), testosterone (T), inhibin B and cortisol were measured using specific ELISA systems. Data were analyzed using student's T test and ANOVA. In non-working boys, concentrations of LH and T gradually increased, attaining first peak at 15 and a second peak at 18 years, whereas concentrations of FSH and inhibin B progressively increased and peaked at 14 years. The concentrations of FSH were maintained thereafter, whereas levels of inhibin B slowly declined to low levels. In working boys, concentrations of LH, which were several fold higher at 10 and 11 years compared to school going boys, increased progressively and reached first peak at 14 and a second peak at 17 years. The levels of FSH steadily increased and attained a plateau at 13 years. The concentrations of T were higher at 11 and 12 years and attained first peak at 13 and a second peak at 17 years. The concentrations of inhibin B gradually reached maximum concentrations at 13 years, declining thereafter to low concentrations at 20 years. The concentrations of cortisol were significantly higher in working boys of all age groups. In conclusion, the present study demonstrates that boys under labor stress experience an earlier reawakening of HPG axis and an advancement of puberty by a year or so.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## OC18.6 The Young Investigator Winner

### Functional characterization of a new mutation in the NKX2.1 in patients with thyroid–lung–brain syndrome

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TTF1/NKX2.1 is a transcription factor expressed in thyroid, lung and brain. Several heterozygous mutations have been described in patients with primary congenital hypothyroidism, respiratory distress and benign hereditary chorea, typical aspects of the 'thyroid–lung–brain syndrome'.
We recently studied a family affected by some of these features, and the direct sequencing of the NKX2.1 gene demonstrated an heterozygous deletion of a cytosine at position 665 that causes a frameshift with the formation of a truncated protein.
Aim of our study was to better characterize the molecular effects determined by the C665del mutation.
The transcriptional properties of both WT and C665del NKX2.1 were investigated by cotransfection assays. As expected, the C665del mutant is unable to transactivate the Tg, the artificial C5 and the SP-C promoters. Interestingly, we demonstrated a dose-dependent dominant negative effect of the C665del on the WT NKX2.1 on Tg or C5 promoters. This dominant negative effect is absent on

the SP-C promoter. To further understand the dominant-negative action, we performed cotransfection experiments with PAX8, observing that the mutant is unable to modify the PAX8 basal activity. Moreover a dominant negative effect is also present when both WT and mutant are cotransfected with PAX8. Same results can be observed in presence of known NKX2-1 coactivators such as P300 or WWTR1.
Then, we evaluated the binding of NKX2.1 to DNA in the presence of NKX2.1 C665del by electrophoretic mobility shift assay (EMSA). This binding remains unaltered but the C665del, alone, is unable to bind to DNA. These data suggest that the mutated NKX2.1 protein produces dominant-negative effects on the wt NKX2.1 in promoter-specific manner and it interferes with the activity of wt, but not with its binding to DNA.
In conclusion, these experiments allow to understand the functional relevance of the different domains in the NKX2.1 protein and show the high variability between genotype-fenotype in patients with TLB syndrome. On the basis of our data it seems reasonable to consider that the pathogenetic mechanisms underlying the effect of the mutations are very complex, and tissue-specific.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## Cardiovascular Endocrinology

### OC19.1

**Both EPA/AA ratio and absolute AA levels constitute an independent risk factor for coronary atherosclerosis in type 2 diabetic patients**

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Background
N-3 polyunsaturated fatty acids (PUFA), such as α linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to modify several key risk factors for cardiovascular disease, although n-6 PUFA arachidonic acid (AA) has important roles in inflammation. However, it is not clear whether the apparent protection of n-3 PUFA against cardiovascular disease has been found in diabetic patients.
Methods
To clarify the effects of PUFA, firstly we calculated fasting serum PUFA, LDL-cholesterol, HDL cholesterol and triglyceride (TG) levels three times in type 2 diabetic patients. Those patients had been never treated with EPA drugs. Next we have followed the patients, focusing on newly onset of acute coronary syndrome, which have been undergoing elective coronary intervention for three years. Then, we divided diabetic patients into two groups in which acute coronary syndrome (ACS, PCI-group, $n=42$) was observed or not (DM-group, $n=44$), whereas 36 normal glucose tolerance subjects (Nor-group) were studied as control. Finally, we analyzed EPA/AA ratio and absolute EPA, AA levels among three groups. These values were analyzed using an ANOVA model.
Results
EPA/AA ratio in PCI-group (0.37) were significantly lower than in DM (0.47, $P<0.05$) and in Nor-group (0.52, $P<0.01$), although absolute AA ratio in PCI-group (207.2 mol%) was statistically higher than in DM (173.9, $P<0.05$) and in Nor-group (162.3, $P<0.01$). Moreover, absolute EPA levels were statistically lower in PCI-group compared to DM and Nor-group ($P<0.05$, $P<0.05$), whereas the average levels of LDL, HDL-cholesterol and HbA1c were not significantly changed among three groups.
Conclusion
A negative correlation was noted between the event of coronary artery disease and both EPA/AA ratio and absolute EPA values in diabetic patients. In addition, constantly high AA levels in PCI-group may contribute the incidence of acute coronary syndrome. We also suggest that both EPA/AA ratio and absolute EPA levels constitute an independent risk factor for coronary atherosclerosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### OC19.2

**Increased arterial stiffness is independently associated with cerebral infarctions and white matter lesions in patients with type 2 diabetes despite good blood pressure and lipid control**

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Aim
Patients with type 2 diabetes (T2DM) have increased risk of cardiovascular disease (CVD) including stroke. The risk of CVD is traditionally assessed using office blood pressure (BP) and lipid profile. Increased arterial stiffness predicts cardiovascular events in the general population. We investigated if arterial stiffness was associated with cerebrovascular disease in patients with T2DM and sex- and age-matched controls indepentl y of classical risk factors.
Methods
patients with newly diagnosed T2DM and Ninety sex- and age- matched controls were examined. Arterial stiffness was assessed by aortic pulse wave velocity (PWV), and cerebrovascular disease by cerebral infarctions and severity of white matter lesions (WMLs) on MRI scans of cerebrum. A blinded reviewer rated WMLs a.m. Breteler (no/slight changes = 0, moderate = 1, severe = 2).
Results
Antihypertensive treatment and lipid lowering treatment was more frequent in diabetic patients, who consequently had lower office BP (126 +/− 12 vs 131 +/− 14 mmHg systolic, $P=0.01$) and lower lipid levels. Despite this, diabetic patients had significantly higher PWV compared to controls, (9.2 +/− 2.0 vs 8.0 +/− 1.6 m/s, $P<0.0001$). PWV was higher in patients with cerebral infarctions (9.9 vs 8.5 m/s, $P=0.002$) and PWV increased across Breteler categories (8.2 +/− 1.7 vs 9.3 +/− 2.0 vs 9.4 +/− 2.1 m/s, $P<0.001$ for trend). PWV remained independently associated with severity of WMLs ($P<0.01$) and cerebral infarctions, ($P<0.02$) after adjustment for the following covariates: age, sex, diabetes, mean arterial pressure, smoking, statins and BMI in multivariate regression.
Conclusion
Despite good BP and lipid control, PWV was substantially higher in T2DM patients. PWV is independently associated with WMLs and cerebral infarctions. PWV may represent a clinically relevant parameter in the evaluation of CVD risk in T2DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details are unavailable.

### OC19.3

**Copeptin and adrenomedullin in a large cohort of patients with coronary heart disease and newly diagnosed glucose intolerance ('Silent diabetes study')**

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Background
Copeptin is used as a diagnostic marker for acute myocardial infarction, adrenomedullin as a prognostic marker for congestive heart failure. Both hormones may be involved in pathophysiology of metabolic syndrome.
Methods
We have used sera of the 'Silent diabetes study' (1). Sera of 920 patients were eligible for this analysis. All patients underwent an oral glucose tolerance test, patients with previously known diabetes were excluded. In coronary angiography, 59 patients had no coronary disease (no CHD), 152 patients had only stenosis <50% (beginning CHD), 164 had 1-vessel disease (1-VD), 172 had 2-vessel (2-VD) and 230 had 3-vessel disease (3-VD). In oGTT 393 patients had normal glucose tolerance (NGT), 279 had impaired glucose tolerance (IGT) and 105 had diabetes mellitus (DM). Copeptin and MR-pro-adrenomedullin (MR-proADM) were measured in the BRAHMS Kryptor assay (Thermo Fisher Scientific). Statistical analysis was performed by ANOVA and Kruskal–Wallis methods.

Results
Patients with no CHD had significantly lower copeptin levels (4.4 + 2.02 pmol/l) compared to patients with beginning CHD (6.94 + 6.29, $P = 0.023$), 1-VD (6.91 + 6.84, $P = 0.03$), 2-VD (6.64 + 5.57, $P = 0.012$) or 3-VD (8.93 + 10.20, $P < 0.0001$), differences between patient groups were not significant. Patients with 3-VD (0.71 + 0.27 nmol/l, $P = 0.0002$) and beginning CHD (0.68 + 0.24, $P = 0.009$) had higher MR-proADM levels compared to no CHD patients (0.57 + 0.13). Both patients with DM (9.8 + 10.15 pmol/l, $P < 0.0001$) and IGT (8.11 + 9.17, $P = 0.0003$) had higher copeptin levels compared to patients with NGT (6.1 + 5.91). There was no significant difference between patients with DM and IGT. Patients with NGT had lower MR-proADM levels (0.63 + 0.17 nmol/l) compared to patients with IGT (0.71 + 0.29, $P < 0.0001$) or patients with DM (0.71 + 0.22, $P = 0.005$).
Discussion
Copeptin is elevated in patients with CHD compared to those with no CHD but there is no grading to the severity of CHD. MR-proADM is elevated in patients with advanced CHD (3-VD) and in beginning CHD compared to no CHD patients. Both marker are elevated in patients with IGT and previously unknown DM. The early elevation of both hormones may argue for an early involvement in the development of the metabolic-vascular syndrome.
1 Doerr *et al.* Diabetologia 2011 **54**2923–2930.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details are unavailable.

## OC19.4

**Oxytocin is cardioprotective hormone**
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We have uncovered the entire functional oxytocin (OT) system in the rat and human heart. In the experimentally-induced myocardial infarction (MI) in rats, the continuous OT delivery improves the cardiac work, reduces inflammation, and stimulates angiogenesis. We hypothesized that these actions of OT are mediated by increase in glucose uptake in cardiomyocytes (CM). Indeed OT (10 nM) increased basal glucose uptake in CM to 4.0 ± 0.2 fmol/mg protein in comparison to 2.2 fmol/mg in control cells ($P < 0.001$). OT had a moderate synergistic effect with 0.1 mM 2, 4-dinitrophenol (a hypoxia-inducing agent, DNP), augmenting basal glucose uptake to 5.5 ± 0.5 fmol/mg. Wortmannin (0.1 μM), an inhibitor of phosphatidylinositol-3-kinase (PI3K), significantly suppressed OT and insulin (10 nM) ($P < 0.001$) effects on glucose uptake, indicating a common pathway. The activation of PI3K pathway suggested beneficial effects of OT in prevention of CM hypertrophy. Analysis of the cells treated with OT revealed accumulation of ANP in perinuclear region and its co-localization with mitochondria without changes of cell size. Further study demonstrated that OT inhibits CM hypertrophy induced by GqPCR agonists such as endothelin-1, inhibits CM apoptosis and decreases oxydative stress after exposure to ischemia-reoxygenation, the conditions, mimicking the myocardial infarction. The pre-treatment of cells with OT (10 nM) completely inhibited the hypertrophic effect induced by ET-1 both in the newborn and adult rat CM. The OT receptor antagonist and cGMP inhibition abrogated the anti-hypertrophic effect of OT. Furthermore, Western blot analysis indicated that OT treatment modulates phosphorylation of proteins downstream of PI3K such as PKB/Akt. In summary, these findings suggest that OT cardioprotective action is due at least in part to stimulation of PI3K signaling pathway and ANP accumulation in CM. Therefore, in pathological conditions, OT plays a cardioprotective role, and improves vascular and metabolic functions, thus OT presents potential for therapeutic use.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details are unavailable.

## OC19.5

**Circulating adrenomedulin levels are associated with atrial natriuretic peptide and brain natriuretic peptide levels in heart failure patients**
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Introduction
Heart failure is characterised by an up-regulation of neurohoromones, and brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) are well-established diagnostic biomarkers with substancial prognostic value. Adrenomedullin is a vasoactive peptide and a potential biomarker of vascular injury in patients with cardiovascular disease (CVD) The aims of the present study were to investigate whether mid-regional pro adrenomedullin (MR-proADM) is predictive of outcome in heart failure (CHF) patients and if MR-proADM is associated with MR-proANP or NT-proBNP.
Materials and methods
Prospective, observational study of 360 unselected CHF patients included at baseline (30% female, mean age 71 years). 63% had CVD. Patients were followed for a median of 17 months with respect to mortality. 184 patients died and 229 were hospitalised.
Results
Mean (S.D.) MR-proADM levels were 0.75 (0.42) nmol/l, mediann (interquartile range) MR-proANP levels were 238 (149–362) nmol/l and NT-proBNP levels were 1138 (469-2636) pg/ml. MR-pro ADM was associated with age ($r = 0.28$, $P < 0.001$), s-creatinine ($r = 0.32$, $P < 0.001$), NT-proBNP ($r = 0.29$, $P < 0.001$) and MR-proANP ($r = 0.42$, $P < 0.001$). Using Cox proportional hazard analysis increasing logarithmic levels of MR-proADM were predictive of mortality HR being 1.32 (1.29–1.35, $P = 0.007$) and MR-proANP were predictive of mortality HR being 1.36 (1.33–1.39, $P = 0.013$) after adjusting for age, gender, CVD, NYHA, systolic blood presure, LVEF and treatment with β-blocker or aldosteron antagonist. When adding NT-proBNP to the model HR of mortality was 1.21 ($P = 0.09$) for MR-proADM and 1.13 ($P = 0.42$) for MR-proANP.
Conclusion
MR-proADM levels were associated with both NT-proBNP and ANP. MR-proADM was predictive of mortality after adjustment for well known risk factors in CHF. Neither MR-proADM or MR-proANP was independently related with outcome after including NT-proBNP in the multivariate model.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## OC19.6

**Endothelial progenitor cells in acromegaly are reduced and responsive to treatment with somatostatin analogues**
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Introduction
Acromegaly is characterized by high cardiovascular morbidity and mortality possibly due to increased prevalence of main traditional cardiovascular risk factors. It is not clear though whether the incidence of atherosclerosis is truly enhanced. Moreover recent *in vitro* studies show a protective role of (GH) and IGF1 (insulin-like growth factor-1) on the endothelium. As expression of endothelial regenerative reserve, endothelial progenitor cells (EPCs) could clarify the complex balance between pro- and anti-atherogenic factors in acromegaly. We therefore aimed to study the distribution of EPCs within a sample of acromegalic patients.
Materials and methods
42 acromegalic patients (female/male ratio = 1.5; mean age = 51 ± 12.75 ys) underwent a clinical evaluation and a blood test including count and typization of peripheral EPCs using monoclonal antibodies CD34+ or CD133+ or KDR+ specific. Data were compared to fourty two control subjects matched by age, gender and class of glucose tolerance. In a group of 9 patients with active disease (normalized IGF1 > 1) the whole evaluation was repeated after 24 weeks of treatment with somatostatin analogues (SSA).

Results

Acromegalic patients show a significantly lower number of CD34+/KDR+ cells than controls ($P=0{,}005$) while CD34+/CD133+/KDR+ cells are reduced in active patients compared to the inactive group ($P=0.00001$) and increase after SSA treatment ($P=0.03$). In acromegalic patients, the number of CD34+/CD133+/KDR+ cells correlates with IGF1 levels ($R=-0.56$, $P=0.0001$), fasting plasma glucose ($R=-0.46$, $P=0.002$), plasma insulin ($R=-0.33$, $P=0.03$) and HOMA index ($R=-0.37$, $P=0.02$), IGF1 showing to be the most important covariate in a multivariate analysis ($\beta=-0.35$, $P=0.03$). In acromegalic and controls the CD34+/KDR+ variability is mainly explained by diastolic blood pressure ($\beta=-0.26$, $P=0.03$).

Conclusions

Acromegalic patients seem to have a reduced endothelial regenerative capacity and an increased cardiovascular risk probably related to IGF-1 levels, glucose metabolism alterations and hypertension. Treatment with SSA could ameliorate EPC peripheral pattern.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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# Nurses Abstracts

## N1

**Clinical practice guideline on linear growth measurement of children**

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Introduction
Growth is an important and sensitive indicator of child health. Abnormal growth is a common consequence of many conditions, therefore its identification acts as a useful warning of possible pathology. Effective growth monitoring requires precise linear growth measurements; however, measurements are often inaccurate and unreliable. Measurement error influences the interpretation of growth patterns resulting in failure to identify underlying pathology or apparent growth divergence in normally growing children.
Purpose
The purpose of this project was to develop a clinical practice guideline to assist professionals in applying evidence-based knowledge in measuring children using standardized instruments and techniques to facilitate accurate and reliable growth assessments.
Methods
Systematic methods were used to identify evidence to answer focused clinical questions about linear growth measurement. A multidisciplinary team of health professionals critically appraised and synthesized the evidence to develop explicit clinical practice recommendations using an evidence-based practice rating scheme. The guideline was prospectively evaluated through internal reviews, external expert reviews, and a pilot study.
Results
Data analyses were used to improve guideline clarity, applicability, and feasibility, while demonstrating validity and intraexaminer and interexaminer reliability ($P<0.0001$). The guideline provides clinical practice recommendations for measurement techniques, measurement instruments, use of less expensive instruments, calibration of instruments, diurnal height variation, measuring height versus length, replicate measurements, and special considerations. Each recommendation is linked with scientific rationale and supporting references. Tools are available to facilitate guideline implementation.
Conclusions
The clinical practice guideline on linear growth measurement is based on the best available evidence and was developed using rigorous methods. Health professionals and parents need accurate and reliable growth information. Guideline use will improve timely recognition, diagnosis, and treatment of growth disorders, while reducing unnecessary and costly evaluations in normally growing children. Widespread dissemination and adoption of the guideline can significantly impact child health around the globe.
Keywords: linear growth, children, evidence-based, clinical practice guideline.

## N2

**The nurse's role in establishing modern testosterone replacement therapy in an endocrine clinic**

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Our nurse-led testosterone replacement clinic in the Waikato Endocrine Service was the only clinic in New Zealand where specialised endocrine nurses established the use of and administered subcutaneous implants using testosterone pellets for hypogonadal men. Information gained through networking with nursing colleagues at the Annual Endocrine Nurses Society of Australasia (ENSA) symposia regarding the introduction of the depot injection Testosterone Undecanoate (Reandron™) has enabled the implants to be superseded by this more modern and patient-friendly therapy. We report on the development of our testosterone replacement programme in which we are currently treating 250 hypogonadal men using intramuscular Reandron™. The results and side-effects of treatment for this large cohort of patients for up to 2 years will be presented. The endocrine nurses have extended the programme to involve the patient's primary health care team, which whilst maintaining good therapeutic practice has improved convenience and access for patients. This presentation demonstrates that sharing best practice and evidence-based research at conferences such as the ENSA symposia can lead to significant changes in nursing practice and improved patient care.

## N3

**Interdisciplinary risk assessment and patient discharge management: combining biomarker & clinical scores, medical & nursing aspects**

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Introduction
Current medical and nursing scores have limited efficiency and safety profiles to assign the most appropriate treatment site to patients with lower respiratory tract infections (LRTIs). We assessed the potential of a combined score of CURB65 with proadrenomedullin (ProADM) levels and the adapted post-acute discharge score (PACD) for triage and discharge management decisions. We aimed to identify the proportions of patients who would be best cared for at different levels of care ranging from home with or without home-health care (Spitex), health ressort, nurse-led unit (NLU), and rehabilitation to acute-care hospitals.
Methods
Consecutive patients with LRTIs presenting to our emergency department were prospectively followed and retrospectively classified according to CURB65 and ProADM levels (CURB65-A) and biopsychosocial risk (PACD). We compared proportions of patients virtually allocated to triage sites with actual triage decisions and assessed the added impact of ProADM and the accuracy of the PACD.
Results
Overall, 93% of 146 patients were hospitalised. Among the 138 patients with available CURB65-A, 17.4% had a low medical risk indicating possible treatment in an outpatient or non-acute medical setting; 34.1% had an intermediate medical risk (short-hospitalisation); and 48.6% had a high medical risk (hospitalisation). Reasons for staying in the acute care setting after resolving medical problems (mean 3.6 days) were predominantly organisational (e.g. waiting for a post-acute care facility, 43 (69%) of the 62 nursing overruling reasons). The PACD identified in 55% ($n=202$) of the LRTI patients a risk for post-acute care needs (sensitivity 82%; specificity 55%).
Conclusion
Current rates of hospitalization are high in patients with LRTI and length of stay frequently extended beyond time of medical stabilization. This suggests a substantial potential of an interdisciplinary and biomarker-enhanced triage. The PACD was able to predict post-acute care needs and qualifies for screening purposes to facilitate early interdisciplinary discharge management.
Keywords: interdisciplinary risk assessment, post-acute care needs, triage, discharge management.

## N4

**Effect of metformin in teenagers with metabolic syndrome**

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Research objective
To estimate efficiency of metformin use in children and teenagers with metabolic syndrome (MetS).
Materials and methods
Eighty children and teenagers aged 10–16 with exogenous-constitutional obesity (ECO) were observed. From them in 19 (23.8%) MetS was diagnosed according to diagnostic criteria of IDF (2007); 15 of them (78.9% – seven boys and eight girls) were prescribed metformin (Siofor, Berlin Chemie) in dose from 1000 to 1500 mg a day on the background of life style change (hypo-calorie diet and physical activity). Middle age was $12.5\pm0.58$ years (Me-12). Duration of treatment and observation was 6 months.
Results and their discussion
In 6 months after treatment in observed group the component of MetS – waist circumference (WC) significantly decreased from $93.7\pm2.82$ to $85.8\pm2.47$ sm ($P=0.04$). Also we observed non-significant reduction of weight from $69.8\pm5.01$ to $66.7\pm4.78$ kg ($P=0.66$), and BMI from $30.1\pm1.37$ to $28.7\pm1.27$ kg/m$^2$ ($P=0.47$).

On the background of the therapy statistically significant decrease in level of TG from 2.62±0.23 mmol/l (95%CI 2.16–3.07) to 1.83±0.20 mmol/l (95%CI 1.45–2.51) ($P=0.02$) was found. Along with it the tendency to significant increase of level of HDL Cholesterol (from 1.14±0.10 to 1.96±0.18 mmol/l, $P=0.0001$) was found.
Level of fasting glycemia (from 4.43±0.19 to 4.14±0.15 mmol/l, $P=0.24$) and HbAlc (from 5.56±0.20 to 5.05±0.22%, $P=0.24$) decreased accordingly by 6.5 and 9.2%. Hemodynamic indicators decreased non-significantly, as they initially were within normal range.
Conclusion
On the background of metformin treatment in six (31.6% – three boys and three girls) of observed diagnosis of MetS was withdrawn; there were no side-effects during metformin use revealed.

## N5

**Translation and cultural adaptation of the Brazilian version of patient assessment of chronic illness care: Pacic-Brazil**

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Background
The Chronic Care Model (CCM), developed by Wagner *et al.*, at MacColl Institute for Heathcare Innovation at Group Health Research Institute, Seattle, Washington, United States, is one approach to improving chronic illness care, including diabetes *mellitus* (DM), that is being used increasingly to assess and improve care. The CCM is based on evidence-based practices and reviews of the literature on effective care. The Patient Assessment of Chronic Illness Care (PACIC) is a 20-item questionnaire assessing the implementation of the CCM from the patient perspective that focuses on the receipt of patient-centered care and self-management behaviors.
Objective
To translate and conduct the cultural adaptation of the Brazilian version of the PACIC in Brazilian patients with DM.
Methods
Methodological study, whose adaptation process cultural included: forward translation, expert committee, back-translation and cognitive interview. The study addresses a sample of 50 Brazilian patients with DM in a Basic Health District Unit in Ribeirao Preto, Sao Paulo, Brazil in 2010.
Results
The former revealed good acceptance of the translated version of the instrument, which participants considered having items of easy understanding. After analysis of the psychometric properties and completion of the validation process, the instrument will become available to Brazilian researchers, enabling its comparison with other cultures.
Conclusion
It is concluded that a culturally adapted instrument in the Brazilian context with a focus on quality of care can provide support for health staff and managers in the planning the health care of patients with chronic illness, especially DM.
Comments/keywords
Comments for the Nurse Abstracts Markers: a culturally adapted instrument in the Brazilian context with a focus on quality of care for patients with DM can ensure continuity of health services and clinical practice of nursing.
Keywords: Chronic Disease, Diabetes Mellitus, Validation Studies, Nursing.

## N6

**Perception of quality of care in patients with pituitary disorders**

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Background
In order to support patients with lifelong chronic illness, such as pituitary disorders, it is a challenge to continuously offer high quality of care.
Aim
The aim of this study was to examine how patients with pituitary disorders of different causes perceive quality of care in contact with a specialized endocrinology reception.
Methods and materials
Randomly selected, patients with chronic pituitary disorders ($n=100$) were asked to participate. Seventy-seven patients (females, $n=44$), 22–82 years of age responded and participated in the study. The questionnaire Quality from the Patient's Perspective (QPP; modified short version) was used to measure patient's perception of quality of care (considered from four dimensions: physical-technical, medical-technical, identity- orientation and socio-cultural atmosphere). Each question was calculated using an action index of each investigated area. Impaired quality of care in specific dimensions above 15% indicates need of improvement. In addition two open-ended questions were asked.
Results
Most of the respondents, 97%, expressed that necessary physical-technical equipment was available while in the medical-technical dimension 26% reported impaired quality of care. In the identity-orientation dimension, impaired quality of care was reported by 25% mainly due to non-participation in care- and treatment decisions as well as concerning information about results of treatments and self-care activities. In the socio-cultural dimension impaired quality of care was reported in 25%. In addition, the patients asked for extended telephone receptions at the clinic and improved information about pituitary disorders.
Conclusion
The patients were satisfied with the technical part of the medical care, but less satisfied with participation in care decisions and information about self-care. In our setting improvements are needed regarding patient information and access to care.
Comments/keywords
Keywords: pituitary disorders, quality of care, participation, patient satisfaction.

## N7

**Neutralization of Flightless I (Flii) using Flii-specific monoclonal antibodies accelerates impaired healing in diabetic wounds through improved cell proliferation**

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Ulceration is a common and severe complication of diabetes affecting 15% of the 200 million patients with diabetes worldwide. A significant proportion of these chronic wounds fail to respond to conventional treatment, hence major amputation is a feared outcome of diabetes. More than 60% of non-traumatic amputations in the western world are performed in the diabetic population. There exists a growing need for effective therapeutic agents to improve healing in these wounds.
The manipulation of actin-remodelling protein Flightless I revealed its important role as a negative regulator of wound healing. Reductions in Flightless produce improvements, whereas elevated expression impairs wound outcomes. We have studied the biopsy tissue recovered from patients with diabetic ulcers. Immunohistochemistry showed increased expression of Flightless in diabetic wounds compared to unwounded skin samples.
Both diabetic wound fluid and Flightless have previously been shown to inhibit cell proliferation and migration. Therefore to determine if the inhibitory effect of wound fluid could be due in part to Flightless, chronic fluids were preincubated

with Flightless neutralizing antibodies (FliiAb) and the effect on fibroblast proliferation determined. FliiAb treated chronic wound fluid ablated the inhibitory effect of chronic wound fluid with fibroblast proliferation being restored to untreated control levels.
It is hypothesized that reduction of Flightless expression in the skin will create permissive environment for improved diabetic wound healing. Using in vivo wound healing models, wounds were created on the back of Type 1 diabetic and non diabetic mice. Diabetic mouse wounds healed faster when Flightless gene expression was reduced. Indeed neutralization of Flightless activity by Flightless specific monoclonal antibodies in WT diabetic wounds lead to higher rate of re-epithelialisation and acceleration of diabetes-impaired wound healing. These results suggest that reducing the activity of Flightless improves diabetic wound healing, which is promising for future development of new therapies for diabetic ulcers.
Comments/keywords
I am a podiatrist with 5 years of experience of working with both Type 1 and Type 2 diabetes patients. I am especially interested in the treatment of non healing diabetic wounds. I am currently undertaking a PhD by research at the University of Adelaide, South Australia. I am in my last year of PhD and I expect to submit my thesis in 3 months. I would be honoured to present the findings of my research results as part of the 'nurse session' of the 15th International Congress of Endocrinology.
Keywords: Wound healing, Flightless protein, diabetes, Flightless neutralizing antibodies, diabetic foot ulcers.

## N8

**Diabetes nurse leadership group: a forum for improving diabetes care in Brooklyn, NY**

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State University of New York (SUNY) Downstate Medical Center, University Hospital of Brooklyn, Brooklyn, New York, USA.

We created a forum to meet on a regular basis to join forces to facilitate providing optimal diabetes care and education for the members of the hospital, academic and communities of Brooklyn.
Learning Objectives:
1. To identify the opportunities where CDE's can have an impact on the education, management and prevention in and out of the hospital and academic setting.
2. To illustrate the use of CDE's in an urban academic medical center to optimize the education of nurses, patients and the community in diabetes management and prevention.
3. To describe the process of using CDE's in diabetes improvement initiatives and education in an academic urban hospital and in the Brooklyn community.
Content Outline
1. CDE Opportunities in an Urban Academic Medical Center
   a. Inpatient
   b. Outpatient
   c. Education of staff
   d. Education of students
   e. Community Health Forums
   f. Research
2. Utilizing CDE's in an Urban Academic Medical Center
   a. In-servicing staff
   b. Precepting students
   c. Conducting Workshops
   d. Representation on Hospital Wide Clinical Committees
   e. Research Activities
   f. Implementing new policies/procedures
   g. Evaluating new policies/procedures
3. The Process of Utilizing CDE's
   A CDE's involvement in:
   a. Community Activities
   b. Patient Clubs
   c. Regulatory Affairs
   d. Nursing Education
   e. Physician Education
   f. Medical Resident/Physician Education in-servicing
   g. Preceptorship of Nurses
Comments/keywords
CDE, diabetes, leadership.

## N9

**Childhood osteoporosis: screening, prevention, treatment and safe handling practices in a tertiary care pediatric hospital**

Nicole Kirouac[1], Leslie Galloway[1], Joanna Gies[4], Raili Macleod[1], Paige McCullough[1], Anna Mendoza[1], Kathy Miller[1], Gina Rempel[1,4], Arlene Stocki[1], Shayne Taback[2,3], Sandy Taylor[1,3] & Brandy Wicklow[1,2]
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Osteoporosis is a challenge facing children of all ages with a variety of health conditions and physical abilities. The reality of this challenge spurred the development of the child health program's interdisciplinary bone health project team in a tertiary care in-patient pediatric hospital. The team's goal was to develop protocols and tools to identify 'at risk' children and ultimately prevent fragility fractures in this group of children. The use of standardized evidence-based diagnosis, treatment, and prevention protocols empowers all care providers to make bone health a priority for their patients. An interdisciplinary approach to bone health and fracture prevention is the key to successful outcomes for all children at risk for osteoporosis. Strategies included the development and implementation of 1) an evidence-based screening tool to allow primary caregivers to quickly recognize the child who is most at risk for osteoporosis, 2) a 'Fragile: Handle with Care' protocol for 'at risk' children along with identifiable signage that alerts caregivers and others who handle the child of the need to do so safely, 3) a bone health algorithm for hospital staff to guide them from screening through diagnosis, prevention or treatment protocols, and 4) a resource for families and caregivers that includes the definition of pediatric osteoporosis, diagnostic criteria, prevention strategies, nutrition and lifestyle recommendations related to activities of daily living, safe handling practices, and tips to prevent injury. Critical success factors and challenges of spreading this hospital wide screening and intervention program will be reviewed. Statistics of the percentage of children admitted to the centre who meet the osteoporosis risk category through this screening will be presented. The team's current focus includes identifying children with osteoporosis in the community by expanding the screening and fracture prevention protocols through the education of pediatric outpatient treatment areas, community care providers and families.
Comments/keywords
This is a poster to share this novel project.
Keywords: Pediatric Osteoporosis, Multidisciplinary Team, Screening, Prevention, Treatment, Bone Health.

## N10

**Endocrine and exocrine function changes in acute pancreatitis**

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Introduction
Acute pancreatitis may cause endocrine and exocrine dysfunction, but in the early phase of acute pancreatitis these changes are poorly documented.
Aim
The aim of this study was to asses the endocrine and exocrine impairment in the early phase of AP and to establish the need for
Patients and methods
We have collected data from 116 patients with AP, with different aetiologies and degrees of severity of the disease. We had 93 male pts (80.17%) and 23 female pts (19.82%). Mean age $45.74 \pm 13.05$ years old (range 20–76 years).
Pancreatic endocrine function was evaluated by fasting blood glucose (FBG), glycosylated haemoglobin. Factors that could influence the endocrine function as obesity, hiperlipidemia were also investigated. Pancreatic exocrine function was evaluated by faecal elastase.
Results
Nine pts (7.76%) had the confirmed diagnosis of diabetes already prior to acute pancreatitis. 42 pts (36.20%) had hyperglycaemia and 65 pts (56.03%) had normal blood glucose level.
The blood glucose levels did not correlate with the severity of the disease. During the hospital stay 9 pts were already in insulin therapy and needed to modify doses, while 17 pts out of them initial hyperglycaemia had to start the insulin therapy recently.
Pancreatic exocrine insufficiency was seen in 21 pts (18.1%).
Conclusions
Endocrine functional impairment was found in pts with AP. Obesity, hyperlipidemia, and diabetes related symptoms increased the likelihood of developing functional impairment after AP.

Hyperglycemia may present in pts with AP, even during the first days of the disease onset, but usually normalizes as the inflammatory process subsides. Blood glucose may fluctuate, and insulin should be administered cautiously.
Comments/keywords
Keywords: Endocrine function, Acute Pancreatitis, Insulin administration.

## N11

### A prospective study of growth and development of children recently adopted from orphanage care

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Background and aims
Over 200 000 international adoptions by US families occurred between 1999–2010. Prior studies suggest that the effects of institutionalized care on growth and development may not be fully reversible. The exact mechanisms through which early life stress affect biobehavioral outcomes have yet to be determined, but environmental influences could regulate both biobehavoral outcomes through an effect on the hypothalamic-pituitary-adrenal (HPA) axis.
Methods
Prospective study of 10 recently adopted children with an average time spent in orphanage care of $23.6 \pm 9$ months. Eligible participants had no history of significant medical, developmental, or behavioral problems. Anthropometric measurements, HPA axis tests, bone age, neurocognitive testing, and behavioral questionnaires were evaluated.
Results
Shortly after adoption by a U.S. family ($1.8 \pm 1$ mos.), height standard deviation unit (Ht SDU) was $-1.6 \pm 0.8$; weight SDU was (Wt SDU) $-0.9 \pm 1.2$; and head circumference SDU (HC SDU) was $-1.8 \pm 1$ Bone age was consistent with chronological age in four, advanced in three, and delayed in three children. Time in orphanage care was positively associated with serum cortisol ($r=0.64$; $P<0.06$) and negatively associated with HT SDU ($r=-0.63$; $P<0.05$). Neurocognitive testing (Bayley-III) showed significant delays in all scores. HC SDU was positively associated with cognitive and receptive language subscales on the Bayley III ($r=0.62$, 0.69; respectively). Response on the Behavior Rating Inventory of Executive Function endorsed clinically significant inhibitory control in half the children, and subscale scores for behavioral regulation were positively associated with HC SDU ($r=0.9$; $P<0.05$).
Conclusion
Prenatal factors and time in orphanage care were associated with negative effects on linear growth, serum cortisol, cognitive, and behavioral outcomes.
Clinical implications
Careful assessment of prenatal and environmental risk factors will help to identify children at risk for untoward effects on biobehavioral outcomes and target early interventions.
Comments/keywords
Keywords: Biobehavioral, Cortisol, Growth, Cognitive, Hypothalamic pituitary adrenal axis.

## N12

### Improving the efficiency and safety of managing children with diabetic keto-acidosis

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Background
The nursing management of DKA in children is labor intensive. The traditional management of DKA involves numerous physician ordered changes of parenteral fluids. This is time consuming for the nurse; challenging for the pharmacy and can lead to delays in proper fluid administration.
Aims
The purpose of this pilot study was to investigate whether a streamlined process using a three-bag-system for treating children with DKA would improve efficiency for nurses, length of hospitalization, cost, and blood glucose levels.
Methods
Standardized orders for fluid solutions and rates of infusion were developed using a three-bag-system.
(1): ¾NSS with 20 mEq/L KCL and 20 mEq/L K-Phosphate
(2): D10% ¾ NSS with 20 mEq KCL/L and 20 mEq/L K-Phosphate.
(3): 250 units regular insulin/250 ml NSS.
Nurses manage IV fluids based on standardized orders with physician communication only as needed for uncovered contingencies. A comparative non-experimental design was used to evaluate the outcomes of children hospitalized with DKA before and after initiation of the three-bag-system. Thirty medical records were reviewed with 16 patients not using three-bag-system (control group) and 14 patients using three-bag-system (study group).
Results
Independent samples *t*-test and $\chi^2$ were used to determine significance. No difference found in glucose between groups. Length of stay, number of IV bags, and cost were reduced in the study group. This change was insignificant. Verbal orders decreased in study group (14%; $P<0.008$) vs control group (68%).
Conclusions
Using the three-bag-system, there was a reduction in IV bags, cost, and length of stay; however, the sample size was too small to demonstrate significance. The reduction in verbal orders was statistically significant.
Clinical implications
Using a three-bag-system within standardized DKA orders creates a streamlined process reducing frequency of verbal orders, creating efficient care. Further studies using larger sample sizes are warranted verifying additional benefits using a three-bag-system.
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Comments/keywords
Keywords: Diabetic Keto-acidosis (DKA).

## N13

### Socio-demographic factors associated with pediatric diabetic ketoacidosis admissions in Southern West Virginia

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Background
Diabetic Ketoacidosis (DKA) is a well known complication in children with Type 1 Diabetes (T1DM) with a mortality rate estimated at 2%. Sparse data is available from the literature describing socio-demographic factors associated with DKA admissions in children. A previous study identified that children of non-Caucasian race and Medicaid, with T1DM, had increased incidence of DKA admissions.
Aims
To identify the socio-demographic factors associated with DKA admissions including type of insurance coverage, income by county, race, gender and HbA1c in West Virginia, a rural part of Appalachia.
Methods
A retrospective chart review of patients with known type 1 diabetes ages 1 to 18 years admitted to the Pediatric ICU with DKA in Charleston WV from January 2007 to December 2010 in comparison to our general type 1 diabetes population. The data collection tool included multiple socio-demographic factors and HbA1c.
Results
We reviewed a total of 167 patients with an admitting diagnosis of DKA; 63 charts were excluded because they did not meet either DKA criteria, age criteria, had new onset diabetes or lived outside of WV. 57% were female, 43% male. Average age was 13.6 years (SD $\pm$ 2.81) 56% were covered by Medicaid or Chips insurance and 44% by commercial payers. 11.5% were African American and 88.5% were Caucasian. The average HbA1c was 10.85%. (SD $\pm$ 2.364).
Conclusions
Salient findings include higher HbA1c, higher rates in African American patients and in those covered by Medicaid.

Clinical implications
This study identifies socio-demographic factors associated with children admitted for DKA in WV. Patients identified at higher risk for DKA include those with elevated HbA1c, African American race and those covered by Medicaid/CHIPS. Findings can be utilized to identify patients at higher risks for DKA and implementation of prevention strategies.

## N14

**Failure to thrive due to inherited congenital isolated growth hormone deficiency**

Lisa Michele Pincham & Paul Kaplowitz
Children's National Medical Center, Washington, Districts of Colombia, USA.

Patient Demographics
22-month-old female, severe failure to thrive.
Past History
Birth weight 5 lb 11 oz at term, grew well for 4 months and then deviating progressively below the curve in height and weight.
Physical exam: Length 66 cm (−5.1 SD), weight 6.6 kg (−6.7 SD). Prominent forehead and midfacial hypoplasia noted. Muscle mass decreased.
Family history: Mother's height of 5 ft 3 inches with menarche at age 13. Father, – 4 SD, diagnosed with isolated growth hormone deficiency at 7 years of age, and treated (5 ft 4 inches). Siblings included a 6-year-old brother who was very small at age 22 months during an endocrine evaluation and a 3-year-old sister with height and weight at both −4 SD below the mean.
Evaluation (studies/assessment)
Free T4 1.28 ng/dL (normal 1.1−1.7), TSH 1.8 uU/mL. IGF-1 <25 ng/mL (44–174) and IGFBP-3 <0.5 ug/mL (1.3–3.5) were both very low. Growth hormone stimulation testing peak 1.1 ng/mL. DNA sequencing of the GH-1 gene found a heterozygous sequence variance.
Interventions (physiologic or psychosocial)
Growth hormone therapy started at 0.27 mg/kg/week and headaches began 5 days later, likely due to increased intracranial pressure, GH stopped and dose reduced by 1/3 which was tolerated. She has grown about 12 cm (first 10 months) but is still −3.5 SD.
Discussion/recommendations
Failure to thrive in the first 2 years of life rarely has an endocrine etiology. In this case, recognizing the importance of the family history and better compliance with follow-up care of the older siblings might have resulted in earlier diagnosis and treatment of these patients. In addition, the headaches likely due to benign intracranial hypertension in the first case suggests that this rare complication of GH therapy might be more common in children with this rare and severe form of GH deficiency, so starting GH at lower doses than usual would be prudent.

## N15

**Diabetic foot in jordan: a qualitative content analysis of self-treated problems**

Ma'en Zaid Abu-Qamar[1] & Anne Wilson[2]
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Diabetes is a chronic condition that predisposes to a wide range of foot problems that potentially end with ulcer formation. The purpose of this manuscript is to report 68 participants' diabetic foot problems self-treated with complementary therapies. Participants were recruited from nine healthcare facilities in Jordan and interviewed via survey. Their responses to an individual question seeking information about complementary therapy usage was analysed using content analysis. The content analysis identified a wide range of foot problems that included: fungus, ulcers, corns, foot pain and other problems. The identified problems are common among sufferers of diabetes, and potentially may develop into ulcers. Accordingly, health education programs need to consider common foot problems among those with diabetes, and lifelong management need to be tailored to reduce the possibility of developing ulcers.
Comments/keywords
Content analysis, complementary therapy, diabetic foot, Jordan, foot problems, prevention, neuropathy.

## N16

**Planning for the future: preparing the endocrine specialist nurse of tomorrow**

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Nursing Studies, The University of Edinburgh and OPD 2 (Endocrine Unit), Edinburgh Royal Infirmary, Scotland, UK.

We have come a long way in the UK over the past ten years towards our goal of meeting the educational needs of nurses specialising in adult endocrinology. Endocrine nurses are now able to access annual training updates and attend specific nurse-led sessions at scientific meetings thanks to the ongoing work undertaken by the Society for Endocrinology's nurse committee.
It is therefore time to turn our attention to how we can encourage nurses to want to specialise in endocrinology and to start to prepare them for such a role before rather than after they find themselves working in this field. One way to do this is to ignite their interest in endocrinology while they are still students. With this aim in mind the first undergraduate course in adult endocrine nursing in the UK was developed and introduced as part of the Bachelor of Nursing (Hons) programme at Edinburgh University for the 2011/12 academic session. The course, which runs over ten weeks, is available as an Honours option to students in their third and fourth years and is delivered as a series of lectures and tutorials. The content covers specific endocrine conditions and alongside these critically explores issues such as compliance with prescribed treatment, quality of life, patient support and patient self-management, the role of the specialist nurse and nurse-led clinics. It is taught primarily by an endocrine nurse/lecturer with some specific input from visiting speakers. In addition to the formal taught aspects students are encouraged to attend endocrine out-patient clinics, observe pituitary surgery and attend local patient support group meetings as well as national patient conferences.
Fifteen students have now successfully completed the course. Course evaluation has been extremely positive. Of the seven final year students, two are now proactively seeking endocrine nursing positions to apply for on graduation.
Comments/keywords
Keywords: Endocrine nurse, education, training.

## N17

**The Swedish pituitary registry**

Maria Forsgren[1], Elisabeth Olsson[1], Christina Dahlgren[2], Olof Ehn[3], Margareta Lindgren[4], Ann-Sofie Nilsson[5], Caroline Westholm[6] & Maria Wärn[7]
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Background
The Swedish Pituitary Registry is a national quality registry founded in 1991 by the Swedish Pituitary Group.
A total of 89 such national quality registries exist in Sweden (2011) that provide important data in the continuous quality assessment and improvement work within the Swedish health care system.
In 2011, a nurse network was started for nurses working with the registry.
Purpose
The purpose of the Swedish Pituitary Registry is to guarantee that all patients with pituitary diseases get equivalent evaluation and treatment, as well as to evaluate given therapy.
Methods
Data from 3955 patients (1893 men/2062 women), median age 52 years (range 0–97), with pituitary diseases were registered 1991–2011.
Data registered in the registry consist of diagnosis, abnormal production of pituitary hormones, impact on vision, tumour extension and given treatment. Follow-ups were done after 1, 5 and 10 years. Since 2011 quality of life (EQ5D) and work ability are included.
Results
Among the pituitary tumours 1744 were non-functioning, 813 produced prolactin, 658 produced GH, 275 produced ACTH, 28 produced TSH and 15 produced FSH/LH.
102 patients had craniopharyngiomas and 320 had other pituitary diseases (including empty sella and Rathke's cleft cyst).
The incidence 1991–2011 for the pituitary tumours overall was 19 cases/million/year. The incidence was 9.3 for non-functioning pituitary tumours, 4.3 for prolactinomas and for the most complete groups in the registry, GH and ACTH, the incidence was 3.5 and 1.5 respectively.

Most of the patients were treated with surgery, radiotherapy or medical treatment, alone or in combination.
Conclusion
The registry gives us possibilities to evaluate given treatments, and to get an indication if there are regional variations in the treatment results. It is also a unique source of acquired knowledge and gained experience for these unusual diseases.
Comments/keywords
Keywords: The Swedish Pituitary Registry, Pituitary diseases.

## N18

**Molecular genetics in turner syndrome**
Kelly Mullholand Behm

The basics of molecular genetics are reviewed with emphasis on introducing terminology frequently encountered in genetic lab results and in lectures focusing on the genetic origins of disease. Mutations thought to be associated with various phenotypical features of Turner syndrome are identified. Specific examples of genetic test results revealing Turner syndrome are reviewed. The new molecular testing available for diagnosing Turner syndrome is presented. Applications to the role of the endocrine nurse are provided.
Objectives
After attending this presentation, attendees will be able to:
1. Describe the possible molecular mechanisms responsible for the TS karyotype.
2. Identify mutations thought to be associated with physical features of TS.
3. Discuss diagnostic testing options for TS.
4. Differentiate between the role of a genetic counselor and a genetics/endocrine nurse in caring for a patient with TS.

## N19

**An Internet based protocol for examining transitional experiences of 16–26 year olds with Turner syndrome (TS)**
Kelly Mullholand Behm[1,2]
[1]Rush University College of Nursing, Chicago, Illinois, USA; [2]Pediatric Endocrinology Nursing Society, Ann Arbor, Michigan, USA.

Problem
Programs for adolescents with Turner syndrome (TS) are currently based on evidence derived from medical surveillance or parental and teacher perceptions. Little is known about how personal perceptions in this population influence behavior and psychosocial outcomes. Facing issues associated with puberty and young adulthood, such as body image, relationships, and careers, can be more challenging when living with TS.
Framework
Unlike frameworks for chronically ill adolescents that focus on medical aspects of care for co-morbidities, the Meleis Transitions Theory integrates concepts, change processes, and multifaceted contexts that permit broader analysis of perceptions of life experiences. The theory encompasses the nature of transition, its facilitators and inhibitors, and process and outcome indicators.
Methodology
This study utilizes a descriptive, qualitative, cross-sectional design with a theory driven thematic analysis. Recruitment is via the internet and referral from health care providers and support groups. Consent occurs through an interactive electronic document. Data are extracted from interviews conducted through sequential e-mails with participants.
Subjects
The sample includes 20 females with TS, ages 16–26. TS results from an arrangement of chromosomes demonstrating complete or partial absence of one X-chromosome.
Anticipated analysis
Coding and thematic analysis of interview data are ongoing using NVivo9 and Excel.
Results
In preliminary analysis, participants describe their individual experiences, conditions surrounding life changes, and patterns of personal response. 'Normal' vs 'not normal' is an overriding theme. Multiple transitions occur simultaneously. There is marked frustration with lack of public awareness of TS. Primary supports are family, friends, and religion rather than health care providers.
Implications
Nurses working with young women with TS need a better understanding of the variables of transition facing this population, as well as factors that enhance or inhibit positive psychosocial outcomes. Strategies for addressing unmet psychosocial and cognitive needs must be developed with the input of affected individuals.
Comments/keywords
Keywords: Turner syndrome, transition, adolescence, adolescent.

## N20

**Self care practice among diabetic patients attending metropolyclinic, Kathmandu**
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Introduction
Diabetes is expected to rise from 177 million today to 370 million in 2030. Self care is a crucial to keep the disease under control. Appropriate self care practice can keep the disease under control.
Objective
The aim of the study was to find out the self care practice among diabetic patients.
Methods
This is a simple descriptive cross-sectional study. A total of 50 respondents, who met eligible criteria were purposively sampled and directly interviewed. Chi-square test was used to see the association. Self care practice of the respondents of this study population was examined using the SPSS version 16.0.
Result
Self care practice among the respondents was not satisfactory. Unsatisfactory self care practice was more than half (56.75%) among adequate knowledge respondents. Among respondents having inadequate knowledge, majority (92.31%) had unsatisfactory practice. Majority (74%) had adequate knowledge and satisfactory self care practice was done by only 34%.
Conclusion
Thus it is concluded that satisfactory self care practice was inadequate. There was significant relationship between self care practice and knowledge regarding diabetes among respondents. Thus the study also concluded that adequate knowledge on Diabetes should be provided to the patients for satisfactory self care practice.
Comments/keywords: self care practice, knowledge, diabetes.

## N21

Abstract withdrawn.

## N22

**Eat, Pray, Love…Yourself**
Shari Liesch

Eat, Pray, Love ….Yourself; the title is modeled after a book (made into a movie) on life exploration and change. As health care providers, we spend a lot of time helping others; but how do we care for our self? We have been challenged to lead by example in our ever changing healthcare climate. To do this, we must nurture the physical, emotional, and relationship parts of our life. Insight into the risks of employment in health care will be used to direct conversations in managing these risks to achieve positive outcomes in longevity of employment and our own lives. In this talk, we will use the tenets of self determination theory (competence, autonomy and relatedness) as we explore personal and professional strategies to increase life satisfaction.

In preparation for this general session, (given at PENS conference, April 2012), Pediatric Endocrine Nurse Society (PENS) nurses were surveyed to determine perceived autonomy, relatedness and competence in their job using Self Determination Theory Work Satisfaction Questionnaire. In addition to this assessment of job satisfaction, it was important to assess their overall Quality of Life. For this, the short version of Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q) was used. This was done to identify correlations between overall quality of life and perceived work satisfaction. Results of the surveys, as well as demographics will be used for the talk. This session will attempt to connect past and present perceptions of workplace and personal factors that impact job and life satisfaction. Through storytelling and exploration we will discuss how nurses can remain healthy as they care for others. Assessment of individual perceptions can direct future research to positively impact group competence, thus impacting nurse roles.

## N23

**Dance for health: implementation of a dance program to improve physical activity of children**

Terri H Lipman[1], Gillian Bazelon[2], Kali Rhodes[1], Carrie Preston[1], Sarah Ratcliffe[3] & Janet A Deatrick[1]
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Background
Sedentary lifestyle, decreased physical activity and poor diet contribute to the increasing problem of childhood obesity and risk for type 2 diabetes.
Aims
The purpose of this study was compare the effect of dance, with unstructured playtime, on the physical activity level of an underserved, urban population of children.
Methods
In this longitudinal study, height, weight and body mass index (BMI) were measured during the first week of the four week program. Every week, heart rates (HR) and pedometer readings (PR) were recorded. Pre activity heart rates were obtained and a dance class was taught for 30 minutes, once a week. Resting HR and PR were measured after the dancing. During the non-dancing days, PR were taken to gauge physical activity during usual activity.
Results
38 children (16 F, 22 M; 4.7–12.9 yr) participated in the study. Average BMI was 18.3 ($\pm$5.5); 20% were $>85^{th}$% for age and sex. Overall, the average PR measurement for dancing days was 1760 ($\pm$945) vs 851 ($+$619) on non-dancing days. The number of steps in dancing days was approximately double those in non-dancing weeks ($P<0.001$). Children 8–10 yr had more steps than younger and older age groups. Age had a quadratic association with PR ($P<0.001$). Males had 37.2% more steps than females ($P=0.026$); BMI was not found to be associated. Resting HR was significantly higher than baseline ($P<0.001$).
Conclusions/clinical implications
Children in this population were not physically fit as evidenced by their elevated resting heart rates after exercise. Implementing dancing increased steps and activity of the children. Dance is a culturally relevant, enjoyable, free and easily accessible method of activity. It is crucial for nurses in pediatric endocrinology to address the obesity epidemic with culturally appropriate interventions and to partner with the community to tackle this public health crisis.
Comments/keywords
Keywords: Children, Obesity, Type 2 diabetes, Activity, Culturally relevant

## N24

**Self-care competence in the case of Brazilian patients with diabetes mellitus in a multiprofessional educational programme**

Camila Aparecida Pinheiro Landim[1], Maria Lucia Zanetti[2], Manoel Antonio Dos Santos[3], Thiago Antonio Moretti De Andrade[4], Tania Alves Canata Becker[5], Ellen Cristina Barbosa Dos Santos[5], Gabriel Guidorizzi Zanetti[6], Beatriz Cardoso Lobato[7], Rosana Cristina Franco[7] & Carla Regina De Souza Teixeira[8]
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Background
Diabetes mellitus is mainly characterized by a dysfunction of glucose metabolism. The growth of diabetes mellitus as a chronic condition requires continuous management and requires health services to implement care strategies. Diabetes education and self-care competence have been recognized over the last few decades as essential for patients with diabetes mellitus to achieve positive treatment results.
Objective
To compare self-care competencies in the physical, cognitive and emotional and motivational dimensions of patients with diabetes mellitus before and after participation in an educational programme.
Design
A prospective, comparative study was used.
Methods
A total of patients with 43 diabetes mellitus participated in this study, before and after a four-month educational programme on diabetes held in Ribeirao Preto, SP, Brazil in 2009. Data were collected through the Scale to Identify diabetes mellitus Patients' Self-Care Competence (Escala para Identificação da Competência do Portador de diabetes mellitus para o Autocuidado) at two points in time: at the beginning (T1) and at the end (T2) of the programme. Linear mixed effects models were used ($P < 0.05$) in the analysis.
Results
Statistically significant differences were not found in relation to physical competence in the comparison between T1 and T2. Statistically significant differences were found in the comparison of cognitive and emotional and motivational competencies, respectively, between T1 and T2.
Conclusion
The scores related to cognitive and emotional and motivational competencies for self-care presented statistically significant differences between T1 and T2.
Relevance to clinical practice: The educational activities implemented in the educational programme favored improved cognitive, emotional and motivational competence for the incorporation of positive self-care actions.
Comments/keywords
Comments for the Nurse Abstracts Markers: health professionals, nurses, psychologists, nutritionists and physical educators, should therefore encourage patients with DM to participate in educational activities, with a view to the incorporation of positive self-care actions.
Keywords: Diabetes Mellitus, Health Education, Nurses, Nursing, Patient Education, Self-Care.

## N25

**Primary hyperparathyroidism, vitamin D deficiency and quality of life**

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Background
The study aimed to evaluate quality-of-life (QoL) aspects and impact of vitamin D deficiency in primary hyperparathyroidism (pHPT).
Method
150 consecutive patients (119 women) with pHPT (mean age 60$\pm$11 years; BMI 27$\pm$5 kg/m$^2$; ionized calcium 1.44$\pm$0.08 mmol/l; PTH 130$\pm$69 ng/l) were included. Six weeks postoperatively, the pHPT patients were reexamined and randomized (double-blind) to daily substitution with calcium carbonate (1g), with or without cholecalciferol (1600 IE). Self-estimation questionnaires (SF-36) were completed at baseline and repeatedly during follow-up. A gender- and age-matched reference group ($n=459$) was randomly selected from the Swedish SF-36 norm database.
Results
Vitamin D deficiency, defined as 25-hydroxyvitamin D $<$ 50 nmol/l, was present preoperatively in 59% of the patients. The 25-hydroxyvitamin D level increased postoperatively (mean 49$\pm$18 vs 52$\pm$17 nmol/L, $P=0.019$). The calcium level normalized after parathyroidectomy in all patients, while the PTH level decreased but was not normalized in 45% of the cases. Compared to the reference population

baseline, the patients scored significantly lower on all eight domains of the SF-36. Postoperatively, patients improved significantly but reached the level of the reference population in only two domains: GH (general health perceptions) and BP (bodily pain). No correlation was found between the SF-36 scores and the calcium, PTH or vitamin D levels at baseline. The baseline scores were inversely correlated to body mass index (BMI; $r=-0.226$–$r=-0.386$; $P<0.01$). The study will be deblinded on January 15 2012 and further follow-up data will be presented.
Conclusions
Patients with pHPT had worse QoL scores than the reference population on all eight domains of the SF-36. The outcome after parathyroid surgery was not predictable from preoperative calcium, PTH- or vitamin D status. The effects of vitamin D supplementation are yet to be evaluated.

## N26

**Evaluation of self management and diabetes education newly diagnosed with Type 2 diabetic patients in Mongolia**

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Introduction
Educating patients about diabetes plays a pivotal role in encouraging people to changing lifestyle and supporting them to improve the quality of life, and actively responsibile for self control of their condition.
Objective
To evaluate self management after receiving diabetes education for newly diagnosed Type 2 diabetes in Mongolia.
Methods
The cross-sectional survey was conducted from May and October 2011. At Diabetes centers and Level II hospitals in Ulaanbaatar. 150 participants with type 2 diabetes was referred within, from May to August as newly diagnos and included only the ones who met inclusion criteria and agreed with informed consent. Each patient's knowledge and psychosocial status were assessed be the internationally accepted questionnaire and collected anthropometric and metabolic indicators according to the protocol. Statistical analysis was performed with the SPSS16 software.
Results
The study involved newly diagnosed Type 2 diabetes 23–64 years were men 43,6% (65),women 56,7% (85). Educated groups participants attended a structured education programme within 0–2 weeks of diagnosis. The diabetes knowledge anthropometric and metabolic indicators were indifferent between groups educated and without diabetes education. However psychosocial status which includes satisfaction of health care service, anxiety of their disease depression related blood glucose reactions eating family and regular self management was significantly different between educated and without education groups.
Conclusion
Psychosocial status which includes satisfaction of health care service, anxiety of their disease, depression related blood glucose reactions eating family and regular self management were significantly different between both groups.
Keywords: Newly diagnosed type 2 diabetes, patient's self management education

## N27

**Individuals with Addison's disease and their experiences of onset and self-care: new knowledge?**

Ylva Wessman

Aim
The aim of the study was to elucidate the experiences of onset and self-care among individuals with Addison's disease.
Background
Addison's disease, primary adrenal insufficiency, is a rare endocrine disorder where the production of glucocorticoids and mineralcorticoids are insufficient. The disease could be difficult to diagnose and the lifelong treatment involves hydrocortisone administration daily. Individuals with Addison's disease have a chronic disease where the different kinds of symptoms and the changing in pharmaceutical treatment affect their condition in daily life.
Design
A qualitative content analysis was used.
Methods
13 individuals were interviewed during 2009.
Results
Two categories emerged from the interviews:' to handle life with the disease related to one's surrounding' and 'to handle life with the disease related to oneself'. The categories are linked to a main theme 'dependent vs independent' which reflects the limitations and strategies to manage the limitations.
Conclusion
The findings show that the individuals could have a long lasting and sometimes even dramatically experiences of onset. They contribute experiences by showing difficulties in managing the hydrocortisone medication in daily life. Their strategies of self-care are mostly based upon an autodidact approach. The individuals express need for more information concerning the adjusting of hydrocortisone medication and of self-care. They also request supplementary knowledge of Addison's disease among health professionals.
Relevance to clinical practice
The study may add new knowledge for health care providers to identifying areas of improvement in the given information to the individuals and to understand the importance of awareness of the symptoms of this rare, and sometimes even life-threatening, disease.
Keywords
Addison's disease, experiences, nursing, onset, qualitative study, self-care.

## N28

**A survey of knowledge related to cystic fibrosis related diabetes**

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Background
Cystic fibrosis (CF) is the most common life threatening autosomal recessive genetic disease in the United States. Cystic fibrosis related diabetes (CFRD) is the most common co-morbidity of CF, and, as patients age, the likelihood of developing diabetes increases. CFRD leads to decreased lung function, poor nutritional status, and decreased survival rates. Symptoms can be subtle and may be overlooked. The extent to which individuals with CF know about CFRD is unknown.
Aims
1. To assess knowledge of CFRD in adults with CF.
2. Describe where information related to their disease is obtained.
3. Examine relationships between measures.
Methods
A cross sectional descriptive design was used. Adults ($>18$ yrs) with CF were recruited during an outpatient CF visit and asked to complete a 15-item CFRD Knowledge Survey. The survey included 10 items on knowledge of CFRD, 2 items on obtaining information about CF, and 3 items on experience with diabetes.
Results
Twenty-six individuals with CF participated, 70% were male, mean (SD) age was 27.73 (10.23). Regarding knowledge, 92% had heard of CFRD, 65% knew symptoms of CFRD, and 58% knew how the diagnosis was made. A majority (65%) reported they seek information related to CF only when necessary. The three most common sources for obtaining information were internet, physician, and clinic. The total knowledge score was significantly correlated with the experience score ($r=0.50$, $P=0.009$), and understanding the importance of knowing that you have CFRD ($r=0.80$, $P<0.001$). The experience score was also significantly correlated with understanding importance of knowing if you have CFRD ($r=0.60$, $P=0.001$). Understanding the importance of knowing if you have CFRD was also significantly correlated with being diagnosed ($r=0.43$, $P=0.028$).
Clinical implications
As the number of patients diagnosed with CFRD continues to increase, both pediatric and adult endocrine nurses need to be educated and equipped to successfully manage this patient population.
Comments/keywords
Cystic Fibrosis, Diabetes, Nursing.

# Poster Presentations

## Adrenal cortex

### P1

**Targeting mutated β-catenin *in vitro* and *in vivo* inhibits cell proliferation and stimulates apoptosis: a promising therapeutic target in adrenocortical carcinoma**

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Adrenocortical carcinoma (ACC) is a rare and highly aggressive endocrine neoplasm, with limited therapeutic option. Activating β-catenin somatic mutations are observed in ACC and associated with a poor outcome. Activation of the Wnt/β-catenin signaling pathway seems to play a major role in ACC aggressiveness, and might be a promising therapeutic target. The H295 cell line derived from an ACC harbors an activating β-catenin mutation. We herein assess the *in vitro* and *in vivo* effect of β-catenin inactivation using doxycyclin (dox) inducible sh-RNA plasmid in H295R adrenocortical cancer cells line (clones named H295R-shβ).
*In vitro*: a dramatic reduction in β-catenin expression was detectable in H295R-shβ after dox treatment compared to control clones (−82%, $P<0.005$). Accordingly, we observed a transcriptional Wnt/β-catenin-dependent luciferase reporter activity decrease (−62%, $P<0.05$) as well as a decreased expression of an ubiquitous target gene, AXIN2 (−76%, $P<0.0001$). β-catenin silencing led to a decreased cell proliferation (−46%, $P<0.05$) and cell cycle alterations with cell accumulation in the G1 phase (+10%, $P<0.05$) and increased apoptosis (+67%, $P<0.05$).
*In vivo*: after subcutaneous induction of tumor xenografts in athymic nude mice, 9 days dox administration was associated with a significant decrease in intra-tumoral β-catenin (−89%, $P=0.007$) and AXIN2 (−87% $P<0.005$) expression in H295R-shβ grafted mice while mice grafted with control H295R clone remained unaffected. Long-term dox administration, starting 3 days after tumor cell inoculation, was associated with a total absence of tumor growth in the H295R-shβ group while tumors were present in all the mice of the control group (median tumor weight after sacrifice 67.4 mg vs 0 mg, $P<0.001$).
In summary, these experiments provide evidences that Wnt/β-catenin pathway inhibition in ACC is a promising therapeutic target.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

### P2

**Pathophysiological significance of CYP11B2 immunohistochemical staining in primary aldosteronism**

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Background
Although primary aldosteronism (PA) is the common cause of hypertension subjected to surgery, methods of pathological confirmation of aldosterone overproduction have not been established. Aim of the study was to investigate immunohistochemically the expression of CYP11B2 in the adrenal tissue of PA.
Methods
Twenty five patients with PA including 20 patients with aldosterone-producing adenoma (APA) and 5 patients without APA (non-APA) were studied. Immunohistochemical staining was performed with anti-CYP11B2 on paraffin-embedded sections. We analyzed the expression of CYP11B2 semi-quantitatively by scoring the staining intensity from (− or ±) to (2+).
Results
Eighty five % of the patients with APA showed positive immunostaining for CYP11B2 in the adrenal tumor. Tumor volume estimated from the diameter was negatively correlated with CYP11B2 score ($P<0.001$) and positively correlated with basal aldosterone to renin ratio (ARR) ($P<0.001$). CYP11B2 score adjusted by the tumor volume was correlated positively with ARR ($P<0.01$) and negatively with serum potassium ($P<0.05$). In addition to the immunostaining in the tumor, there existed cell clusters of positive immunostaining of CYP11B2 (APCC) in the adjacent tissues. Although the APCC was seen in the adjacent tissue of APA, the number of APCC was significantly larger in patients with non-APA than those with APA ($P<0.001$). Serum potassium was significantly lower ($P=0.01$) and number of anti-hypertensive drug ($P<0.05$), prevalence of hypokalemia (P=0.001), plasma aldosterone concentration ($P=0.001$), and ARR ($P<0.01$) were significantly higher in patients with APA than those with non-APA.
Conclusions
The present study clearly demonstrated that CYP11B2 immunostaining is useful for the histopathological confirmation of aldosterone production and that both CYP11B2 expression and tumor volume could contribute to the extent of hyperaldosteronism in APA. In addition, increased number of APCC in the non-tumorous adrenocortical tissues could cause aldosterone excess in patients with non-APA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### P3

**The role of salivary cortisol measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the diagnosis of subclinical hypercortisolism (SH) in patients with adrenal incidentaloma (AI).**

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Introduction
For defining SH in AI patients at least 2 altered criteria among serum cortisol after 1 mg dexamethasone test (1 mgDST), ACTH levels, 24 h urinary free cortisol (UFC) and midnight serum cortisol levels are generally required. Midnight salivary cortisol (MSC), a sensitive and easy-to-perform marker of overt hypercortisolism, appears to be of limited utility in these patients. Data on the role of salivary cortisol using LC-MS/MS, the gold standard procedure for steroids measurement, are lacking.
Methods/design
In 50 AI patients (F/M 27/23) we evaluated morning salivary cortisol (CS8, normal values. 1.5–15 nmol/L), salivary cortisol after 1mg-DST (Sa-DST, cut-off $<1.2$ nmol/L), MSC (cut off $<2.8$ nmol/L) using LC-MS/MS, serum cortisol after 1 mgDST (Se-DST), UFC and ACTH levels. We defined SH in the presence of at least 2 out of: Se-DST $>83$ nmol/L, ACTH $<10$ pg/ml, UFC $>193$ nmol/24 h. In all patients, the presence of diabetes, hypertension and dyslipidemia was detected as an indirect marker of SH.
Results
MSC levels were higher in patients with SH ($8.8\pm9.1$ nmol/L) than in those without ($4.5\pm3.3$ nmol/L, p=0.03). CS8 and Sa-DST were comparable between patients with and without SH ($22.9\pm23.2$ vs $18.2\pm14.3$ nmol/L, $P=0.4$; $6.6\pm7.2$ vs $4.6\pm3.9$ nmol/L, $P=0.06$; respectively). The CS8 and MSC levels were associated with Se-DST ($R=0.4$ and 0.5, respectively, $P<0.01$). The presence of chronic complications was related to Se-DST ($R=0.3$, $P=0.03$) but not to any salivary parameter. Using the MSC cut-off of 2.8 nmol/L the sensitivity (Se) and the specificity (Sp) for diagnosing SH was 25% and 81% respectively. MSC values $<1.0$ nmol/L excluded the presence of SH (Sp 38%), while MSC $>8.0$ nmol/L had the 100% Sp (Se 13%).
Conclusion
In AI patients, CS8 and Sa-DST are of limited utility, while MSC, even measured by LC-MS/MS, cannot be used for the SH screening, though may be useful as a confirmative test.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### P4

**Semen parameters in men with congenital adrenal hyperplasia (CAH) due to 21-hydroxilase deficiency (21-HD)**

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CAH has been described to be associated to infertility and semen abnormalities in 40–70% of adult male patients, because of hypogonodotropic hypogonadism, consequent to the increased levels of sex steroids, but mostly because of the

frequent presence of testicular adrenal rest tumors. The aim of the current study was to evaluate semen parameters and hormonal setting in a subset of men with CAH. Fourteen patients with classical CAH due to 21-HD (six with simple virilizing, and eight with salt-wasting CAH) and 28 controls entered the study. All patients were under treatment with glucocorticoids and, if necessary, mineralcorticoids at the moment of the study; every patients but one were well-controlled by medical therapy. Semen analysis and hormone assessment were performed in the totality of patients and controls. A significant decrease in sperm concentration ($P=0.002$), total motility ($P=0.002$), progressive motility ($P=0.001$) and sperm morphology ($P<0.001$) was found in patients compared with controls. Serum testosterone, LH and DHEA-S levels were directly correlated with sperm morphology ($P=0.002$) whereas serum 17-OH progesterone, testosterone, LH and androstenedione levels were inversely correlated with either total or progressive motility ($P=0.04$). In conclusion, the results of the current study confirmed the evidence of an impairment of semen parameters in men with CAH despite the appropriate treatment for the adrenal disease and seem to suggest that an uncontrolled disease might affect total and progressive sperm motility while an excessive glucocorticoid treatment might impair sperm morphology.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P5

**Non-radioactive strategies on the diagnosis of congenital adrenal hyperplasia due to 21 hydroxylase deficiency (CAH - 21OHD).**

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Introduction

Defects in the pseudogene, CYP21A1P, can be transferred to the functional CYP21A2 gene by recombination and account for approximately 95% of CYP21A2 mutations, leading to CAH-21OHD. We conducted a comprehensive genetic analysis to assess whether Multiplex Ligation dependent Probes Amplification (MLPA) could substitute southern blotting with radioactive probes without compromising reliability of the diagnosis.

Patients and Methods

We studied 90 families/99 patients presenting salt-wasting (SW; $n=32$), simple-virilizing (SV; $n=29$), and non-classical (NC; $n=29$) phenotypes. Molecular analysis was sequentially performed by detecting the 8 most frequent point mutations by allele specific oligonucleotide polymerase chain reaction (ASO-PCR), large deletions and conversions by MLPA (KIT-P050-B2-CAH), and rare mutations by direct sequencing. Parental segregation was evaluated.

Results

Mutated alleles were elucidated by ASO-PCR in 92.2%, by MLPA in 5.0% and by direct sequencing in 2.8%. In SW group, the most frequent mutations detected by ASO-PCR were IVS2-13A/C>G (43.7%), p.R356W (12.5%), and p.Q318X (11.0%), large conversions in 12.5% by MLPA, and the 1762_1763InsT (1.6%) by direct sequencing. In SV patients, the most frequent mutations detected by ASO-PCR were p.I172N (55.2%), p.R356W (10.3%) and IVS2-13A/C>G (6.9%), large conversion in one single allele (1.7%) by MLPA, and the p.G424S mutation was found in 1.7% by direct sequencing. In NC patients, the most frequent mutations detected by ASO-PCR were p.V281L (72.4%), p.I172N (5.2%), IVS2-13A/C>G (5.2%). Direct sequencing revealed rare mutations (p.P453S, p.R408C, and p.A265V) in one allele each (5.2%). No large conversions were found in this group.

Conclusion

Although MLPA false positive results could arise due to mutations/polymorphisms close to the probe binding regions and due to probe hybridization and ligation, these problems can be overcome by the association of MLPA with ASO-PCR and parental segregation. Using these approaches, we can successfully substitute southern blotting in a cost-effective laboratory routine.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This work was supported, however funding details unavailable.

## P6

**Diagnostic value of urinary steroid profiling in the evaluation of adrenal incidentaloma.**

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Objective

An adrenal incidentaloma is discovered in about 3–4% of patients undergoing radiological examination (CT/MRI) of the abdominal region. Current diagnostic algorithms for differentiating between benign and malignant lesions largely depend on imaging studies, which lack in specificity. This often leads to extended radiological follow-up of benign lesions, which is time-consuming, expensive and carries a health risk. It was recently suggested that urinary steroid profiling using gas-chromatography/mass-spectrometry (GC/MS) might be a valuable diagnostic test regarding this clinical problem. We present a large clinical series to test this hypothesis.

Methods

Urinary steroid profiles from patients with adrenal enlargement evaluated between 01-01-2000 and 01-11-2011 were collected. The concentrations of 22 metabolites were measured using GC/MS in 24-hour urine samples. Patient's records were studied and information was collected regarding symptoms and signs, laboratory measurements, imaging studies, treatment, pathology reports and clinical outcome. Steroid profiles were analyzed by calculating receiver operating characteristics (ROC) for every individual metabolite.

Results

In our population of 155 patients, we found significantly higher concentrations of eighteen metabolites in patients with ACC ($n=18$) compared to patients with other adrenal conditions. Tetra-hydro-deoxycortisol (THS) distinguishes ACC from other adrenal disorders with 100% sensitivity and specificity at a cut-off value of 2.95 μmol/24 h.

Conclusion

Our results confirm earlier findings, which suggest that measuring urinary excretion of THS can be useful in differentiating adrenocortical carcinoma from other adrenal tumors. The technique of GC/MS is reliable, non-invasive and less expensive than repeated imaging studies. The next step is prospective validation of this diagnostic tool.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P7

**The combined use of nocturnal salivary cortisol and urinary cortisol to creatinine ratio in the evaluation of cycling in patients with Cushing's syndrome**

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Cyclical Cushing's syndrome is typically detected by collecting sequential daily early morning urine (EMU) samples for cortisol to creatinine ratio over a 28 day period. More recently nocturnal salivary cortisol (NSC) measurement has been shown to be a sensitive means of screening for Cushing's syndrome. The Endocrine Society have suggested that NSC may be used to assess patients for cyclical Cushing's however there is limited evidence that it correlates with the present standard of EMU testing or that it demonstrates a cyclical pattern over 28 days. In this study we sought to correlate NSC with EMU results collected the following morning and to determine whether NSC could be used to detect cycles in patients with cyclical Cushing's syndrome.

A sequential 28 day collection of NSC and EMU was performed on 11 occasions in ten patients with confirmed or suspected Cushing's syndrome. One patient with cyclical Cushing's completed the collection both before and after cabergoline therapy. Seven collections were from patients with active Cushing's disease (3 with cyclical form), three were from patients in remission (1 with cyclical Cushing's and 2 with clinical features suspicious of recurrence) and one was from a patient eventually shown not to have hypercortisolism.

In total there were 270 matched salivary and urinary results. The Spearman's rank correlation coefficient was 0.79 ($P=<0.001$). In two patients with cyclical

Cushing's, EMU and NSC followed a similar cyclical pattern. In one patient with recurrent cyclical Cushing's, cortisol was elevated in both saliva and urine but with more prominent cycles in saliva.
We found that NSC correlated reasonably well with EMU collected the following morning. NSC detected all three cases of cyclical Cushing's. If these results are replicated in larger numbers NSC may prove to be an additional option or replacement for EMU in detecting cyclical Cushing's syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P8

### Defining 17α-hydroxylase in zebrafish: expression pattern of two paralog genes (*zCyp17a1*; *zCyp17a2*) and comparative *in vitro* and *in silico* analysis

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Background
Zebrafish is emerging as a comprehensive model system in endocrinology. Zebrafish synthesise steroid hormones in the interrenal (counterpart of the mammalian adrenal), gonad and brain. Data on steroidogenic pathways is patchy and steroidogenic enzymes in zebrafish have not been well characterised. Human 17α-hydroxylase (hCYP17A1) facilitates two conversion steps, 17α-hydroxylase and 17,20-lyase reactions in the adrenal and gonad. Two zCyp17a (zCyp17a1; zCyp17a2) enzymes exist in zebrafish.
Aim
To characterise the expression pattern and the catalytic activity of zebrafish Cyp17a enzymes.
Methods
*zCyp17a* expression was determined by RT-PCR from whole embryos and adult tissues. Functional assays were performed using transiently transfected COS7 cells expressing *zCyp17a* or *hCYP17A1*. 17α-hydroxylase activity was assessed by the conversion of pregnenolone into 17-hydroxypregenenolone and progesterone into 17α-hydroxyprogesterone. The 17,20-lyase activity was measured by the conversion of 17α-hydroxypregenenolone into DHEA and 17α-hydroxyprogesterone into androstenedione.
Results
*zCyp17a1* and *zCyp17a2* expression was observed from fecundation. In adult fish, both enzymes are expressed in the interrenal, gonad and brain. zCyp17a1 and zCyp17a2 17α-hydroxylated substrates 1.5–3 and 6–11 times more efficiently than human CYP17A1. Furthermore, zCyp17a1 showed a preference for the Δ4-pathway, while zCyp17a2 converted Δ5-steroids more efficiently. In contrast to hCYP17A1, zCyp17a1 efficiently synthesised both, DHEA and androstenedione. zCyp17a2 completely lacks 17,20-lyase activity. We conducted comparative *in silico* analysis between two newly developed three-dimensional zCyp17a models and our human CYP17A1 model. Residue divergence within the substrate and redox interaction domains may explain our *in vitro* findings.
Conclusions
Herein, we demonstrated that both zCyp17a enzymes facilitate 17α-hydroxylation more efficiently than hCYP17A1. Catalytic efficiency is substrate specific. zCyp17a2 cannot synthesise androgen precursors. Expression data suggest a role of zCyp17a enzymes already during early embryologic development. Importantly, our data provides novel insights into zebrafish steroidogenesis and will help to establish a novel cutting edge tool for *in vivo* studies of steroidogenic disorders.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P9

### Progressive adrenal insufficiency in a patient with 46,XY DSD caused by two novel mutations in the cytochrome P450 side-chain cleavage (*CYP11A1*) gene

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Background
Cytochrome P450 side-chain cleavage enzyme (CYP11A1) catalyses the first and rate-limiting step of steroidogenesis. CYP11A1 firstly converts cholesterol into 22R-hydroxycholesterol, which relies on mitochondrial steroidogenic acute regulatory protein (StAR)-mediated cholesterol import. Two further StAR-independent CYP11A1 reactions facilitate pregnenolone biosynthesis. CYP11A1 deficiency is rare and manifests with adrenal insufficiency (AI), and, in 46,XY individuals, with normal male genital development or disorder of sex development (DSD).
Patient
We describe a 46,XY patient born with hyperpigmentation, micropenis and penoscrotal hypospadias. At birth, biochemical and hormonal findings were normal except for low testosterone concentrations. Development was unremarkable apart from an episode labelled as sepsis (day 15 of life) with documented hyponatraemia and hyperkalaemia. Finally, AI was diagnosed at the age 2.8 yr with raised ACTH and renin, low aldosterone and normal baseline cortisol, not responding to ACTH-stimulation. Ultrasound showed normal-sized adrenals and testicular microlithiasis.
Results
Molecular genetic analysis showed mutations neither in the *SF1* nor *StAR* gene. However, two novel *CYP11A1* mutations (p.Arg360Trp (g.27921C>T); p.Arg405X (g.28327C>T)) were found. Segregation analysis confirmed compound heterozygosity. Functional *in vitro* analysis was performed in COS7 cells transfected with wild-type or mutant *CYP11A1* cDNA, with or without wild-type *StAR* cDNA. Transfected cells were incubated with either cholesterol or 22R-hydroxycholesterol. Pregnenolone was quantified by liquid chromatography/tandem mass spectrometry. Our *in vitro* assays showed that p.Arg360Trp retains by 30–40% of wild-type activity. Our *in silico* analysis confirmed these findings and suggest that p.Arg405X completely abolishes CYP11A1 activity.
Conclusions
Our data demonstrates that *CYP11A1* mutations can differently impact on adrenal and gonadal sterodoidogenesis, which is exemplified by progressive AI during infancy but impaired gonadal function already during prenatal life. The delayed onset of adrenal insufficiency may hinder early diagnosis of CYP11A1 deficiency. Thus, a short synacthen test should be performed in patients with DSD to assess adrenal function and prevent life-threatening adrenal crisis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P10

### The mTOR-pathway in normal and tumoral human adrenocortical tissues

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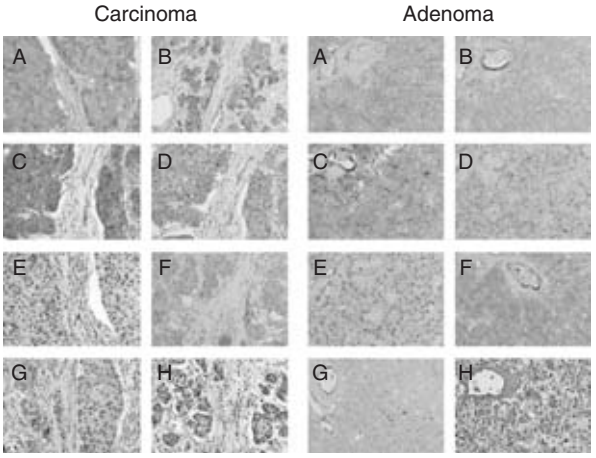
Background
Novel treatment options are required for patients with adrenocortical carcinomas (ACCs). mTOR-inhibitors are anti-neoplastic drugs that target the mTOR-pathway.
Aim
To describe the expression of the mTOR-pathway in normal and pathological adrenocortical tissues.
Methods
We evaluated mRNA expression levels of mTOR, S6K and 4EBP1 in 10 normal adrenals (NA), 11 adrenal hyperplasias (AH), 19 adrenal adenomas (ACA) and 26 ACCs (24 adults, 2 children) by qRT-PCR and determined protein expression of total/phospho-mTOR; total-S6K/phospho-S6K and total/phospho-4EBP1 in 3

NAs, 3 AHs, 6 ACAs and 17 ACCs (15 adults, 2 children) by immunohistochemistry (IHC).
Results
The expression levels of mTOR and 4EBP-1 mRNA were not significantly different in the different tissues evaluated. The S6K mRNA levels were significantly lower in ACCs compared to the other groups (ACC: $0.10 \pm 0.08$; ACA: $0.20 \pm 0.11$; AH: $0.29 \pm 0.07$; NA: $0.23 \pm 0.08$; median $\pm$ SD; $P < 0.01$). In the NA and AH a stronger IHC staining of the evaluated proteins was observed in the glomerulosa and reticularis layers. The majority of ACCs and ACAs presented a significant IHC staining of total-mTOR (59%; 83%); total-4EBP1 (88%; 100%); phospho-4EBP1 (59%; 83%) and phospho-S6K (59%; 83%). A significant staining of phospho-mTOR and total-S6K were observed in 6% and 24% of ACCs and in 33% and 66% of ACAs, respectively.
Compressively a significant staining of both phospho-S6K and phospho-4EBP1 was observed in 41% ACCs and 84% ACAs. A significant expression of at least one of these proteins was observed in 13/17 ACCs (76%).
In ACCs, none of the evaluated mTOR-pathway components was correlated with the Weiss's score or the hormonal status.
Conclusion
This study demonstrates a layer-specific expression of the major components of the mTOR-pathways in NA and the presence of an activated mTOR-pathway in the majority of adrenal tumors. These results support a potential role of mTOR-inhibitors in the treatment of selected patients with ACCs.




Immunocytochemical detection of total-mTOR (A), phospho-mTOR (B), total-4EBP1 (C), phospho-4EBP1 (D), total-S6K (E) and phospho-S6K (F) in a case of human adrenocortical carcinoma (ACC [left panel]) and a case of human adrenocortical adenoma (ACA [right panel]). Pictures "G" shows the absence of staining in the negative controls, and picture "H" the HE staining in both panels Magnification, X100.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P11

**Measurement of cortisol in scalp hair can be a new tool in the diagnosis and follow-up of patients with Addison's Disease and (cyclic) Cushing's Syndrome**

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Introduction
The diagnosis of Cushing's Syndrome (CS) and in particular cyclic CS can be complicated. Standard screening tests for CS are the measurement of cortisol in 24-hours urine collections and in midnight saliva. In case of cyclic CS, results of these tests can be normal in between periods of hypercortisolism. The development of a method to measure cortisol in scalp hair provides the opportunity to investigate historical cortisol levels of months to years ago, with each cm of hair corresponding to a period of one month. This method has been well validated in healthy individuals and might contribute enormously in the diagnosis and follow up of patients with (cyclic) CS. Furthermore, in patients with Addison's Disease (AD), the measurement of historical cortisol levels could provide useful information concerning the disease course and effect of hydrocortisone replacement therapy. Our aim was to study whether hair cortisol levels correspond with clinical course in patients with (cyclic) CS and AD.
Methods
Hair samples were collected from 16 CS patients, 5 cyclic CS patients and 3 AD patients. Cortisol was extracted from these samples with methanol and cortisol levels were measured using an ELISA. A group of 195 healthy individuals were used as control group.
Results
Cortisol levels were significantly elevated in CS patients ($P < 0.0001$) and decreased in patients with AD ($P = 0.002$) compared to healthy individuals. Hair cortisol timelines of patients with CS, cyclic CS and AD corresponded with clinical course.
Conclusion
Scalp hair can be used to evaluate the clinical course in patients with CS and AD and provides valuable information about previous cortisol exposure. This can contribute significantly in the diagnosis of cyclic CS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P12

**Pattern of adrenal hormonal secretion in patients with adrenal adenomas: the relevance of aldosterone in arterial hypertension**

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Introduction
Autonomous aldosterone secretion (AAS) is present in approximately 10% of hypertensives. Adrenal incidentalomas (AI) can be found in up to 19% of hypertensive individuals. However, data on the incidence of AAS in hypertensive patients with AI is scarce. Our aim was to evaluate the adrenal aldosterone secretory profile in patients with adrenal adenomas with and without arterial hypertension.

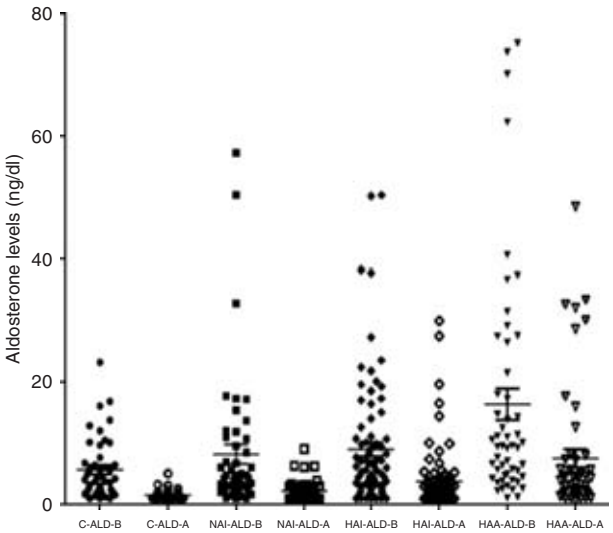


**Figure 1** Comparison of aldosterone levels among the control and the three patients' groups. ALD-B: basal aldosterone levels. ALD-A: aldosterone levels following the modified saline infusion test. C: control group. NAI: normotensive patients with adrenal incidentalomas. HAI: hypertensive patients with adrenal incidentalomas. HAA: hypertensive patients with adrenal adenomas identified during investigation for arterial hypertension.

Patients and methods
We investigated 72 normotensive subjects with normal adrenal morphology and 191 subjects divided in three groups: 46 normotensive individuals with an AI (NAI), 89 hypertensive patients with an AI (HAI) and 56 hypertensive patients with an adrenal adenoma identified after investigation for arterial hypertension (HAA). All patients underwent a low dose dexamethasone suppression test to assess the presence of autonomous cortisol secretion. The diagnosis of autonomous aldosterone secretion was based on a modified saline infusion test (MSI), i.e. following overnight dexamethasone administration to suppress ACTH-induced aldosterone secretion.
Results
To evaluate the prevalence of AAS, we applied the following cut-offs: post MSI aldosterone (ALD) levels: 2.41 ng/dl and the aldosterone/renin (ALD/REN) ratio: 0.35 ng/dl/μU/ml). Based on these cut-offs, 12% of NAI, 36.4% of HAI and 54.2% of HAA patients had AAS. The prevalence of autonomous cortisol secretion did not differ among the three groups. Post MSI aldosterone levels and the aldosterone/renin ratios were significantly elevated in HAI and HAA patients compared to NAI subjects (Figure 1).
Conclusions
Using the MSI test, we found a remarkably increased prevalence of AAS in hypertensive patients with adrenal adenomas, even when the latter present an incidental finding.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P13

**Influence of unilateral adrenalectomy on set point of Hypothalamic-pituitary-adrenal-axis in long-term survivors of childhood nephroblastoma and neuroblastoma**

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Introduction
Adrenal insufficiency, or relative insufficiency, might partly explain increased mortality rates in nephroblastoma and neuroblastoma survivors after unilateral adrenalectomy. Aim of this study was to assess adrenal function and its metabolic effects after unilateral adrenalectomy.
Patients and Methods
Cross-sectional study with a socio-demographically similar control group, conducted between October 2009-March 2011 at the Erasmus University Medical Centre, the Netherlands. Sixty-seven adult long-term survivors of nephroblastoma, 36 survivors of neuroblastoma and 49 control subjects were included in this study. Adrenal function was assessed by a 1 μg short Synacthen-test. Cortisol, adrenocorticotrophic hormone (ACTH), low (LDL-C) and high-density lipoprotein-cholesterol (HDL-C), triglycerides, apolipoprotein-B, glucose and insulin levels were assessed in blood samples taken at baseline. In addition, cortisol levels were assessed after 30 ($t=30$) and 60 minutes. Homeostatic Model Assessment (HOMA) was calculated.
Results
Adrenal insufficiency was not present in survivors. Interestingly, baseline serum cortisol levels were higher in survivors after unilateral adrenalectomy (mean 503 nmol/l) ($N=46$) than in survivors with both adrenals intact (mean 393 nmol/l, $P=0.002$) ($N=52$), and than in controls (mean 399 nmol/l, $P=0.013$) ($N=49$). After correcting for age, sex, and use of oral estrogens, unilateral adrenalectomy was independently associated with elevated baseline cortisol and ACTH levels. Baseline cortisol levels were positively associated with triglycerides ($P<0.001$), LDL-C ($P=0.004$), apolipoprotein-B ($P<0.001$) and HOMA ($P=0.008$).
Conclusions
No adrenal insufficiency was observed in survivors of nephroblastoma and neuroblastoma. Survivors treated with unilateral adrenalectomy had relatively high basal cortisol and ACTH levels, indicating a higher central setpoint of the hypothalamic-pituitary-adrenal axis. This higher setpoint was associated with lipid concentrations and insulin resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This work was supported, however funding details unavailable.

## P14

**PET-tracers for differential diagnosis in primary hyperaldosteronism – *in vitro* studies**

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Objective
The major diagnostic problem in primary aldosteronism is the differentiation between bilateral hyperplasia and aldosterone producing adenoma which is essential for further treatment. Adrenal vein sampling is regarded as the current gold standard, however it is an invasive, highly examiner-dependent method. Molecular imaging targeting the aldosterone synthase (CYP11B2) which is expressed specifically in aldosterone producing adrenal tissue may be an useful alternative. CYP11B2 is highly homologous to 11β-hydroxylase (CYP11B1) (93%). We, therefore, aimed to develop a PET tracer which binds to CYP11B2 with both high affinity and high selectivity.
Methods
We have synthesized more than 90 new compounds so far mostly containing a fluorine atom which enables radiolabelling with 18F for PET imaging. Compounds were tested for inhibition of aldosterone/cortisol (corticosterone) in NCI-H295 cells, murine Y1 cells expressing human CYP11B1/CYP11B2 and in V79 chinese hamster fibroblasts expressing rat CYP11B1/CYP11B2.
Results
After structural optimization, 7 fluorinated CYP11B2 inhibitors could be identified (IC50 values for inhibition of aldosterone synthesis up to 5.5 nM, selectivity factors for inhibition of aldosterone vs cortisol synthesis in murine Y1 cells mostly $>100$).
Conclusion
We developed several fluorinated inhibitors of CYP11B2 exhibiting high affinity and selectivity binding to the target enzyme. These compounds may be suitable for specific molecular imaging in primary hyperaldosteronism. Establishment of radiosynthesis and in vivo evaluation is subject of ongoing studies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P15

**Role of Endogenous Somatostatin and Cortistatin in Regulating Adrenal Gland Function**

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Neuroendocrine balance of the hypothalamo-pituitary-adrenal axis (HPA) is a critical component in the control of metabolic homeostasis, and its dysregulation can contribute to severe pathologies, like obesity, where the HPA is significantly altered at the central and systemic levels. Somatostatin and cortistatin have been shown to reduce circulating ACTH and glucocorticoid-levels in rodents and humans in vivo. However, to date, no studies have thoroughly investigated and compared the precise actions and potential physiological relevance of somatostatin/cortistatin at the adrenal gland level. Accordingly, here we studied the specific roles of endogenous somatostatin/cortistatin on the regulation of the adrenal gland by using somatostatin-knockout (KO) and cortistatin-KO mice and their wildtype-littermate controls, both under normal or obesity-conditions. Results revealed that circulating corticosterone levels were elevated in male/female somatostatin/cortistatin-KO as compared to normal controls. Similar results were observed in obesity conditions, although male plasma corticosterone levels were lower in obese cortistatin-KO. Interestingly, at the adrenal level, major gender- and genotype-dependent differences were found in the expression of key components potentially involved in glucocorticoid regulation. Specifically, MCR2 (main ACTH-receptor in adrenal gland) was not altered in cortistatin-KO while it was decreased in female somatostatin-KO under normal conditions, and increased in obese somatostatin-KO male mice. Interestingly, expression of 11β-hydroxysteroid-dehydrogenase and tyrosine-hydroxylase, two key markers associated with glucocorticoid synthesis were reduced in male somatostatin/cortistatin-KO mice under normal conditions, but were increased in female

cortistatin-KO under normal conditions and in obese somatostatin-KO mice. These results, together with data showing major differences in somatostatin-, CRF-, IGFI- and leptin-receptors in the adrenal gland, reveal that endogenous somatostatin and cortistatin exert unique, gender-dependent actions in the control of adrenal function, which are tightly regulated in extreme metabolic-conditions (obesity), thereby inviting to investigate further the hitherto poorly explored relevance of both peptides in the (patho)physiological regulation of HPA axis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P16

**Cyclical Cushing's syndrome masquerading as Polycystic Ovarian Syndrome – pitfalls in diagnosis**

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Introduction
We present a case of probable cyclical Cushing's syndrome (CS) masquerading as polycystic ovarian syndrome (PCOS) which went undetected for almost a decade. Our case highlights the subtleties and complexities of interpreting diagnostic tests in patients with cyclical endogenous hypercorticolism.
Case
A 33-year old female with presumed PCOS presented with a seven year history of persistent hirsutism and acne. However there was no menstrual disturbance and she had two spontaneous pregnancies. She worked as an advisor for a Slimming Company and reported that both weight and hirsutism fluctuated significantly in a cyclical fashion despite a healthy lifestyle.
Investigations
On presentation BMI was 28 kg/m$^2$ without marked truncal obesity. Blood pressure was 132/90 mmHg and there were no other clinical stigmata of hypercortisolaemia. The diagnosis of PCOS was revisited and CS was queried in view of the absence of menstrual disturbance and fluctuating weight-gain. 17-OHP and serum testosterone was normal. 24-hour urinary free cortisol excretion was raised at 221 (normal $<$200 nmol/24 h). 48 hr low dose Dexamethasone suppression test (DST)showed non suppression of cortisol (Baseline 430 nmol/l; 48 hr 156 nmol/l) confirming endogenous hypercortisolaemia. There was also disruption of diurnal cortisol variation(Midnight cortisol 538 nmol/l). High dose DST suppressed cortisol to 51 nmol/l confirming ACTH dependent CS(ACTH 28 ng/L; normal 0.1 to 47.0). Pituitary MRI demonstrated an 18$\times$10 mm adenoma with cystic degeneration. Transphenoidal pituitary surgery was undertaken without complications and was curative.
Conclusion
Cyclical CS is a rare entity and often misdiagnosed. Cyclical manifestation of the pathognomonic signs and symptoms of hypercortisolism are suggestive of the diagnosis and endocrine testing during symptoms may aid the diagnosis. PCOS shares common symptoms with CS like hyperandrogenism and weight gain and is often over diagnosed by non-specialists. A high index of suspicion remains the cornerstone of diagnosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P17

**Prolonged zona glomerulosa insufficiency causing hyperkalemia in primary aldosteronism following adrenalectomy**

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Context
Unilateral adrenalectomy is the therapy of first choice in aldosterone producing adenoma (APA). Improvement of blood pressure (BP) and hypokalemia is achieved in the majority of patients. Because of hypoaldosteronism, hyperkalemia can develop in the postoperative course. Our aim was to analyze the frequency of hyperkalemia, to determine the cause of hypoaldosteronism and to assess the influence of preoperative mineralocorticoid antagonist (MRA) therapy at our center.
Patients
We analyzed retrospectively data from 69 patients with APA after adrenalectomy. Hyperkalemia was defined as potassium $>$5.0 mmol/L. Patients were seen at the out-patient clinic. BP, aldosterone, renin, serum potassium, creatinine, microalbuminuria and the number of antihypertensive medications were recorded. Dosage and duration of preoperative treatment with MRA were assessed.
Results
We observed postoperative hyperkalemia in 16 patients (23.2%). In 12 of these patients, hyperkalemia was mild without need for further treatment. In 4 patients, the hyperkalemia persisted for 15, 13, 11 and 10 months. The maximum serum potassium observed was 7.1 mmol/L. These patients needed dietary advice, treatment with fludrocortisone (0.1–0.3 µg/day), forced diuresis and bicarbonate administration. They suffered from secondary hypoaldosteronism as plasma renin concentrations remained postoperatively suppressed. In univariate analysis, postoperative hyperkalemia was significantly associated with higher age at diagnosis (57.5+3.2 vs. 49.7+1.5 years, $P$=0.021) and with a worse renal function postoperatively (creatinine 1.29+0.11 vs. 0.98+0.04 mg/dl, $P$=0.025). Although 49 patients received spironolactone in a dose of 51.6+3.0 mg (treatment time 2.3+0.3 months) and 5 patients eplerenone in a dose of 30.0+5 mg (5.0+3.3 months) prior surgery, this did not have a significant impact on postoperative hyperkalemia.
Conclusion
Clinically meaningful postoperative hyperkalemia occurs in 5.8% of adrenalectomized PA patients, caused by prolonged secondary zona glomerulosa insufficiency. Potassium levels after adrenalectomy must be monitored to avoid life-threatening hyperkalemia. Pre-treatment with MRA does not appear to be effective in preventing hyperkalemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P18

**Use of the 250 mcg short synacthen test in the diagnosis of primary aldosteronism**

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Aberrant and upregulated eutopic receptors have been identified *in vitro* in patients with primary aldosteronism (PA). We previously identified an exaggerated aldosterone response to synacthen in patients with PA versus healthy controls. In this study we aimed to evaluate whether the synacthen test differentiates between patients with PA and essential hypertension (EH).
The 250mcg intramuscular synacthen test was performed after 30 minutes recumbency in the morning and off interfering medications in ten patients with PA (7 aldosterone producing adenoma (APA), 3 bilateral adrenal hyperplasia (BAH)), 14 with EH (normal aldosterone to renin ratio) and 14 normotensives. PA was confirmed with saline suppression testing and classified using CT imaging and adrenal venous sampling. Serum aldosterone and cortisol were measured at 0, 30 and 60 minutes. Differences were compared using one way analysis of variance.
Aldosterone was significantly higher in the PA group compared to the EH and normotensive groups at all three time points ($P\leq$0.001 at 0, 30 and 60 minutes). The area under the curve (AUC) on receiver operating curve analysis was greatest at T=60 minutes (AUC: 0.96, $P\leq$0.001). There was also maximal separation between groups at this time point. There was no difference in cortisol response between groups and no difference in response between APA and BAH patients.
This study demonstrated an exaggerated aldosterone response to ACTH in patients with PA compared to those with essential hypertension and normotensive controls. This was a small study however if confirmed in larger numbers and in patients taking anti-hypertensive medications, the synacthen test may prove to be a useful diagnostic tool for PA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P19

**Evaluation of sexual function and psychological attitudes in adult women with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase Deficiency (21-HD)**

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Women with CAH have decreased fertility and sexual activity because of psychological aspects and genital status. The aim of this study was to evaluate sexual function and psychological attitudes in 18 women with CAH due to 21-HD (12 with non-classical, four with salt-wasting and two with simple virilizing CAH; 20–48 yrs) under standard treatment, and 18 age-matched healthy women. Sexual function was evaluated by a validated Female Sexual Function Index (FSFI) questionnaire for the assessment of six domains of female sexual function (desire, arousal, lubrication, orgasm, satisfaction, and pain) as well as an experimental questionnaire (SIEDY), evaluating the same FSFI domains together with three more domains including body image, relationship with partner and masturbation. Psychological attitudes were investigated through the validated Middlesex Hospital Questionnaire (MHQ), evaluating six domains (anxiety, phobia, obsession, somatisation, depression, hysteria). A significantly lower FSFI score, particularly in lubrication and pain domains, was found in patients than controls ($P<0.05$). This evidence was confirmed by SIEDY questionnaire, which demonstrated a significant difference also in body image and relationship with partner domains ($P<0.05$). A significantly higher score ($P<0.05$) was also found in all MHQ domains except for hysteria domain. Serum testosterone, 17-OH progesterone and androstenedione levels were directly correlated with anxiety, phobia, obsession, somatisation, depression ($P<0.05$) and inversely correlated with desire, arousal, lubrication, orgasm, satisfaction, and pain ($P<0.05$). Conversely, DHEA-S was not correlated with MHQ domains, but inversely correlated with arousal, lubrication, orgasm and satisfaction ($P<0.05$).
In conclusion, this study demonstrated that women with CAH are affected not only by disturbed psychological balance but also to a pathological sexual function, as demonstrated by both a standard and an experimental questionnaire, despite an appropriate treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P20

**Role of adrenal vein sampling in primary aldosteronism. Impact of different diagnostic criteria on subtype diagnosis**

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In patients with primary aldosteronism (PA), adrenal vein sampling (AVS) is considered the gold standard to distinguish between unilateral and bilateral disease, while diagnostic imaging tests (CT/MRI) are often inconclusive for the diagnosis of PA. To date agreement is lacking on the best criteria indicating successful cannulation and lateralization. Aim of the study was to evaluate the impact of different diagnostic criteria for the successful cannulation and lateralization on subtype diagnosis and to compare the difference of the findings between adrenal CT scan and AVS. 67 patients with confirmed PA underwent AVS. The different diagnosis of PA subtype reached using AVS data assessed by more permissive (type 1) and strict (type 2) criteria were compared. All patients performed CT scan and imaging results were compared with results of AVSs. Using Type 1 criteria AVSs were successful in 86% of patients;using type 2 criteria only 64.5%. Type 1 criteria led to a higher rate of diagnosis of unilateral PA (85% of successful procedures) than type 2 (75%). There was considerable disparity in the diagnosis reached using the 2 different criteria, with a concordance in only 45% of patients. In conclusion more permissive criteria for successful cannulation and lateralization on AVS can lead to incorrect diagnosis and accordingly to inappropriate treatment options. In the selected group of patients with successful AVS, CT findings correlated with AVSs findings and correctly identified unilateral or bilateral disease in 58,5% of patients using type 1 criteria and in 47.5% using type 2 criteria. Finale diagnosis was based on histological results in 32 patients (48%) which underwent adrenalectomy based on AVSs findings. On the basis of CT findings alone 17% of patients from the first group and 32,5% of patients of the second group probably would have been incorrectly bypassed as candidates for adrenalectomy. CT scanning lacks sensitivity and specificity and should, therefore, be followed by AVS, which is the only reliable means of differentiating unilateral from bilateral PA and lateralizing APAs preoperatively. However, there are still controversies to be solved by large prospective studies on the criteria to adopt for defining the most appropriate cut off for correct cannulation and lateralization.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P21

**Predicting factors of the post-surgical decline in renal function in patients with primary aldosteronism**

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Primary aldosteronism (PA) is the most common cause of secondary hypertension. Although decline in renal function especially that experienced after adrenalectomy (ADX) has been demonstrated, details of the mechanism remain to be elucidated. Aim of the study was to investigate the factors predicting renal outcome after ADX in PA. Twenty patients with PA and four patients with non-functioning adrenal tumor (NFT) as control were studied. eGFR, serum potassium, plasma aldosterone concentration (PAC), and plasma renin activity (PRA) before and 1 month after ADX were analyzed. The study was approved by the institutional ethical committee. Baseline characteristics ($M\pm SD$, PA vs. NFT) were as follows: age; $51\pm9$ vs. $65\pm9$ yrs, blood pressure; $138\pm13$ (estimated duration of hypertension $10.3\pm9.1$ yrs) vs. $114\pm10$ (mmHg), eGFR; $79.2\pm18.4$ vs. $75.3\pm19.2$ (ml/min/1.73 $m^2$), serum potassium; $3.1\pm0.9$ vs. $4.4\pm0.3$ (mEq/L), PRA; $0.3\pm0.3$ vs. $1.0\pm0.6$ (ng/ml/h), PAC; $336\pm238$ vs. $87\pm35$ (pg/mL), and ARR; $2089\pm2728$ vs. $131\pm102$. Eighty % of PA patients (16/20) showed decline in eGFR after ADX ($79.2\pm18.4$ to $72.2\pm21.5$, $P=0.002$). Post-ADX eGFR was correlated positively to baseline eGFR ($r=0.82$) and ΔPAC ($r=0.46$) and negatively to ΔK ($r=0.44$) and estimated duration of hypertension ($r=0.63$). Of the 13 patients with PA with hypokalemia before ADX, twelve patients showed normokalemia and 1 patient showed hyperkalemia (5.6 mEq/L) after ADX. Post-ADX serum potassium was correlated positively to baseline PAC ($r=0.70$) and ARR ($r=0.75$) and negatively to ΔPAC ($r=0.63$) and ΔARR ($r=0.76$). By contrast to the changes in PA, there were no changes in eGFR and serum potassium in NFT after ADX. These results suggest that duration of hypertension and PAC is the predicting factors for a significant decline in eGFR and hyperkalemia after ADX. Early diagnosis with correction of hypertension and hyperaldosteronism is essential for a better renal outcome in PA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P22

**Specialized surgery for adrenocortical carcinoma in The Netherlands: analysis of national cancer registry data**

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Introduction
Carcinoma of the adrenal cortex (ACC) is a rare disease with an estimated incidence of 1–2 per 1 million population. Optimal treatment of ACC is multidisciplinary and has complex aspects such as adrenal surgery, adjuvant therapy with mitotane and cytotoxic chemotherapy in advanced stages. The Dutch Adrenal Network (DAN), a collaboration between all university centers and Máxima Medical Center, was founded with the objective of improving patient care and stimulating scientific research regarding ACC. Currently, not all patients

with ACC are treated in such a specialized DAN-center. The objective of our current investigation is to determine whether there are differences in survival between patients operated on in DAN-centers and non-DAN hospitals.
Methods
Data on all adult ACC patients diagnosed between 1999 and 2009 were obtained from The Netherlands Cancer Registry (NCR). Overall survival was calculated and a comparison was made between DAN- and non-DAN hospitals.
Results
The registry contained data on 191 patients, ACC was histologically confirmed in 185 patients (97%). Median survival was significantly different between patients with ENS@T disease stage I-III operated on in a DAN hospital ($n=40$) compared to patients operated on in a non-DAN hospital ($n=33$, median survival not reached versus 45 months (95% CI 16–74 months), $P=0.026$). This difference remained significant when corrected for gender, age, year of diagnosis, chemotherapy and stage of disease in multivariate analysis ($P=0.026$, hazard ratio 0.44 (95% CI 0.21–0.90)).
Conclusion
The results display that surgery in a DAN-center yields a survival benefit for patients with local or locally advanced adrenocortical carcinoma. These findings should be carefully considered in the strive for further centralization of ACC-treatment in The Netherlands.
Declaration of interest
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## P23

**Long term follow-up of patients with adrenal incidentalomas – a prospective study**

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Introduction
Adrenal incidentalomas (AIs) are increasingly detected due to the widespread use of abdominal imaging for diagnostic purposes. AIs are usually benign non-functioning adenomas but few prospective studies exist regarding their evolution. The aim of this study was to investigate the morphological and hormonal changes of AIs in a cohort of patients with long-term follow-up.
Materials and methods
This was a prospective observational study conducted at the department of Endocrinology in Hippokration General Hospital (Thessaloniki, Greece) from 2008. The following tests were performed: serum cortisol at 08:00 and 23:00, 24h-urinary free cortisol, 1-mg dexamethasone-suppression-test, plasma adreno-corticotrophic hormone (ACTH), serum aldosterone (ALDO), plasma renin activity (PRA), ALDO/PRA ratio and 24 h-urinary total metanephrines and catecholamines. Radiological assessment was repeated at 6–12 months and yearly thereafter.
Results
Forty patients (12 males and 28 females; mean age $58.9 \pm 11.6$ years), with 51 AIs and follow-up of $19.8 \pm 18.3$ months (range 0–48) were evaluated. The mean diameter was $27.5 \pm 11.5$ mm, being unilateral in 29 [15 (37.5%) in the right and 14 (35%) in the left gland] and bilateral in 11 patients (27.5%). Two patients underwent adrenalectomy, revealing one benign cortical adenoma and one pheochromocytoma. Three patients (7.5%) had subclinical Cushing's syndrome (SCS), 1 (2.5%) pheochromocytoma and 2 (5%) aldosteronoma. The remainder had non-secretory masses. One patient with SCS who underwent adrenalectomy experienced imrovement of dyslipidemia and normalization of blood pressure. Although 6 AIs were $>40$ mm, there were no malignancies detected.
Mass enlargement (5–10 mm) was observed in 6 AIs (15%) (from the first year of follow-up in 5), while mass shrinkage (27 mm) in 1 (2.5%). No hormonal evolution was noticed.
Conclusions
The vast majority of AIs involved benign, non-secretory lesions. Increase in size occurred early on follow-up and was more frequent than previously reported. Adrenalectomy led to amelioration of cardiovascular risk factors in SCS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P24

**Effects of sugar cane extract (SCE) supplementation on steroidogenesis of the adrenal cortex in male rats**

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Effects of sugar cane extract (SCE) on the adrenal corticosterone and progesterone secretion in male rats were investigated using an *in vitro* cell culture system. Three-week old male rats were fed SCE free diet or 2.16% SCE diet for 7 weeks. After 7 weeks of treatment, animals were decapitated, and adrenal cells were cultured in the absence or presence of rat ACTH (10–15 to 10-10 M) for 4 hours. Body and adrenal gland weight, plasma concentration of ACTH, corticosterone, progesterone, testosterone and basal mRNAs levels of StAR, 5 steroidogenic enzymes (P450Scc, 3β-HSD, P450c21, P450c11, 17βHSD) and ACTH receptor (MC-2) in adrenal gland were measured. There were no differences in body and adrenal gland weight between two groups. The plasma concentrations of ACTH, corticosterone progesterone were significantly high in the SCE supplemented group as compared with the control group. Adrenal cells in the SCE supplemented rat exhibited significantly higher basal level of corticosterone and progesterone than the control rat. The response of ACTH on corticosterone and progesterone release from adrenal cells was significantly higher in the SCE supplemented rat than the control rat. Basal level of P450c21 mRNA in adrenal glands was significantly higher in the SCE supplemented rats than the control rats, whereas the basal mRNAs levels of StAR, P450Scc and P450c11 in the SCE supplemented rat were significantly low as compared with the control rat. The level of 3β-HSD was non-significantly higher in the SCE supplemented rat than the control rat. In addition, the level of MC-2 mRNA was significantly high as compared with the control rat. These results clearly demonstrated that SCE supplementation stimulates basal and ACTH stimulated secretion of corticosterone and progesterone from adrenal cells in male rats. These results also suggest that stimulatory effect of SCE on adrenal steroidogenesis may result in resistance against stress.
Declaration of interest
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## P25

**Evaluation of renal function of primary aldosteronism between pre- and post-treatment**

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Objective
The prevalence of primary aldosteronism (PA) occures in approximately 5–10% of patients with hypertension. Aldosterone induces fluid retention and a variety of angiopathy. We investigated the course of hypertension and renal function in the post-treatment period.
Methods
Nine patients with PA were performed adrenalectomy and 21 patients were administered medications including mineralocorticoid receptor brocker. Blood pressure, serum potassium, aldosterone, plasma rennin-activity (PRA), the aldosterone/renin ratio (ARR), and renal function were evaluated between pre- and post-treatment.
Results
Systolic blood pressure and ARR were significantly decreased and serum potassium level was significantly increased in both groups. However, eGFR was decreased 1 year after treatment in both groups. Drug utilization for hypertension was increased in the group of medication, whereas was not in the group of operation.
Conclusion
Blood pressure was significantly decreased both the treatment of operation and medication. However, renal function was decreased despite the treatment of operation or medication. Fluid retention and vascular distance caused by hypersecretion of aldosterone induced hypertension. The treatments would induce efferent arteriole dilatation and decreased renal blood flow by reduction of blood pressure. The renal dysfunction is considered to be influenced by renal blood flow. Careful long-term follow-up was necessary, because differences of the PA duration and the treatment may affect the course of renal function.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P26

**Follow-up of nonsurgical nonfunctioning adrenal adenomas at five years**

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The management of nonfunctioning adrenal masses is controversial. The size is usually the only criterion for surgical treatment. The objective was to study the progression of the tumor size and the follow-up of several cardiovascular risk parameters in patients with nonsurgical nonfunctioning adrenal masses.
Retrospective longitudinal observational study of 24 patients (54.2% men) with nonfunctioning adenomas followed for five years without surgical treatment. Age, sex, size and location of tumor, blood pressure (BP), body mass index (BMI), lipids, fasting glucose, ions (Na and K), cortisol levels, 24 hours cortisoluria, ACTH, S-DHEAS, aldosterone, urine catecholamines and metanephrines, were analyzed at the time of diagnosis and after five years. LDL and TG were classified according to ATP -III criteria, and diabetes or prediabetes were diagnosed according to the ADA criteria. Adrenal CT was performed every 6 months. Data are presented as mean (standard deviation) and percentage.
At baseline, mean age was 55.5 (11.8) years, BMI 30.1 (5.3) kg/m$^2$. The adenoma's size was 2.1 (0.9) cm. There was hypertension in 50% of patients, hypercholesterolemia in 29.2%, hypertriglyceridemia in 8.3%, mixed dyslipidemia in 12.5%, prediabetes in 29.2% and diabetes in 4.2%.
The mean tumor growth at 5 years was 0.4 (0.5) cm. 45.8% of patients showed a deterioration on the LDL levels, 37.5% worsened the TG levels, and 37.5% aggravated their carbohydrate metabolism. 29.2% of patients required to increase the antihypertensive treatment and 12.5% of nonhypertensive patients developed hypertension ($P < 0.01$). Only one of the adenomas became functioning (hipercortisoluria, lack of cortisol suppression with dexamethasone), confirmed by biopsy after surgery.
In our serie, the growth of nonfunctioning adrenal adenomas is stable after 5 years since diagnosis. However, there is a tendency to a deterioration of BP, carbohydrate metabolism and lipid profile.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P27

**Importance of the genetic study in fertile women with nonclassical congenital adrenal hyperplasia**

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The Nonclassical Congenital Adrenal Hyperplasia (NCCAH) is an autosomal recessive disorder. The importance of the diagnosis in fertile women relies in its therapeutic implications, genetic counseling and antenatal care during pregnancy. Diagnosis is established by the presence of basal 17-OH-progesterone (17-OHP) levels $\geqq 6$ ng/ml or stimulated after ACTH test $\geqq 15$ ng/ml. Basal values between 2-6 ng/ml or stimulated between 10–15 ng/ml would require further confirmation. To study the correlation between the result of basal and/or stimulated 17-OHP and the presence and type of mutation in the 21-hydroxylase's gene.
Descriptive study of the genetics of 11 women diagnosed analytically of NCCAH with basal 17-OHP$\geqq 6$ ng/ml or stimulated $\geqq 10$ ng/ml. Molecular genetic study of 21-hidroxilase gene was requested. Patients were divided into 2 groups. A: those with basal 17-OHP $\geqq 6$ ng/ml and/or stimulated $\geqq 15$ ng/ml (highly suggestive of diagnosis of NCCAH). B: those with basal 17-OHP $< 6$ ng/ml and/or stimulated 10–15 ng/ml (uncertain diagnosis).
Results are shown on table.
- In our serie with an uncertain analytical diagnosis of NCCAH, the genetic study did not confirm the disease and it revealed three carriers of a mutated allele (mild mutation).
- A patient with basal 17-OHP of 7.5 ng/mL had a severe mutation.
- The genetic study is necessary to identify those pregnant women who would benefit from antenatal treatment.

| Number of participants N = 11 | Group A | Group B |
|---|---|---|
| Basal 17-OH-P ng/mL (mean ± SD) n = 11 | 7.9 ± 8.3 | 2.3 ± 1.1 |
| S timulated 17-OH-P ng/mL (mean ± SD) n = 9 | 50.8 ± 22.7 | 12.1 ± 1.0 |
| Genetics | | |
| Normal | 0 | 3 |
| 1 mutated allele | 1 | 0 |
| 2 mild mutants alleles | 3 | 0 |
| 1 mild mutant allele and 1 severe mutant allele | 1 | 0 |

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The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P28

**Aldosterone-producing cells regenerated from mesenchymal stem cells**

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Introduction
Mesenchymal bone marrow cells (BMCs) contain pluripotent progenitor cells, which differentiate into multiple lineages. In a previous study, we reported that adenovirus-mediated forced expression of steroidogenic factor-1/adrenal 4 binding protein (SF-1/Ad4BP), an essential nuclear receptor for steroidogenesis, could transform human BMCs into steroidogenic cells, suggesting a possibility of cell transplantation therapy in patients with steroid deficiency. In this study, we focused on the capability of the cells to produce aldosterone and characterized the mode of production.
Methods
Primary cultured human BMCs were infected with lentivirus containing human SF-1 cDNA and the steroid production was examined in various conditions.
Results
In human BMCs, endogenous expression of AII receptor type 1 was detected. Introduction of SF-1 into human BMCs caused dramatic inductions of most steroidogenic enzymes including CYP11B1 as well as ACTH receptor. CYP11B2 was barely detectable by SF-1 introduction. However, under the condition of SF-1 introduction, it was dramatically induced by AII treatment, thus leading to a good responsiveness of the cells to ACTH and AII, respectively and final production of corticosterone, aldosterone and cortisol. AII blocker, Losartan canceled the increase of those steroids secretion by AII treatment.
Conclusion
These data suggest that SF-1 can transform BMCs into aldosterone-producing cells which can be regulated by AII-AII receptor system.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P29

**The ACTH-independent macronodular adrenal hyperplasia gene hunt: from candidate genes to a pangenomic strategy**

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ACTH-independent macronodular hyperplasia (AIMAH) affects both adrenals, and familial forms are reported, suggesting a genetic origin. Rare mutations have been reported in several genes, including Gs alpha (GNAS), Phosphodiesterase 11A (PDE11A), Fumarate Hydratase (FH), and the Glucocorticoids receptor (GR).
Objective
To assess the prevalence known genes mutations, and identify new candidate genes in AIMAH.
Design and methods
Germline and/or tumor DNA of 62 AIMAH patients was studied by direct sequencing of candidate genes (GNAS, PDE11A, FH and GR), and by pangenomic genotyping using Affymetrix SNP6 arrays in search for chromosomal alterations in germline and in AIMAH nodules.
Results
Missense PDE11A mutations were more common in patients (18/62, 29%) than in controls (7% in 279 controls). Mutations in FH were found in 4/49 patients (8%). No germline GR mutation, and no somatic GNAS mutation were found.
Chromosomal gains and losses are uncommon in AIMAH nodules; a recurrent gain was found in 1q (2/29); recurrent losses included chromosomes 1p (3/29), 3, 17 and 18q (2/29). No large recurrent gain or loss was found in germline; one loss in 6q, and in 16p, and one gain in 9q were found. Copy neutral loss of heterozygosity in chromosome 16p was found in 10 nodules from 6 out of 29 patients studied.
Conclusions
Candidate genes and pangenomic approaches are complementary methods to decipher the complexity of AIMAH genetics. PDE11A mutations seem to predispose to AIMAH. Copy neutral loss of heterozygosity in chromosome 16p is the most common alteration in AIMAH nodules, suggesting the implication of gene(s) in this region.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P30

**Superselective adrenal venous sampling for intra-adrenal localization of aldosterone hypersecretion.**

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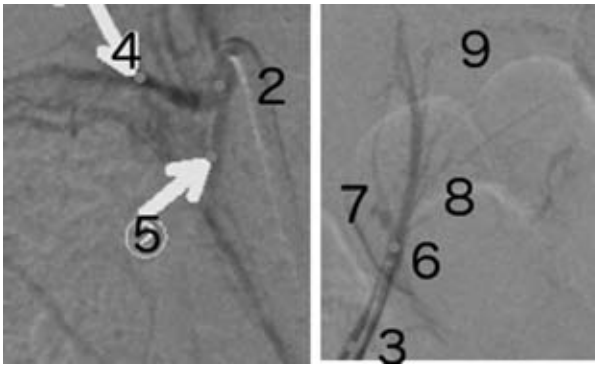
Introduction
Unilateral aldosterone hypersecretion can be adequately treated based on the localization by adrenal venous sampling. However, bilateral aldosterone producing adenoma or primary aldosteronism with preclinical Cushing syndrome cannot be treated with simple unilateral adrenalectomy. Diagnosis of intra-adrenal localization of aldosterone hypersecretion is necessary for surgical treatment of these types of primary aldosteronism.
Method
From 2009 to 2011, we performed superselective adrenal sampling of 68 patients with primary aldosteronism. A 6.5-Fr catheter was inserted from the left femoral vein to select the orifice of the left adrenal vein. Then a 6.5-Fr catheter designed for various types of right adrenal vein ("Adselect series", Hanako medical, Tokyo, Japan) was inserted from the right femoral vein into the right adrenal vein. MDCT information was used as a reference of catheter manipulations. Simultaneous venous sampling was repeated 15 minutes after intravenous one-shot injection of the 0.25 mg of ACTH. Then superselective adrenal venous sampling was performed by catheterization into each 3 tributaries of bilateral adrenal vein using high-fro-type microcatheter.
Results
In all cases, superselecive adrenal venous sampling was successfully perfrmed. In 27 cases, unilateral aldosterone hypersecretion was diagnosed, followed by unilateral adrenalectomy. Superselective sampling provides very high absolute concentration of aldosterone in at least one adrenal venous tributary of unilateral adrenal gland, confirming the diagnosis of aldosterone producing adenoma. In 4 cases, bilateral aldosterone hypersecretion with normal aldosterone level in at least one tributary was diagnosed showing bilateral aldosterone producing adenoma, which enabled surgical treatment of unilateral total adrenarectomy combined with contrarateral partial adrenarectomy. In the remaining 37 cases, blood sample from all the tributaries of bilateral adrenal vein showed high aldosterone concentration showing idiopathic hyperaldosteronism, followed by medical treatment.
Conclusion
Superselective adrenal venous sampling allows primary aldosteronism with bilateral aldosterone producing adenoma treated by surgery.




Bilateral superselective adrenal venous sampling. Blood were sampled from 9 points shown in the figure. Intra-adrenal localization of aldosterone hypersecretion could be diagnosed: bilateral aldosterone hypersecretion with normal aldosterone content in one of the tributaries. This patient could be treated by unilateral total adrenarectomy combined with contralateral partial adrenarectomy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P31

**Analysis of the glucocorticoid receptors polymorphism in patients with adrenal incidentaloma and patients with Cushing's syndrome.**

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The effects of glucocorticoids are mediated by the glucocorticoid receptors (GR) and some single nucleotide polymorphisms (SNPs) have been associated to enhanced sensitivity (N363S, BclI) or reduced sensitivity (ER22/23EK) to glucocorticoids.
Aim of the present study was: 1) to assess the frequency of N363S, BclI and ER22/23EK in 46 patients with Cushing's syndrome (CS) and 73 patients with adrenal incidentaloma (AI) compared to 186 healthy subjects; 2) to evaluate a possible correlation between the different SNPs and patient phenotype.
In all patients we evaluated clinical and demographic parameters, urinary free cortisol (UFC) and cortisol after 1 mg dexamethasone suppression test (DST). DNA was extracted from peripheral blood leukocytes using PCR. The PCR product was digested with 1 U of TasI at 65 °C overnight, and the accuracy of genotyping was confirmed by sequencing analysis.
We did not observe any differences between the frequency of N363S, BclI and ER22/23EK in patients and controls. Although not statistically different, we observed a reduced frequency of BclI in patients with AI and cortisol $>5$ mcg/dl after DST. Moreover, 4 out 5 patients with AI carrying the N363S SNP had an overt metabolic syndrome despite normal UFC and cortisol levels after DST.
Our data suggest that the evaluated SNPs are not involved in the pathogenesis of CS or AI. In patients with CS, we did not observe any genotype/phenotype correlation, since the overt hypercortisolism likely masks any modulatory effect

of the SNPs on clinical parameters. In AI patients, who present only a mild hypercortisolism, BclI may affect cortisol suppression after DST, while N363S may be associated to a worsen metabolic profile.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P32

### KCNJ5 Mutations in European Families with Non-Glucocorticoid Remediable Familial Hyperaldosteronism

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Primary Aldosteronism (PA) is the most frequent cause of endocrine hypertension. Three forms of familial hyperaldosteronism (FH) have been described, named FH-I to -III. Recently, a mutation of KCNJ5 has been shown to be associated with FH-III, whereas the cause of FH-II is still unknown. In this study we searched for mutations in KCNJ5 in 46 patients from 21 families with FH, in which FH-I was excluded. We identified a new germline G151E mutation in two PA affected subjects from an Italian family and three somatic mutations in aldosterone-producing adenomas (APA), T158A previously described as a germline mutation associated with FH-III, and G151R and L168R both described as somatic mutations in APA. The phenotype of the family with the G151E mutation was remarkably milder compared to the previously described American family, in terms of both clinical and biochemical parameters. Furthermore, patients with somatic KCNJ5 mutations displayed a phenotype indistinguishable from that of sporadic PA. The functional characterization of the effects of the G151E mutation in vitro, showed a profound alteration of the channel function, with loss of $K^+$ selectivity, $Na^+$ influx and membrane depolarization. These alterations have been postulated to be responsible for voltage gate $Ca^{2+}$ channel activation, increase in cytosolic calcium and stimulation of aldosterone production and adrenal cells proliferation. In conclusion, we describe herein a new mutation in the KCNJ5 potassium channel associated with FH-III, responsible for marked alterations of channel function but associated with a mild clinical and hormonal phenotype.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P33

### Applicability of the guideline for the diagnosis of primary hyperaldosteronism in patients with hypertension in Japan (PHAS-J2): Prospective multi-center study of national hospital organization

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Primary aldosteronism (PA) is the most major cause of secondary hypertension, with its prevalence ranging from 3 to 20% of the hypertensive patients. Although guideline for diagnosis of PA has been established by the Endocrine Society, details of its applicability remain to be elucidated. In this study, we investigated the applicability of each step of the guideline in hypertensive patients by the multi-center collaborative study of National Hospital Organization (NHO) in Japan (PHAS-J2). Total 25 NHO hospitals participated into the study. The study was performed in accordance with the Guidelines for Human Research of Japanese Government and approved by the institutional ethical committee. Written informed consent was obtained from each patient. Patients with hypertension ranging from 20 to 75 yrs were enrolled. Plasma aldosterone to renin activity ratio (ARR) with a cutoff of 20 was used for case detection and captopril test was used as a confirmatory testing. Captopril-positive patients were subjected to subtype testing with adrenal CT, 131I-adosterol scintigraphy, and adrenal venous sampling (AVS). Total 1237 patients were enrolled to the study. Case detection was positive in 21% of the patients and captopril test was positive in 58% of the screening-positive patients. Collectively, prevalence of PA was 12.2% of the total patients with hypertension. Adrenal tumor was visualized in 32% on CT. Applicability of each step was 85% in confirmatory testing, 93% in adrenal CT, 14.9% in adrenal scintigraphy, and 39% in AVS. Subtype diagnosis was achieved in 46% in those patients subjected to adrenal scintigraphy and/or AVS. These results suggest that applicability of the PA guideline in its subtype testing is not high enough to make definite diagnosis. Given the high prevalence of PA in hypertension, modification of the PA guideline as well as further advances in subtype testing are mandatory.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P34

### Copy number alterations and loss of heterozygosity in cortisol-secreting adrenocortical adenomas using SNP arrays: evidence of new candidate genes and pathways.

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Introduction
The genetic mechanisms underlying adrenocortical tumor development are still largely unknown. We used high-resolution single nucleotide polymorphism (SNP) microarrays to detect copy number alterations (CNAs) and copy neutral losses of heterozygosity (cnLOH) in cortisol-secreting adrenocortical adenomas (ACAs). We focused on microalterations aiming to discover new candidate genes involved in early tumorigenesis and/or autonomous cortisol secretion.
Methods
15 cortisol-secreting ACAs with matched blood samples were investigated. The SNP arrays were performed by Affymetrix SNP 6.0 and paired data analysis by Partek Genomics Suite software. Gene ontology annotation, pathway, gene and protein network analyses were used to identify candidate genes.
Results
We detected 962 CNAs with a median of 18 CNAs per sample. Half of them involved non-coding chromosomal regions, 89% were $<100$ Kb and 28% were found in at least two samples. Most frequently gained segments were 5p15.33, 6q16.1, 7p22.3-22.2, 8q24.3, 9q34.2-34.3, 11p15.5, 11q11, 12q12, 16q24.3, 20p11.1-20q21.11, and Xq28 ($\geqq$20% of cases), most of them being identified in the same three ACAs. These regions contained among others genes like NOTCH1, CYP11B2, HRAS, and IGF2. Recurrent losses were less common and smaller than gains, being mostly localized at 1p, 6q and 11q. Pathway analysis revealed that Notch signaling was the most frequently altered. We identified 46 recurrent microalterations that each affected a single gene (31 gains and 15 losses), including some genes involved in steroidogenesis (CYP11B1) or tumorigenesis (CTNNB1, EPHA7, SGK1, STIL, FHIT). Finally, 20 small cnLOH in four cases affecting 15 known genes were found.
Conclusion
Our findings provide the first high-resolution genome-wide view of chromosomal changes in cortisol-secreting ACAs and identify novel candidate genes, such as HRAS, EPHA7, and SGK1. Furthermore, they implicate that the Notch1 signaling pathway might be involved in the molecular pathogenesis of adrenocortical tumors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P35

### Differential DNA methylation of the CYP11B1 and CYP11B2 genes in association with human adrenal zonation

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Background
The human adrenal cortex is composed of 3 distinct zones: the zona glomerulosa (ZG), the zona fasciculata (ZF), and the zona reticularis (ZR). Steroidogenic enzymes are expressed in a zone-specific manner. The promoters of CYP11B1 and CYP11B2 possess a number of target sites for DNA methylation that is closely associated with gene silencing.
Methods and Materials
Adrenals from 5 autopsy subjects were assessed for immunohistrochemically defined adrenal zonation, using antibodies for 17alpha-hydroxylase/17,20-lyase (CYP17), 3beta-hydroxysteroid dehydrogenase (HSD3B), and dehydroepiandrosterone (DHEA)-sulfotransferase (SULT2A1). CYP17, HSD3B, and SULT2A1 are entirely expressed in ZF/ZR, ZG/ZF, and ZR, respectively. We used laser capture microscopy to isolate DNA from each zone in adrenal tissues and performed an analysis of DNA methylation. Promoter methylation patterns of CYP11B1 and CYP11B2 in adrenal zones were examined by means of bisulfite sequencing.
Results and Discussions
CYP11B1 was completely hypomethylated in ZF while completely hypermethylated in both ZG and ZR. In contrast, CYP11B2 in ZG was hypomethylated compared with those in ZF and ZR. Inter-individual diversity was observed in DNA methylation of CYP11B2 in ZR. The important differences in DNA methylation were that CYP11B1 and CYP11B2 were hypomethylated in ZF and ZG, respectively compared with other zones and that hypomethylation of CYP11B2 in ZG was incomplete whereas that of CYP11B1 in ZF was complete. 11beta-hydroxylase (CYP11B1) has been reported to be expressed highly in ZF and weakly in ZR while aldosterone synthase (CYP11B2) has been sporadically expressed only in ZG. NR4A receptor family involved in the regulation of CYP11B2 has been shown to be differentially expressed in a zone-specific manner.
Conclusions
Taken together, DNA methylation patterns of the CYP11B1 and CYP11B2 promoters were closely associated with adrenal zonation. The DNA methylation patterns, however, do not fully explain zone-specific expression patterns of 11beta-hydroxylase and aldosterone synthase. DNA methylation is considered important for adrenal zonation in conjunction with zone-specific transcription factors. Since the expression of aldosterone synthase depends on salt intake, incomplete DNA hypomethylation of CYP11B2 in ZG is ascribed to high-sodium diets in contemporary society.
Declaration of interest
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## P36

### Super-selective ACTH-stimulated adrenal venous sampling can discriminate the main lesion from the normal tissue for doing partial adrenalectomy in unilateral aldosterone-producing adenoma

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Introduction
Primary aldosteronism (PA) is one of the common diseases, and aldosterone-producing adenoma (APA) is a surgically curable type, which is usually treated by unilateral total adrenalectomy. We had recently developed a new method of supper-selective ACTH-stimulated adrenal venous sampling (SS-ACTH-AVS) for exactly detecting the main lesion(s) of hyperaldosteronemia. Then we attempted to partially remove the unilateral lesion of hyperaldosteronism detected by SS-ACTH-AVS without resecting normal tissues.
Methods
We developed a new micro-catheter to obtain adrenal effluent at intra-adrenal tributary veins. By using that catheter, SS-ACTH-AVS was performed in 50 patients with PA demonstrating unilateral CT-detectable adrenal nodule. Adrenal effluents were sampled at more than 2 intra-adrenal first-degree tributary veins in each adrenal gland after ACTH stimulation. When concentration of aldosterone in effluent sampled at one of the tributary veins connecting to any nodule was $>1400$ ng/dl and the others were $<1400$ ng/dl, the nodule was diagnosed as APA. Then, the patients with APA were treated by laparoscopic unilateral partial resection of the nodule. When aldosterone was $>1400$ ng/dl in all effluents sampled at tributaries of the unilateral adrenal, showing the presence of the nodule and $<1400$ ng/dl in all effluents sampled at each tributary of the opposite adrenal gland, the patients were also diagnosed as unilateral hyperaldosteronism and treated by unilateral total adrenalectomy.
Results
Thirty patients with CT-detectable APA exactly diagnosed by SS-ACTH-AVS were treated by unilateral partial adrenalectomy. Pathological examinations of resected adrenal glands demonstrate that APAs were completely removed without destruction of their capsules. One year after adrenalectomy, concentrations of aldosterone in peripheral blood samples were normalized in 30 patients treated by partial adrenalectomy as well as in 20 treated by total one.
Discussion
SS-ACTH-AVS is promising for choosing how to remove the adrenal lesion inducing hyperaldosteroneima, such as unilateral partial and total adrenalectomy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P37

### Deletion of TASK3 $K^+$ channels leads to hyperaldosteronism in neonatal mice

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Depolarisation of adrenal zona glomerulosa cells is a pivotal event for the secretion of aldosterone. Potassium channels like TASK1 and TASK3 are highly expressed in the adrenal cortex and determine the membrane voltage. Deletion of TASK3 $K^+$ channels in mice caused a severe age-dependent hyperaldosteronism. The aim of this study was to identify the mechanisms underlying this phenotype.
Neonatal TASK3-/- mice (ko) showed higher plasma aldosterone levels compared to wildtype (wt) animals correlating with an increased adrenal mRNA expression level of the aldosterone-synthase. These high aldosterone levels decreased within the first two weeks of life. Plasma concentrations of progesterone and corticosterone showed the same age-dependent dysregulation. These results pointed to a broader adrenal dysfunction in newborn ko mice. A chip-based analysis was performed to measure differential gene expression in adrenals of 1 and 12 day old mice. Interestingly, adrenal renin mRNA was strongly upregulated in newborn ko mice, but decreased to normal wt levels in 12 day old mice. The high renin gene expression was confirmed by realtime PCR and by renin-specific immunofluorescence.
These data suggest that transient activation of the local adrenal renin-angiotensin-system contributes to the hyperaldosteronism of neonatal TASK3-/- mice. Future studies are needed to investigate the signalling pathways resulting in increased adrenal renin production and to identify the compensation mechanisms present in adult mice.
Declaration of interest
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## P38

**Expression of kisspeptin and its receptor in aldosterone-producing adrenal adenomas**

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Kisspeptins are the product of the KiSS-1 gene, a metastasis suppressor gene, and play important physiological roles in the hypothalamo-pituitary gonadal axis and reproduction. It was reported that kisspeptin-10 stimulated the aldosterone synthesis in H295R human adrenal cancer cells (Nakamura et al., 2007). However, expression of kisspeptin and its receptor has not been studied in tumor tissues of adrenocortical tumors. The aim of the present study was therefore to clarify expression of kisspeptin and its receptor in tumor tissues of adrenocortical tumors, particularly aldosterone-producing adenomas (APAs). Immunocytochemisty of kisspeptin and kisspeptin receptor was performed by the ABP method in tumor tissues of adrenal tumors obtained at surgery, including 31 APAs. The antiserum against human kisspeptin-10 was raised in a rabbit (Takahashi et al., J Mol Neurosci 41:138;2010). The antibody against kisspeptin receptor (GPR54) was obtained from Santa Cruz Biotechnology, Inc. (sc-48220). Kisspeptin and kisspeptin receptor were immunostained in tumor tissues of all cases of APAs examined. Kisspeptin and kisspeptin receptor were also immunostained in normal adrenal medulla of attached adrenals in all 19 cases examined, and very weakly in normal adrenal cortex of 6 out of 19 cases examined. Furthermore, positive immunostaining of kisspeptin and kisspeptin receptor was observed in other types of adrenal tumors, including cortisol-producing adenomas, non-functioning adenomas and adrenal cancers. Western blot analysis confirmed a band of 43 kDa representing kisspeptin receptor in tumor tissue extracts of the adrenal tumors. The present study has shown expression of kisspeptin and kisspeptin receptor in tumor tissues of adrenal tumors including APAs, raising the possibility that kisspeptins act as autocrine or paracrine regulators for adrenal hormone synthesis and proliferation of adrenal tumor cells.

Declaration of interest

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## P39

**Reduction of the 10-year probability of fracture predicted by FRAX® and bone mass recovery in patients with Cushing's Syndrome after 24 months from cure of hypercortisolism**

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Introduction

Cushing's Syndrome (CS) has been associated with bone mass abnormalities and with an increased risk of osteoporotic fractures.

Objective

Evaluate the effect of treatment on the 10-year probability of fracture predicted by FRAX® and on the overall bone profile, in patients with CS.

Patients and Methods

We evaluated 36 patients (6 M, 30 F, 12 post-menopausal, mean-age 43.6 ± 13.5), 22 with Cushing's disease (CD), 10 with ACTH-independent CS (ACS) and 4 with ectopic ACTH syndrome. BMD, T-score and Z-score at lumbar spine and left femur and prevalence of vertebral fractures using dual-energy X-ray absorptiometry (DEXA) and the 10-year probability of fracture predicted by FRAX® were assessed at baseline and after a 24 months median follow-up from cure of hypercortisolism. Six patients were on bisphosphonates (BSFs) treatment.

Results

At baseline 60% of patients showed bone mass abnormalities and 22% had vertebral fractures. Bone parameters did not differ between CD, ACS and ectopic CS. Bone mass abnormalities were not related to age, degree and duration of hypercortisolism. After a 24 months median follow-up from cure of hypercortisolism, a significant improvement in BMD, T-score and Z-score was observed with normalization in 7% of patients. There was no difference in percent increase at spine and femur between patients treated and untreated with BSFs. The 10-year probability of hip fracture (FRAX® Hip) and of a major osteoporotic fracture (FRAX® Major) was significantly reduced (respectively of 57% and of 48%) without difference between patients treated and untreated with BSFs. The FRAX® Hip and FRAX® Major percent reduction was related respectively with baseline neck femur and spine Z-score.

Conclusions

In CS patients, the cure of hypercortisolism is associated with recovery of bone mass and with significant reduction of the 10-year probability of osteoporotic fractures evaluated by FRAX®. BSFs seem not to affect bone improvement.

Declaration of interest

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## P40

**The Wnt/Beta-catenin and Ras/Raf/MEK/ERK signaling pathways alterations in adrenocortical tumors**

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Adrenocortical tumors (ACT) include benign and malignant tumors. Adrenocortical carcinomas (ACC) are highly malignant neoplasms with a poor prognosis, but their genetic alterations to date identified are limited. Laboratory studies on ACT have revealed a wide variety of signaling pathways involved in these tumors, among these Wnt/β-catenin signaling pathway and Ras/Raf/MEK/ERK pathway resulted often disregulated. Another important factor in many signaling pathways is the epidermal growth factor receptor (EGFR), responsible for proliferation and overexpressed in many adrenocortical tumors.

The objective of our study was to evaluate genetic alterations in key components of Wnt/β-catenin and Ras/Raf/MEK/ERK signaling pathways in order to better understand the pathogenesis of sporadic adrenocortical tumors and provide light onto new possible prognostic factors.

We performed high resolution melting (HRM) analysis for evaluating the presence of activating mutations in EGFR (exons 18, 19, 20, 21), BRAF (exons 11 and 15), H-RAS (exons 1 and 2), N-RAS (exons 1 and 2), K-RAS (exons 1 and 2), CTNNB1 (exon 3), AXIN2 (exon 7). We analyzed a series of 92 sporadic samples: 21 ACC, 38 aldosterone producing adenomas (APA), 29 cortisol producing adenomas (CPA) and 4 normal adrenocortical tissues. Only samples resulted with altered melting curves were direct sequencing.

We found 2 different BRAF mutations in 2 ACC, 4 H-RAS silent mutations in 1 APA, 1 CPA and in 2 ACC, 16 CTNNB1 alterations in 5 APA, 6 CPA and 5 ACC. No alteration in EGFR, N-RAS, K-RAS.

These results suggest that abnormalities in Ras/Raf/MEK/ERK pathway do not represent a frequent pathogenetic mechanism of adrenocortical tumorigenesis, while alterations in Wnt/β-catenin pathway seem to be a more common event. Nevertheless this study identified diverse genetic alterations, whose role in adrenocortical tumors should be further investigated.

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## P41

**Cancer induces adrenal and pituitary alterations during the development of cachexia-anorexia syndrome**

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Introduction

Tumour cachexia-anorexia syndrome (CAS) is a complex disorder characterized by progressive nutritional and metabolic alterations that lead to loss of muscle and adipose tissue observed in advanced-stage cancer. CAS indicates unfavourable prognosis, regardless of cancer type. Under the influence of permanent aggression, we have evaluated the effects of the cancer cachexia development on the hypothalamic-pituitary-adrenal (HPA) axis.

Methods

Experimental cancer CAS was induced by subcutaneous inoculation of the Walker-256 carcinosarcoma cells in Wistar rats. Histopathology, functional and nutritional parameters were studied in tumour-bearing rats, compared with paired

feeding or free-fed control rats (PBS inoculation). Adrenals and pituitary weights were corrected for body weights.

Results

The onset of the anorexia and body weight reduction was observed from the 5th day in the tumour group (Fig. 1), followed by gradual catabolic changes. Although pair-feeding decreased body weight, it did not affect body mass index; both control groups were similar at necropsy. On days 5 and 12 after tumour inoculation, cachexia index (%) was 10.3(±1.2) and 14.7(±0.9), respectively. In the tumour group, adrenals and pituitary weighed 2.25-fold(±0.15) and 2.17-fold(±0.5) greater than control on day 5, respectively. On day 12, adrenals and pituitary weight significantly decreased to 1.62(±0.09) and 1.45(±0.08) fold change from control, respectively. Adrenal cortex of rats with cancer CAS exhibited hypertrophy of the zona fasciculata; mean cortical width measured 1.22(±0.04) and 1.13(±0.03) mm on days 5 and 12, respectively (control, 0.92±0.02, $P<0.001$). After acute stimulation of tumour-bearing rats (12 days) with supraphysiological doses of exogenous ACTH, plasma corticosterone levels was 146.7±18.3, while the normal response in control rats was 193.3±21.3 ($P<0.01$).

Conclusion

Adrenal and pituitary undergo morphological alterations during the development of cancer CAS indicating the HPA axis activation. Results suggest a biphasic adaptive response to cancer as stressful aggression, which starts before the day 5 of tumour inoculation.

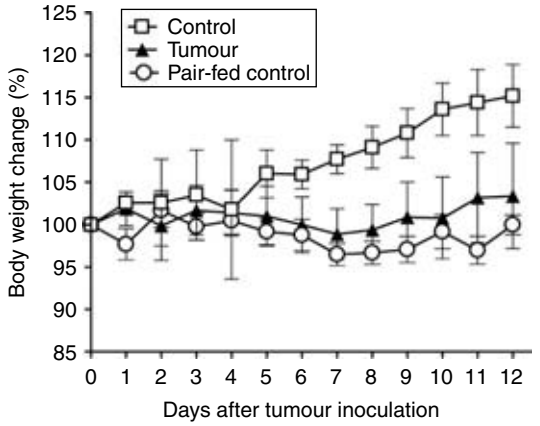


**Figure 1** Weight loss associated with tumour-induced cachexia in control (free-fed), tumour-bearing and pair-fed rats ($n=6$ in each group). $P<0.05$ (ANOVA) and $P<0.001$ for control group compared with each other (Student-Newman-Keuls post-hoc test).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P42

**A revolution in the diagnosis of adrenal disorders: rapid multiplex quantitation of serum steroids by means of tandem mass spectrometry (MSMS)**

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Context

The biological diagnosis of adrenal disorders is currently based on the quantitation of serum steroids using automated immunoassays. These direct assays often lack the required accuracy and precision and interlaboratory surveys show that performance characteristics of MSMS are far better than those of automated immunoassays.

Method

After liquid chromatography of serum extracts, steroids were quantified on Waters QuattroPremier mass-spectrometer by either targeting several informative steroids for the diagnosis of congenital adrenal hyperplasia (CAH), or by establishing a complete steroid profile using the multisteroid-CHS-kit from PerkinElmer.

Subjects

Group 1: 10 subjects with suspected adrenal tumour (hirsutism and abnormal ultrasound imaging), Group 2: 26 neonates with suspected CAH (positive screening and/or hypoglycemia), Group 3: 10 preterm neonates positive at neonatal screening), Group 4: 10 female neonates with isolated clitoris enlargement.

Results

Group 1: the complete profile including glucocorticoids, mineralocorticoids, and androgens identified in a single run the pathological secretory pattern, with increased levels of DHEA-sulfate, androstenedione and cortisol precursors. Group 2: 19 neonates had normal levels of cortisol and cortisol precursors, precluding the diagnosis of CAH, 6 neonates had excessive 17-hydroxyprogesterone levels, with low cortisol levels, suggesting a 21-hydroxylase defect, 1 neonate had increased 11-deoxycortisol and androstenedione levels, whith low-normal cortisol level, data consistent with 11-hydroxylase blockade. Group 3: the multisteroid assay showed moderately elevated 17-hydroxyprogesterone relative to term neonates, with very high DHEA-sulfate levels and normal cortisol levels. The same subjects had normal steroid profile at 3 months of age. Group 4: the profile of 10 serum steroids was normal, precluding a disorder of sex steroid secretion.

Conclusion

Rapid multisteroid assays in a single run using MSMS allow confirming or rejecting the diagnosis of adrenal tumour or CAH within a few minutes. At the same time, that avoids unnecessary therapy, shortens the delay of treatment starting, and drastically cut the investigational costs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P43

**Outcome of 232 patients with monolateral adrenal incidentaloma from a single centre**

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Unsuspected adrenal masses, or incidentalomas, are increasingly found with the widespread use of thoracic and abdominal imaging. These masses may be hormonally active or nonfunctional and benign or malignant. Clinicians must determine the nature of the mass to decide what treatment, if any, is needed.

In our 17 year experience, we observed 232 patients (158 females, 74 males aged 22-91, mean age 69) affected with monolateral incidentaloma. 58 of them (25%) underwent adrenal surgery and pathology revealed: 40 cortical adenomas, 12 pheocromocitomas, and 6 primary or metastatic carcinoma. In patients with cortical adenoma, 20 patients presented hormonal findings consistents with Cushing disease, 10 with Conn disease, and 10 presented no hormonal alterations with a size mass >4 cm. In patients with feocromocitoma, only 6 patients presented elevations of cathecolamines/metanephrines. In patients with neoplastic disease, the size of the mass was, in our experience, predictive for malignancy. Among the patients under clinical follow up, 15 of them presented biochemical findings suggestive for subclinical cortisol hypersecretion, but they do not still meet the criteria for surgery, and only 7 patients switched from a silent/subclinical to a hyperfunctional hormonal condition, and/or showed a significant increase of the mass, that required surgery. In 6 of them pathology revealed a cortical adenoma, and in 1 metastasis of an occult lung carcinoma.

Our data suggest that the management of incidental adrenal masses is hampered by the limited studies of their natural history. The low prevalence of adrenal cortical carcinomas and the relatively low incidence of progression to hyperfunction call into question the advisability of current practice of intense, long-term clinical follow-up.

Declaration of interest

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## P44

**Female patients with primary aldosteronism are diagnosed earlier and have a better outcome**

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Introduction
Primary aldosteronism (PA) is the most frequent curable form of hypertension. Hypokalemia is a late symptom of PA. Consequently, PA often is not diagnosed for many years. Our aim was to identify clinical and laboratory parameters in a large cohort of PA patients obtained during their first assessment.
Methods
96 consecutive patients prospectively studied since 2008 at the German Conn's Registry Center in Munich were eligible for this study. Diagnosis of PA was established using the criteria recommended by the Endocrine Society. Clinical data were analysed according to gender. Adrenal vein sampling revealed that 62.5% patients had a unilateral adenoma and 31.1% bilateral hyperplasia.
Results
Mean age at diagnosis was 52 years. Female patients ($n=34$, 35.4%) were diagnosed 10 years earlier than males (44.8 vs. 54.8 years). The time from initial recognition of hypertension to PA diagnosis was 12.8 years, 9.8 in females vs. 14.5 years in males. 33% of the females vs. 16.4% of the males presented with pre-hypertension (120-139/80-89 mmHg).
The prevalence of coronary heart disease was 5.2%, thrombosis 3.1%, stroke 2.1% and peripheral arterial occlusive disease 1%. Combined cardiovascular morbidity was similar in females and males (11.8 vs. 11.3%); women, however, had a higher risk of thrombosis (6% vs. 1.6%).
Specific treatment (adrenalectomy or mineralocorticoid antagonist treatment) reduced mean 24h-blood pressure from 150/94 to 134/83 mmHg ($P<0.01$).
Graded according to the WHO classification, 60% of all patients became normotensive, 67% of the operated patients still requiring antihypertensive medication (50% in females, 73% in males).
Conclusion
Women with PA have a high risk of cardiovascular diseases although they are diagnosed 10 years earlier than men and hypertension is often not as obvious. They have a 50% chance of reaching normotension without antihypertensive medication.
Declaration of interest
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## P45

**Influencing factors on quality of life in patients with Addison's disease**

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Introduction
Several previous studies showed a reduced quality of life (QoL) in patients with Addison's disease, but only few data on the potential influencing factors are available so far.
Methods
We determined current QoL in 200 patients with Addison's disease using AddiQoL-36, a questionnaire developed in collaboration with the European consortium Euradrenal. Furthermore, data about symptoms, diagnosis and medication were collected by questionnaires and serum concentration of vitamin D 25-OH was measured.
Results
With increasing latency between first symptoms and diagnosis of adrenal insufficiency, QoL decreased highly significant ($P=<0.001$). Patients diagnosed in the first month after onset of symptoms had a significantly higher QoL compared to patients with a diagnosis delayed more than 12 months ($P=0.01$). Age at manifestation correlated negatively with QoL ($P=0.01$). Another important influencing factor was the incidence of additional autoimmunopathies. QoL decreased significantly with increasing autoimmune morbidity ($P=0,01$). Coeliac disease ($P=0.05$), atrophic gastritis ($P=0.01$) and especially primary ovarian failure ($P=0.01$) were highly correlated with reduced QoL. Vitamin D 25-OH serum level seemed not to be of influence. QoL did not vary significantly between patients with normal ($>20$ ng/ml), low ($<20$ ng/ml) or extremely low ($<10$ ng/ml) vitamin D concentrations.
Conclusions
Quality of life in patients with Addison's disease is impaired significantly in patients with autoimmune polyglandular syndrome and in those with manifestation at higher ages. Latency between first symptoms and diagnosis revealed as the most important factor influencing the patients quality of life even years after manifestation of the disease. Similar results have been seen in another study recently, emphasizing the relevance of this point.
A higher attention for this rare disease and an earlier diagnosis might lead to a sustained improvement of quality of life in Addison patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P46

**Differential methylation signature in benign and malignant cortisol producing adrenocortical tumors**

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Background
Adrenocortical carcinomas (ACC) are rare tumors with an incidence of 1–2 per million. Although rare, it is an aggressive endocrine cancer with poor prognosis. As of today there is no reliable diagnostic biomarker to distinguish benign tumors from malignant tumors. Global changes in DNA methylation and DNA promoter hypermethylation are important events in tumorigenesis. Promoter hypermethylation can cause silencing of tumour suppressor genes or lead to abnormal function of important cellular pathways. The purpose of this study was to investigate the changes in DNA methylation in cortisol producing adrenocortical carcinoma compared to benign cortisol producing adenomas.
Methods
DNA was extracted from 16 adrenocortical tumors and methylataion status was analyzed using the Infinium Human Methylation 450K BeadChip. Total RNA was isolated from 30 tumors, converted to cDNA and mRNA expression was analyzed by q-PCR using SYBR Green and GAPDH as internal control.
Results and discussion
Unsupervised hierarchical clustering showed an altered DNA methylation profile in ACCs compared to adenomas. CYP1B1, OTX1 and RASAL1 were identified as some of the most differentially hypermethylated genes. Aberrant methylation of CYP1B1 has been previously reported in nodular goiter, colorectal cancer and gastric carcinomas. CYP1B1 mRNA expression was significantly reduced in ACCs compared to adenomas. However, no significant difference in mRNA expression was observed for OTX1 and RASAL1. In conclusion, mRNA expression of CYP1B1 was significantly reduced in cortisol producing adrenocortical carcinomas compared to adenomas, expression of CYP1B1 might be used as a biomarker in distinguishing aggressive tumors from benign ones and also provide outlines for choosing the appropriate treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P47

**18-Hydroxycorticosterone, 18-hydroxycortisol and 18-oxocortisol in the diagnosis of primary aldosteronism and its subtypes.**

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Diagnosis of primary aldosteronism (PA) is made by screening, confirmation testing and subtype diagnosis (CT scan and adrenal vein sampling, AVS). However, some tests are costly and unavailable in most hospitals. We evaluated

the role of serum 18-hydroxycorticosterone (s18OHB) and urinary and serum 18-hydroxycortisol (u and s18OHF) and 18-oxocortisol (u and s18oxoF) in the diagnosis of PA and its subtypes, aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia (BAH). We studied 62 patients with low-renin essential hypertension (EH), 81 PA (20 APA, 61 BAH), 24 patients with glucocorticoid-remediable aldosteronism, 16 patients with adrenal incidentaloma and 30 normotensives. We measured s18OHB, s18OHF and s18oxoF before and after saline load (SLT) and 24hour u18OHF and u18oxoF. PA patients displayed significantly higher levels of s18OHB, u18OHF and u18oxoF compared to EH and normals; APA patients displayed s18OHB, u18OHF and u18oxoF levels significantly higher than BAH patients. Similar results were obtained for s18OHF and s18oxoF. SLT significantly reduced s18OHB, s18OHF and s18oxoF in all groups but steroid reduction was much less for APA patients compared to BAH and EH. The s18OHB/aldosterone ratio after SLT more than doubled in EH, but remained unchanged in APA patients. In conclusions: u18OHF, u18oxoF and s18OHB measurements in patients with a positive ARR correlate with confirmatory tests and AVS in PA patients. If verified, these steroid assays would refine the diagnostic work-up for PA.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P48

**MicroRNA profiling of benign and malignant adrenocortical tumors reveals potential biomarkers of recurrence**

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Objective

To identify miRNAs predictors of poor prognosis in adrenocortical cancer.

Methods

Using microarrays, we evaluated the expression of 728 human miRNAs in six adenomas (ACAs) and twelve carcinomas (ACCs). The ACC group was composed of two subgroups A and B consisting of six recurrent (subgroup A) and six non-recurrent tumors (subgroup B). These two distinct subgroups have been characterized recently (de Reynies et al, 2009) on the basis of distinct gene expression profiles: comparison of global survival revealed a major difference in outcome in these two subtypes of ACC, with a very high rate of relapse and death within two years following surgery in the A subgroup.

Results

Twelve miRNAs were differentially expressed between ACCs and ACAs, 5 of which were down-regulated and 7 up-regulated in ACCs. The best discriminatory miRNAs between ACCs and ACAs were miR-195 and miR-335 which were down-regulated in ACCs. 29 miRNAs were differentially expressed between the two ACC subgroups A and B, with all discriminatory miRNAs more strongly expressed in A than in B carcinoma samples. Among them, miR-139-5p was the most powerful discriminatory miRNA between A and B subtypes with consistent up-regulation in recurrent carcinoma (A). Quantitative RT-PCR revealed that the levels of expression of miR-195 and miR-335 were similar in ACAs and normal adrenal cortex while strongly repressed in ACCs. Overexpression of miR-139-5p was confirmed in ACCs type A. These results were validated in a separate cohort of ten benign and twenty malignant samples (10 ACCs type A and 10 ACCs type B) using quantitative RT-PCR. Target prediction analysis revealed that predicted targets of these miRNAs are involved in biological processes which enhance tumor progression.

Conclusions

Our data suggest that adrenocortical cancer cells progressively switch from a high miR-195 and miR-335 status to a low miR-195 and miR-335 phenotype. miR-139-5P is a potential prognostic biomarker of recurrent ACCs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P49

**Prevalence and Characteristics of Familial Hyperaldosteronism: the PATOGEN study (Primary Aldosteronism in TOrino - GENetic forms).**

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Primary Aldosteronism (PA) is the most frequent cause of secondary hypertension and patients display an increased prevalence of cardiovascular events compared to essential hypertensives. To date, three familial forms of PA have been described and termed, familial hyperaldosteronism types I, II and III (FH-I to -III). The aim of this study was to investigate the prevalence and clinical characteristics of the three forms of FH in a large population of PA patients. Three hundred consecutive PA patients diagnosed in our unit were tested by long-PCR of the CYP11B1/CYP11B2 hybrid gene that causes FH-I and all available relatives of PA patients were screened to confirm or exclude PA and thus FH-II. Urinary 18-hydroxycortisol and 18-oxocortisol were measured in all familial PA patients. Two patients were diagnosed with FH-I (prevalence 0.66%) as well as 21 of their relatives, and clinical phenotypes of the 2 affected families varied markedly. After exclusion of families who refused testing and those that were not-informative, 199 families were investigated of which 12 were diagnosed with FH-II (6%) and an additional 15 individuals had confirmed PA; clinical and biochemical phenotypes of FH-II families were not significantly different from sporadic PA patients. None of the families displayed a phenotype compatible with FH-III diagnosis. Our study demonstrates that familial forms of hyperaldosteronism are more frequent than previously expected and reinforces the recommendation of the Endocrine Society Guidelines to screen all first-degree hypertensive relatives of PA patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P50

**Intercurrent Illness Dose Regimen in Adrenal Insufficiency with a Dual Release Hydrocortisone Formulation Derived from Population Pharmacokinetic Modelling**

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Patients with adrenal insufficiency need adjustment of the hydrocortisone (HC) dose during an intercurrent illness. Current recommendation to double the daily HC dose at each occasion does not consider the non-linear dose proportionality in bioavailability i.e. doubling the dose gives less than 100% increase in exposure. We have developed an intercurrent illness dose regimen for a once daily dual release HC tablet using population pharmacokinetic (POPPK) modeling.

Methods

Cortisol serum concentration time profiles were obtained in 62 patients after administraton of oral once daily dual release HC tablets (20–60 mg) on 116 occasions, and in healthy volunteers (after betametasone suppression) after single 5 ($n=14$) and 20 mg ($n=16$) doses. All data was simultaneously modeled using a POPPK modeling approach in NONMEM. Simulations were made in order to investigate optimal intercurrent illness dosing regimens. The safety of the optimal regimen was thereafter studied in a prospective study in patients with Addison's disease.

Results

Simulated data demonstrated that adding an additional dose at $8\pm2$ hours after the morning dose resulted in slightly higher peak concentrations for the second dose and increased cortisol levels during the evening and early night time. Giving three daily doses with 8-hour intervals resulted in no dose accumulation on the day after, but with cortisol levels during the night. The dual release HC b.i.d. or t.i.d. intercurrent illness regimen gives less fluctuations and higher cortisol concentrations in the afternoon compared to immediate release. Prospective collection of data in 173 patient years demonstrated that changing the dosing interval of the dual release tablets from o.d. to b.i.d. (or t.i.d.) during undercurrent illness was safe.

Conclusion

The daily dual release HC dose given twice or thrice daily with $8\pm2$ hours intervals during intercurrent illness increases the 24 h cortisol coverage and has been documented to be safe.

Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P51

### Cornell product left ventricular hypertrophy in electrocardiogram in patients with primary aldosteronism

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Introduction
Left ventricular hypertrophy (LVH) is associated with increased morbidity and mortality. LVH defined by ECG (ECG-LVH) has been evaluated using standard voltage criteria by Sokolow and Lyon (SL-LVH) and more recently using the Cornell product criteria (CP-LVH). Evaluation of CP-LVH is beneficial in hypertensive patients: however, in patients with primary aldosteronism (PA) whose incidence of cardiovascular events is higher than essential hypertension (EHT), there are no data on CP-LVH. Our aim was to show the higher rate of ECG-LVH, both CP-LVH and SL-LVH, in PA.
Methods
During a 10-year period, the diagnosis of PA was made in 127 patients. During the same period, clinical characteristics and ECG of this group were compared with those of 127 patients with EHT matched for age, gender, and blood pressure.
Results
The prevalence of CP-LVH was 38% for PA, 22% for EHT (odds ratio (OR) = 2.1; 95% CI 1.2 to 3.6), and the prevalence of either CP-LVH or SL-LVH (CP/SL-LVH) was 54 and 34% (OR = 2.2; 95% CI 1.3 to 3.7), respectively. However, the prevalence of SL-LVH was 29 and 23% (OR = 1.3; 95% CI 0.75 to 2.3), respectively. The rate of ECG-LVH (CP-LVH, CP/SL-LVH, but not SL-LVH) increased with decreasing quartile of estimated GFR among PA (Cochran-Armitage $P$ trend = 0.0018, 0.0222), but not for EHT, indicating the strong 'kidney–heart interaction' in PA. In a multivariate logistic regression analysis, the presence of PA was an independent predictor of CP-LVH, CP/SL-LVH, but not for SL-LVH.
Conclusion
Patients with PA exhibit more CP-LVH than did EHT patients independent of blood pressure. CP-LVH clearly reveals the 'kidney-heart interaction' in PA. Thus, screening for CP-LVH to detect higher-risk patients should become a routine part of the evaluation of PA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P52

### Efficacy of long-term treatment with retinoic acid in patients with Cushing's disease

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Cushing's disease, i.e. cortisol excess due to an ACTH-secreting pituitary adenoma, is a rare disorder with considerable morbidity and mortality. Current therapeutic strategies include pituitary surgery, radiation, adrenalectomy and medical treatment with steroidogenesis inhibitors, as drugs aimed at the pituitary adenoma are as yet under investigation. Experimental data showed that retinoic acid restrains ACTH secretion by tumoral corticotrophes thus we decided to evaluate the efficacy of retinoic acid treatment in patients with Cushing's disease.
Methods
Seven patients with Cushing's disease (two men, five postmenopausal women) were started on 10 mg retinoic acid daily and dosage increased up to 80 mg/day for 6–12 months. ACTH, UFC and cortisol as well as clinical features of hypercortisolism were evaluated at baseline, during retinoic acid administration and after drug withdrawal.
Results
A marked decrease in UFC levels was observed in five patients: mean UFC levels on retinoic acid were 47–79% of baseline values and normalization in UFC was achieved in four patients. Plasma ACTH decreased by 10–35% in four responsive patients, including patients in whom UFC normalized while levels increased up to 130% of pretreatment values in one patient. Serum cortisol was unchanged during retinoic acid treatment except for a 40% decrease observed in one patient. Blood pressure, glycaemia and symptoms of hypercortisolism, e.g. facial plethora, ameliorated to a variable extent on treatment. Patients reported mild adverse effects, e.g. xerophthalmia, arthralgias.
Conclusions
Prolonged treatment with retinoic acid proved beneficial and well tolerated in five out of seven patients with Cushing's disease. This represents a novel, promising approach to medical treatment with Cushing's disease.
This study was funded by AIFA grant #FARM78F3YR.
Declaration of interest
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## P53

### Relationship of current glucocorticoid dose with metabolic outcomes in CAH: analysis of the United Kingdom congenital adrenal hyperplasia adult study executive (CaHASE) cohort

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We have previously reported the following metabolic abnormalities were common in 203 adult patients with CAH: obesity (41%), hypercholesterolemia (46%), insulin resistance (29%), osteopenia (40%) and osteoporosis (7%) (Arlt *et al. JCEM* 2010 **95** 5110–21). The CAH patients were taking different glucocorticoid therapies at various doses ($n$ = 196): hydrocortisone ($n$ = 25 M, 26 W), prednisolone ($n$ = 21M,67W), dexamethasone ($n$ = 15M,22W) or combination therapy ($n$ = 4 M, 16 W). To test the hypothesis that glucocorticoid exposure mediates adverse metabolic outcomes, we converted current treatment regimens to prednisolone dose equivalents using three published models: British National Formulary (BNF), Rivkees (*Pediatrics* 2000 **106** 767–73) and Arlt (*JCEM* 2009 **94** 1059–67). The respective ratios for prednisolone:hydrocortisone:dexamethasone were 1:5:0.15, 1:4:0.057, and 1:5:0.125. The relationship of glucocorticoid dose and metabolic parameters was tested by treating dose as a continuous variable using regression analysis and as a categorised variable using ANOVA (with prednisolone dose equivalents in four categories: $<$2.5 mg ($n$ = 4 M, 16 W), 2.5$\leq$5.0 mg ($n$ = 12 M, 27 W), 5.0$\leq$7.5 mg ($n$ = 24 M, 57 W) and $>$7.5 mg ($n$ = 25 M, 31 W)). The mean prednisolone equivalent dose (men: 6.1:7.5:5.7, women: 5.1:6.0:5.0 mg/day) was different for the three models for both sexes ($P < 0.01$). There was no effect of dose in any of the three models as judged by ANOVA or univariate regression analysis on; BMI, waist circumference, triglycerides, HDL-cholesterol, insulin resistance (HOMA-IR) and femoral or lumbar bone mineral density. Partial correlation analysis (age and sex adjustments) showed significant relationships between dose and systolic (BNF: $r$ = 0.188, $P$ = 0.01; Rivkees: $r$ = 0.147, $P$ = 0.04; Arlt: $r$ = 0.189, $P$ = 0.01) and diastolic blood pressure (BNF: $r$ = 0.170, $P$ = 0.02; Arlt: $r$ = 0.154, $P$ = 0.03; except Rivkees: $r$ = 0.070, $P$ = 0.37) and for Rivkees model only, between dose and HDL-cholesterol ($r$ = 0.186, $P$ = 0.02) and HOMA-IR ($r$ = 0.175, $P$ = 0.04). Confounding factors in our analysis may include variable patient compliance with drug and duration of treatment. In conclusion, no published method for comparing glucocorticoid dose equivalence is superior to another. We found little impact of dose on individual metabolic parameters in adults with CAH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P54

**Frequency and causes of adrenal crises over life-time in patients with 21-hydroxylase deficiency**

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Background
Adrenal crisis (AC) is a life-threatening complication in patients with congenital adrenal hyperplasia due to classical 21-hydroxylase deficiency (21-OHD). AC was defined as an acute state of health impairment which required i.v. glucocorticoid administration and hospital admission. No data on AC over life-time in 21-OHD is available.
Study design
In a retrospective study AC was studied following two approaches: a) questionnaire-based: 122 adult 21-OHD patients (50 men, 72 women, median age 35 years, range 18–69) completed a disease-specific questionnaire, b) patient chart based: charts of 67 21-OHD patients (32 males, 35 females, median age 31 years, range 20–66) were analyzed from diagnosis to last follow-up with regard to frequency and causes of AC since diagnosis.
Results
Evaluation of questionnaires revealed 257 AC in 4456 patient years (frequency 5.8 crises/100 patient years), while patient charts documented 106 AC in 2181 patient years (4.9 crises/100 patient years). The chart-based evaluation showed that gastrointestinal infections (29%) and salt-wasting crisis (18%) were the main causes of AC. In 14% the cause remained uncertain. There was no difference in the overall frequency of AC in males and females. AC mostly occurred during childhood, with more than 70% of AC in the first 10 years of life and one third of AC in the first year of life. Still 20% of cases of AC were observed in adults (>18 years).
Conclusion
Our longitudinal analysis demonstrates a significant risk of AC in patients with 21-OHD over life-time. Specific age-adapted and repeated crisis prevention training may help to reduce morbidity due to AC in 21-OHD.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P55

**Urinary cortisol metabolites in corticotroph and adrenal tumours**

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We investigated whether urinary cortisol metabolites could help to diagnose patients with cortisol oversecretion either with benign adrenal or corticotroph tumours.
Liquid chromatography tandem mass spectrometric assay (LC-MSMS) of cortisol (F)m, cortisone, tetra-hydrocortisol (THF), allo-THF, tetra-hydrocortisone (THE), α-cortol, β-cortol, α-cortolone, α-cortolone and internal standard 6-α-methylprednisolone were assayed after deconjugaison and dichloromethane extraction. LC-MSMS was performed with an Acquity UPLC separative module and aTQD detector (Waters, Millport, USA).
In-patients of the endocrine department ($n=253$, 196F, 49 (20–74)years (median (2.5th–97.5th percentile) were control subjects ($n=175$ (N)), patients with active Cushing disease's ($n=35$, (CD)) or with adrenocortical tumours ($n=43$, (AS)). The latter were either secreting or non-secreting tumours (the former were subsequently operated and the latter followed-up). Initial diagnosis was made using routine tools including cortisol assays (Fr) performed by RIA (Diasorin, France).
Analytes excretions of the groups are presented in the table (ng/day). Log-transformed cortisol concentrations determined were significantly linearly correlated with a significant bias (Passing-Bablock regression).
Areas under the ROC curves (AUC) were >0.950 for cortisol, THF, α-cortol for the diagnosis of (CD) with an equal 39% sensitivity at 100% specificity for cortisol and α-cortol. Combined analysis of cortisol and α-cortol improved the sensitivity.
AUC were >0.700 for cortisol and β-cortolone for the diagnosis of whole population of (AS). Based on urinary cortisol, 0800 h ACTH and cortisol in plasma, AS tumours could be non-secreting or secreting (at least two positive criteria). AUC were >0.800 for cortisol, β-cortol and β-cortolone for the diagnosis of secreting (AS).
(F) and β-cortolone and to a minor extent both cortols are useful parameter to investigate benign (AS) and (CD). Whether their combined use can facilitate the diagnosis of these diseases has to be investigated in prospective studies.

**Table 1**

| | cortisol | corti-sone | THF | alloTHF | THE | α-cortol | β-cortol | α-corto-lone | β-cortolone |
|---|---|---|---|---|---|---|---|---|---|
| [N] | 101 (43 –533) | 124 (28 –429) | 3064 (1305 –11839) | 350 (11 –680) | 3947 (781 –9924) | 162 (65 –519) | 289 (130 –946) | 714 (210 –2191) | 423 (108 –1180) |
| [CD] | 357 (181 –2012) | 297 (138 –618) | 8637 (4613 –36884) | 400 (49 –2052) | 8561 (1837 –22976) | 609 (229 –3266) | 872 (274 –6783) | 2643 (1228 –5375) | 835 (201 –3245) |
| [AS] | 66 (17 –216) | 89 (18 –277) | 2467 (521 –8220) | 275 (27 –871) | 3264 (714 –8973) | 162 (36 –453) | 227 (43 –683) | 815 (143 –1997) | 187 (36 –497) |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P56

**Soundwave's effect on hematic cortisol level: a pilot study**

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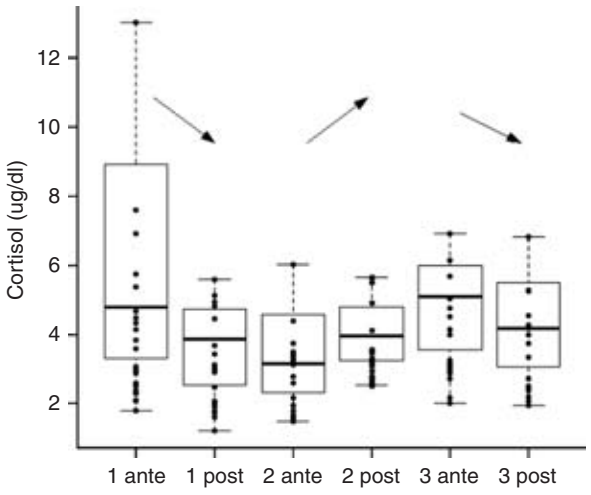
In order to prove that different sound-waves trigger different human hormones release by the way of their vibratile impact on the bodies, we exposed 30 testers (heterogeneous for gender, age and lifestyle) to different frequencies of noisy sound-waves (not melodic and rhythmic music). We organized three sessions in different days and with different waves frequencies: 1st at 40–115 Hz, 2nd at 8200–8500 Hz, 3rd with mixed radio-waves.
Blood cortisol level was measured with the Access Cortisol Kit (Beckman).
An hour of exposure to a low frequency sound (40–115 Hz) diminishes the hematic cortisol concentration in 86% of the testers. On the other hand, high frequencies (8200–8500 Hz). raise cortisol values in 65% of the testers in just 30-40 min.



After each session, testers expressed their opinion on the experience. Appreciation of low frequencies corresponds to lowered cortisol levels. Dislike of high frequencies corresponds to increased cortisol values, while displeasure of mixed radio-waves (low frequencies) is not associated to higher cortisol levels. This finding suggests that the emotional status does not interfere with cortisol release, as much as the physical properties of sound-waves do.
This pilot study gave several interesting clues, to be confirmed in a larger study: i) high frequencies raise cortisol level, while low frequencies diminish it; ii) this change is not correlated to the emotional response to the exposure; iii) older testers seem to respond in a different way.
Each column refers to one sample collection. The black dots represent each individual cortisol value in Ug/dl. The black bar shows the media of the distribution. The lower limit of the box indicates the middle point between the lowest value and the media while the higher limit indicates the middle point between the higher value and the media.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P57

### Reevaluation of the 4 mg intravenous dexamethasone suppression test for differentiation of Cushing's disease from pseudo-Cushing's syndrome

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Context
Differentiating Cushing's disease (CD) from pseudo-Cushing's syndrome (PCS) is one of the most challenging problems since no biological test is yet perfect. Almost 30 years ago our group demonstrated the interest of 4 mg i.v. dexamethasone suppressing test (DST) to differentiate obese patients to CD.
Objective
To reevaluate the diagnostic accuracy of the 4 mg i.v. DST in carefully selected patients with PCS and CD.
Design
Patients recruited from November 2008 to July 2011 were retrospectively studied. The criteria for PCS were: presence of clinical and biochemical (Urinary Free Cortisol and/or 1 mg DST) features compatible with CS, normal pituitary MRI and at least one year of clinical and biochemical follow-up.
Diagnosis of CD has been confirmed by pathology in 29 patients operated, three patients with abnormal MRI were medically treated.
Patients underwent 4 mg dexamethasone infusion according to Abou-Samra *et al* (*JCEM* 1985). The diagnosis of CD is based on absence of ACTH and cortisol suppression at 8 am on day 2.
Results
68 patients (54F/14M), 32 with CD and 36 with PCS were included. Age and BMI were similar between groups but hirsutism, proximal amyotrophy, and vascular weakness were significantly more frequent in the CD group ($P<0.001$).
Midnight plasma cortisol, 8 am cortisol and ACTH after 4 mg DST were respectively associated with a 95.4% (86.5–99), 90.5% (81.8–96.7) and 98.4% (92.1–99.6) diagnostic accuracy. Cortisol and ACTH threshold used in our endocrine units to affirm the CD (respectively 100 nmol/l and 5 ng/l) yielded more accurate sensibility (93.8%) and specifity (86.1%) than midnight plasma cortisol cut-off of 207 nmol/l retained by the Endocrine and European Societies which was associated with a 96.8% of sensibility and 58.1% of specificity.
Conclusion
4 mg i.v. DST is a very easy and accurate test to distinguish CD from PCS and deserve a place in the CD diagnosis assessment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P58

### Treatment with mineralocorticoid receptor antagonists in 'subclinical' cases with adrenal adenomas and autonomous aldosterone secretion

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Introduction
Patients with adrenal adenomas appear to exhibit an appreciable prevalence of autonomous aldosterone secretion (AAS). Our aim was to evaluate the anti-hypertensive effect of targeted therapy with mineralocorticoid receptor (MR) antagonists in AAS patients.
Patients and methods
We studied 60 hypertensive patients, 21 males and 39 females, harboring an adrenal adenoma with AAS. All patients were receiving combination of anti-hypertensive treatment with inadequate blood pressure (BP) control. Subjects of Conn's syndrome were excluded. The diagnosis of AAS was established using a modified saline infusion test (MSI), i.e. saline infusion after dexamethasone administration. Cut-off levels were developed using 72 normotensive controls with normal adrenal imaging (post MSI aldosterone levels 67 pmol/l). Following hormonal evaluation, patients were switched to receive aldosterone antagonist treatment (spironolactone or eplerenone) and followed up for eight weeks. Self-reported systolic and diastolic BP values were recorded before and after treatment modification.
Results
The mean (S.D.) age of our study population was 54.80 (10.35) years and the body mass index 30.35 (4.33) kg/m$^2$. The serum potassium concentration was 3.8 (0.4) mEq/l and the post MSI aldosterone levels 226.7 (214.1) pmol/l. The use of MSI led to the identification of 30/60 AAS patients that would be undiagnosed using the Endocrine Society guidelines. Treatment with MR antagonists resulted in a marked decrease of maximum BP values (systolic BP: 161.4 (23.6) after vs 126.3 (10.2) mmHg before and diastolic BP: 97.9 (12.5) after vs 79.7 (7.4) mmHg before respectively, $P<0.001$ in both cases; Fig. 1).
Conclusions
We showed a remarkable anti-hypertensive effect of MR antagonists in adrenal adenoma patients with AAS. These patients would have been unrecognized without the use of the recently proposed cut-offs for AAS diagnosis. Our findings await validation with further cohorts. Targeted therapy is expected not only to adequately control BP, but also reduce the adverse aldosterone-mediated cardiovascular actions.

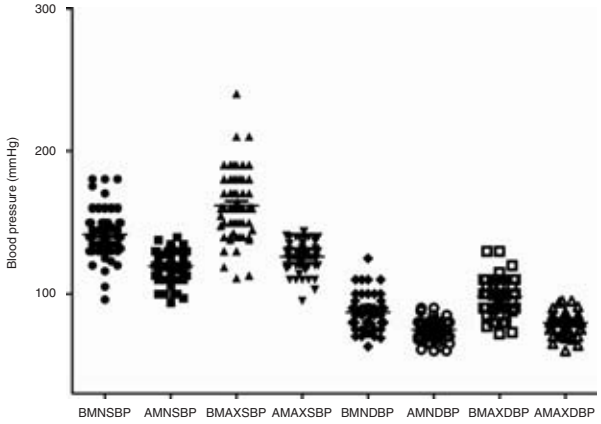


**Figure 1** Minimum (MIN) and maximum (MAX) SBP and DBP values before (B) and on mineralocorticoid receptor antagonist treatment (A) in 60 patients with AAS, $P<0.001$ in all cases. SBP: systolic blood pressure, DBP: diastolic blood pressure.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P59

**The effects of the acute administration of alprazolam, a benzodiazepine, in patients with subclinical Cushing's syndrome**

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Subclinical Cushing's syndrome (SCS) is a status of altered hypothalamo-pituitary-adrenal (HPA)-axis secretion in the absence of the classical signs or symptoms of overt cortisol excess. Among the various tests used for the diagnosis of SCS, the 1-mg dexamethasone test (DST) is the most used. Alprazolam (ALP), a benzodiazepine activating GABAergic receptors, possesses clear centrally-mediated inhibitory effects on ACTH and cortisol secretion in normal subjects, while it does not modify ACTH and cortisol hypersecretion in patients with overt hypercortisolism.

Aim of the study was to verify the effect of alprazolam (1 mg p.o. at 23.00 h) on the cortisol response to DST test (1 mg at 24.00 h) in 22 patients with adrenal adenomas (AA) and SCS (14♀ and 8♂, age: 66.3±1.8 years), 11 patients with non-functioning AA (NF, 7♀ and 4♂;55.9±2.9), 10 patients with overt Cushing's syndrome (five of adrenal nature, CS, 4♀ and 1♂,42.0±3.8; 5 with Cushing's disease, CD, 4♀ and 1♂,58.0±5.6). 14 normal subjects (NS, 9♀ and 5♂;55.8±2.2) were enrolled as controls. SCS was defined by a post-DST cortisol level $>1.8$ μg/dl and one of the following: midnight serum cortisol $>7.5$ μg/dl, ACTH $<5$ pg/ml or UFC $>100$ μg/24 h. ALP reduced cortisol secretion after DST in patients with SCS ($P<0.05$), while it was ineffective in CS and CD patients. ALP pre-treatment reduced cortisol values $<1.8$ μg/dl in five patients with SCS but in none CS or CD. All NF and NS showed cortisol levels $<1.8$ μg/dl after DST and ALP+DST.

These data demonstrate that GABAergic activation by ALP still modulates adrenal hyperfunction in patients with subclinical hypercortisolism but not in those with overt disease, suggesting only a partial autonomous adrenal cortisol secretion in SCS. The clinical usefulness of ALP to increase the sensitivity of 1-mg DST in distinguishing functional from organic subclinical hypercortisolism should be confirmed.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P60

**Is isolated secondary adrenal insufficiency more frequent than autoimmune Addison's disease?**

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Similarly as in autoimmune Addison's disease (AAD), diagnosed in about 80% of primary adrenal insufficiency, idiopathic isolated secondary adrenal insufficiency (ISAI) seems to have an autoimmune origin.

Within the last 35 years we have registered in our Department 325 patients with ISAI (F/M ratio 10.9) aged 17–87 years. In comparison, we have in our registry 241 patients with AAD, collected within over 40 years. The diagnosis of ISAI was based on clinical examination and hormonal, immunological and imaging studies. The hormonal investigations: cortisol and plasma ACTH (circadian rhythm), TSH, LH, FSH, PRL, fT4, androgens, E2, testosterone, Synacthen-stimulation test, urinary excretion of cortisol. Immunological studies:antithyroid and pituitary autoantibodies with an immunoblotting assay (with human pituitary cytosol as an autoantigen in 65 patients. A corticotroph-specific transcription factor (Tpit) was investigated as a candidate autoantigen. MRI of the pituitary – in a part of cases.

Results

In all ISAI patients basal cortisol and ACTH levels were found to be below the lower normal limit while Synacthen significantly stimulated cortisol secretion. Various autoimmune disorders were detected in 215 patients, the most frequently thyroid diseases (mainly hypothyroidism), vitiligo, premature ovarian failure, pernicious anemia and T1DM. The thyroid autoantibodies were found in 65%, while pituitary autoantibodies in 34% of patients under study. Tpit was identified as a minor antigen in 10.5% of of the investigated patients. Partially enpty sella was the most frequent imaging finding.

Conclusions

ISAI appeared to be more frequent than AAD. 2/ Association of various autoimmune disorders in 66% of ISAI patients suggests autoimmune origin of the idiopathic isolated secondary adrenal insufficiency.

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P61

**Dyslipidemia in patients with adrenal incidentaloma with and without subclinical hypercortisolism and diabetes mellitus.**

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Introduction

Patients with adrenal incidentalomas (AI) and subclinical hypercortisolism (SH) are thought to have increased incidence of cardiovascular risk factors, including diabetes mellitus (DM) and hypertension. Data regarding dyslipidemia are scarce. The aim of this study is to evaluate the possible influence of SH on lipid pattern in relation to the presence/absence of DM.

Methods and design

276 AI patients were enrolled (168 F, 108 M), 55 with diabetes (AIDM+) and 221 without DM (AIDM−). As the presence of dyslipidemia is a common condition associated to DM, 83 age-, BMI- and gender-matched type 2 DM patients (CDM+) and 77 euglycemic subjects (CDM−) served as controls. SH was diagnosed in 49 patients in the presence of at least two out of these three parameters: cortisol levels after 1 mg dexamethasone suppression test $>3$ μg/dl, ACTH levels $<10$ pg/ml, and 24 h urinary free cortisol levels $>70$ μg/24 h. The prevalence of dyslipidemia was evaluated according to ATP III criteria. No subject was taking anti-dyslipidemic drugs.

Results

The prevalence of dyslipidemia tended to be higher, but not statistically significant, in AIDM+ in respect to CDM+ subjects (63.6 vs 54.2%, respectively, $P=0.27$), and was similar between AIDM− and CDM− (23.0 vs 20.8%, respectively, $P=0.68$). No difference was observed between AI patients with and without SH, in both DM and euglycemic groups. Considering the whole group of AI patients, the presence of dyslipidemia was associated with DM (OR 4.88, 95%CI 2.43–9.80, $P=0.0001$) and age (OR 1.03, 95%CI 1.00–1.06, $P=0.047$) and not with BMI (OR 1.02, 95%CI 0.96–1.08, $P=0.55$), and SH (OR 0.83, 95%CI 0.39–1.76, $P=0.62$).

Conclusions

In patients with adrenal incidentaloma, dyslipidemia seems to be associated to diabetes mellitus and not to subclinical hypercortisolism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P62

**Prevalence of primary aldosteronism in Chinese patients with resistant hypertension**

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Background

Studies across the world have unveiled different prevalence of primary aldosteronism (PA) in distinct geographical areas and human races. However, owing to disparities in methodology and recruitment, the true incidence is still open. Especially information in Asian resistant hypertension is rare, so we determined to investigate the prevalence of PA in this population.

Methods

We launched a large-scale, hospital-based national survey and enrolled 1656 patients with resistant hypertension finally. Serum aldosterone and plasma renin

activity were measured in every subject and ARR (aldosterone-to-renin ratio) was calculated to screen PA. Positive patient ARR > 20 underwent intravenous saline infusion test, and diagnosis of PA was established if post-saline aldosterone was above 8 ng/dl.
Results
Among 1656 patients with resistant hypertension, 494 (29.8%) were screened positive on the basis of ARR > 20 and underwent intravenous saline infusion. The diagnosis of PA was established in 118 (7.1%) subjects according to an unsuppressed post-saline aldosterone (>8 ng/dl). Prevalence of PA decreases with aging and so do aldosterone and renin activity level. Data show no significant gender difference in prevalence, however, women intrinsically have lower PRA level and thus higher ARR, due to which some screening strategies may require further modification.
Conclusion
The prevalence of primary aldosteronism in Chinese patients with resistant hypertension is relatively lower compared with previously reported data from other populations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P63

**Genetic, anthropometric and metabolic features of adult norwegian patients with 21-hydroxylase deficiency**

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Objective
The aim of this study was to determine genetic, anthropometric and metabolic features in an unselected population of adult Norwegian patients with classical 21-hydroxylase deficiency (21OHD).
Patients,
Methods, and design
Sixty-four 21OHD-patients participated (23 men, 41 women; mean age 40.3 (range 19–72) in a cross-sectional study including DNA sequencing of the CYP21A1P-CYP21A2 locus, anthropometric measurements including dual X-ray absorptiometry (DXA) scanning, and biochemical analyses. The results were compared with reference cohorts from the general population.
Results
We identified four novel and plausibly disease-causing CYP21A2 mutations. Gene deletions/conversions (42.1% of alleles), the point mutations I2splice (23.0%) and I172 N (22.2%) were common. The genotype corresponded to clinical phenotype in 89% of the patients. Reduced final height was more pronounced in the men; 168.2 cm (151–186) (mean and range) than in the women 159.1 cm (144–173). The prevalence of osteopenia was 44% in the men and 29% in the women. Both men and women had normal body mass index, but markedly increased fat mass compared with the normal population. Diastolic blood pressure was higher than normal. Only 20% of the women had testosterone levels in the normal range; 13% of the men had testosterone levels below normal.
Conclusion
In this population-based survey of 21OHD we identified four novel mutations and high concordance between genotype and phenotype. The patients had reduced final height, with high frequency of osteopenia in the men, increased fat mass, and increased diastolic blood pressure. These results indicate unfavourable metabolic status and need for improvement of the therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P64

**Relationship between plasma dexamethasone level and cortisol in the overnight dexamethasone suppression test**

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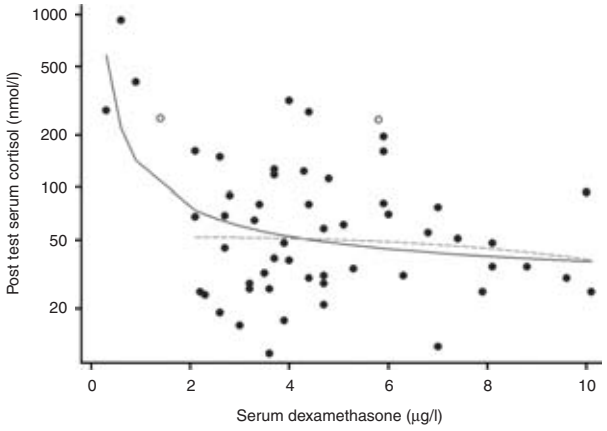
The 1-mg overnight dexamethasone suppression test (DST) is a screening test for endogenous Cushing's syndrome (CS). The principle is that dexamethasone (D) will suppress ACTH and cortisol (C) secretion in healthy individuals, but not in CS. We have studied the associations between D and C concentrations in patients without CS after 1-mg DST.
Methods
On suspicion of CS, 59 patients received 1-mg D at 23 p.m. and next day at 8 a.m. blood was drawn for C and D. Of 56 subjects with successful measurements, three were diagnosed with CS., C (reference 8 a.m. 142–651 nmol/l) and D were measured with in-house methods. We aimed to investigate if there was a cut-off for plasma D below which C was not suppressed using polynomial regression. Data were analyzed using Stata version 10.1 (StataCorp, etc.). The study was exempted from ethical review by the regional medical ethics committee and approved without need for informed consent.
Results
Median post-test C in the 53 subjects without CS was 48 nmol/l (IQR 28–81) (to convert to μg/l, divide by 2.548). Mean post-test D was 11.7 nmol/l (SD 5.9; range 0.8–25.7). Three subjects had D < 5.0 nmol/l, and in these, C was not suppressed. Among 50 subjects with -D > 5.0 nmol/l, no association of D with C was found. In the regression model, post-test D was associated with C ($P < 0.001$ Figure 1A), and the regression line suggested a strong negative association when D was <5.0 nmol/l, but no association when D was >5.0 nmol/l ($P = 0.55$; Figure 1A).
Conclusions
This study of the 1-mg DST suggests that 8 a.m. plasma dexamethasone <5.0 nmol/l is insufficient to suppress serum cortisol. Among people who achieved 8 a.m. plasma dexamethasone >5 nmol/l, there was no association between post-test dexamethasone and cortisol.




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P65

**Mechanisms of gastroprotective action of corticotropin-releasing factor (CRF): involvement of CRF receptor subtypes 1 and 2**

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Exogenous corticotropin-releasing factor (CRF) induces an increase in glucocorticoid production and also may protect the gastric mucosa against stress-induced injury. However, it remained unknown whether glucocorticoids released in response to CRF injection contribute to the gastroprotective effect of CRF. In the present study we investigated whether exogenous CRF may protect

the gastric mucosa against gastric injury through involvement of glucocorticoids and, consequently, through CRF receptor subtype 1. Gastric injury was induced by 3 h cold-restraint stress (at 10 °C) or indomethacin (35 mg/kg) in conscious rats as well as by 3.5 h gastric ischemia-reperfusion in anaesthetized animals. We compared the effects of CRF administration (1.25 and 2.5 μg/kg, i.p.) on the gastric erosion in rats with normal and deficient corticosterone production as well as in rats with normal and occupied glucocorticoid receptors (by RU-38486, 20 mg/kg). Glucocorticoid deficiency was created by metyrapone (30 mg/kg). The selective CRF receptor subtype 2 antagonist, astressin2-B, was also used. Administration of CRF markedly increased plasma corticosterone levels and significantly suppressed the occurrence of gastric erosion induced by each stimulus. Metyrapone injected shortly before CRF administration caused a fast inhibition of CRF-induced corticosterone response and attenuated the protective effect of CRF on the gastric mucosa against the stress- and indomethacin-induced erosion. The gastroprotective effect of CRF was also attenuated by the pretreatment rats with glucocorticoid receptor antagonist RU-38486 in stress and indomethacin ulcerogenic models. Metyrapone and RU-38486 did not influence the protective effect of CRF on the gastric mucosa against ischemia-reperfusion-induced lesion. At the same time the protective effect of CRF on the gastric mucosa against ischemia-reperfusion-induced lesion was prevented by astressin2-B. The results obtained suggest that exogenous CRF may protect the gastric mucosa through involvement of glucocorticoids and, consequently, through CRF receptor subtype 1 as well as through CRF receptor subtype 2.
Supported by RFBR grants-10–04–00605.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P66

**Comparison of salivary cortysol by chemiluminensce and mass spectometry in hypercortilosism**

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BACKGROUND
The determination of Night Salivary Cortisol (NSC) is a useful survey in the screening for hypercortilosism. Study data about its usefulness, even when different methods of dosage are used, are anyway scarce.
OBJECTIVE
To compare the diagnostic performance in the NSC dosage of the chemiluminescence method (CLIA) and of mass spectrometry (LC-MS/MS).
METHODS
Saliva samples were taken at 11:00 pm in 36 healthy volunteers and in 10 patients with Cushing's syndrome at the diagnosis. Each subject provided 2 saliva samples which were used for the NCS dosage by CLIA and with LC-MS/MS.
RESULTS
The controls had NSC values in LC-MS/MS (0.89l ± 0.37 ng/ml; range 0.10–1.89) significantly lower than the Cushing ones (3.18l ± 1.86 ng/ml; range 0.98–6.19); the same using CLIA: the NCS in the controls (0.40 ± 0.22 mcg/dl; range 0.18–1.05) was significantly lower than the Cushing ones (1.43 ± 0.66 mcg/dl; range 0.02–2.29). The diagnostic performance in LC-MS/MS [AUC 0.94 (0.86–1.02] was a bit higher than that of the CLIA [AUC 0.89 (0.71–1.07)]. The cutoff able to guarantee the highest sensitivity and specificity was calculated through the ROC curve, and it was to 1.2 ng/ml in LC-MS and 0.58 mcg/dl in CLIA. In LC-MS/MS a 90% sensitivity and a 88% specificity were obtained; for CLIA, a 90% sensitivity and a 84.8% specificity; if the cut off provided from the lab was used (0.2 mcg/dl), an unacceptably low performance was obtained. The values in CLIA related significantly to those in LCMS/MS ($r$ 0.9).
CONCLUSIONS
This is the first study which compares a CLIA method with a method such as LC-MS/MS. Our data demonstrate that the cutoffs are highly influenced by the method being used. The analyzed methods can have a similar performance, provided the cutoffs are redefined in the single labs.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P67

**Renin-angiotensin-aldosterone system and inflammation - new aspects of an old system: results from the study of health in pomerania**

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Objective
The main physiological function of the renin-angiotensin-aldosterone system (RAAS) is the regulation of water and salt homeostasis as well as arterial blood pressure. In addition, the RAAS is proposed to be involved in the pathophysiology of several diseases like hypertension, vascular remodelling, atherosclerosis, insulin resistance and type 2 diabetes mellitus. In a cohort study in north-east Germany, we investigated the association of plasma renin- (PRC) or plasma aldosterone concentrations (PAC) with inflammatory biomarkers.
Design/Measurements
Our study population included 2,892 individuals from the first follow-up of the population-based Study of Health in Pomerania (SHIP-1). The associations of PRC or PAC with the inflammatory biomarkers fibrinogen (FIB), white blood cells (WBC) and high sensitive C-reactive protein (hs-CRP) were determined. Multivariable logistic regression models were calculated.
Results
Subjects with high PAC (>75th percentile) had higher odd ratios (OR) for increased plasma FIB levels (>4.28 g/l, OR 1.55; 95% CI 1.08–2.22) compared to those with low PAC (<25th percentile). Similar results were found for the association of PRC with FIB levels (>4.28 g/l, OR 2.36; 95% CI 1.62–3.45). Furthermore, subjects with high PRC had higher odd ratios for increased WBC counts (>9.1 Gpt/l, OR 2.57; 95% CI 1.79–3.69) and hs-CRP levels (>5.54 mg/l, OR 1.48; 95% CI 1.02–2.13). In a subsample analysis including normotensive individuals who did not take RAAS-altering medication, high PRC (>75th percentile) were associated with higher odd ratios for increased FIB levels (>4.0 g/l, OR 1.92; 95% CI 1.14–3.23) or WBC counts (>9.3 Gpt/l, OR 2.62; 95% CI 1.53–4.47).
Conclusion
High PAC or PRC are associated with inflammatory biomarkers, indicating a link to inflammatory processes within the general population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P68

**MGMT expression in human adrenocortical carcinoma cell lines and tissues and effects of temozolomide on tumor cell growth.**

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Background
In case of inoperable disease or tumor recurrence, there are only a few medical treatment options for patients with adrenocortical carcinoma (ACC). The oral alkylating drug Temozolomide (TMZ) shows efficacy in a subset of patients with melanoma, glioblastoma or pancreatic neurendocrine tumours. Response to TMZ seems determined by the tumoral expression level of the DNA repair enzyme O(6)-Methylguanine-DNA methyltransferase (MGMT).
Aims
To evaluate the effects of TMZ on human ACC.
Methods
The human ACC cell lines H295, HAC-15 and SW13 were treated with increasing concentrations of TMZ (range: 1–100 μM). Cells were harvested for determination of cell number (after 72 and 144 h of incubation) or for DNA fragmentation (apoptosis; after 72 h). By Colony Forming Assay (CFA) we determined the effect of TMZ on surviving fraction (SF) and colony size (CS). MGMT gene promoter methylation was determined with Cobra technique in the cell lines, 4 normal adrenals (NA) and 16 ACC. Expression of MGMT mRNA was determined by RT-qPCR (mRNA) and by Immunohistochemistry (protein) in the cell lines, 4 NA and 7 ACC.
Results
H295, HAC-15 and SW13 cell lines showed a time- and dose-dependent inhibitory response to TMZ (EC50 17.6 ± 1.0 μM; 17.0 ± 1.1 μM and 41.6 ± 1.1 μM, resp.). In addition, TMZ induced a dose-dependent stimulation of DNA-fragmentation. CFA showed that TMZ decreased SF in H295, HAC15 and SW13 (−85 ± 8%, −82 ± 10% and −57 ± 12%, resp). CS was significantly decreased

in H295 and SW13 (−45±7% and −31±10%, resp.). Partial methylation of the MGMT promoter was found in the 3 cell lines, in 1/4 NA and in 3/16 ACC. MGMT mRNA expression was present in all cell lines, NA and in 6/7 ACC. The cell lines expressed MGMT protein homogeneously, with the strongest staining in the SW13 cells. NA and 3/7 ACC expressed MGMT in a heterogeneous manner.
Conclusions
ACC variably express MGMT mRNA and protein. TMZ, at pharmacological concentrations, is effective in ACC cell lines expressing MGMT, suggesting that other factors determine sensitivity to TMZ as well. However, the cell line with the highest MGMT mRNA and protein level showed the lowest sensitivity to TMZ. One of the effects of TMZ on ACC cell growth is the induction of apoptosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P69

**New quick cortisol assay during adrenal vein sampling in patients with primary aldosteronism**

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Background
Adrenal vein sampling (AVS) is the gold standard test for identification of unilateral primary aldosteronism (PA) which is curable by surgery, but is a difficult procedure with low success rates. Rapid cortisol assays during AVS improved AVS success rates.
Methods
We have developed a gold nanoparticles based immunochromatographic quick cortisol assay (QCA) in which cortisol concentration could be measured both quantitatively and semi-quantitatively within 6 minutes. We performed a prospective randomized controlled study to evaluate the success rate of AVS incorporating our new QCAs at Kanazawa University Hospital. Ninety PA patients were randomly assigned to undergo AVS using quantitative QCA (quantitative QCA group, $n=30$), AVS using semi-quantitative QCA (semi-quantitative QCA group, $n=30$) or AVS without QCA (control group, $n=30$). We next evaluated the usefulness of semi-quantitative QCA at 4 centers: Kanazawa University Hospital and 3 centers with initial low AVS success rates. We randomly assigned 151 PA patients to undergo AVS using semi-quantitative QCA (semi-quantitative QCA group, $n=77$) or AVS without QCA (control group, $n=74$).
Results
AVS success rate in the quantitative QCA group (28 of 30 (93%)) did not differ from that in the semi-quantitative QCA group (28 of 30 (93%)), which were significantly higher than that in the control group (21 of 30 (70%)). The total AVS success rate in the semi- quantitative QCA group at 4 centers was 71 of 77 (92%), significantly higher than that in control group (43 of 74 (58%). Even at 3 centers with initial low AVS success rates, the AVS success rate in the semi-quantitative QCA group was 39 of 43(91%), significantly higher than that in control group (21 of 43 (49%).
Conclusions
Our new quick cortisol assay was very fast, simple, and easy and was proved to improve the AVS success rates.
Declaration of interest
I fully declare a conflict of interest. Details below.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector

## P70

**Primary aldosteronism is very frequent in resistant hypertension and is associated to early renal vascular damage**

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Background
Data on the prevalence of primary aldosteronism (PA) in subjects with resistant hypertension (inadequate pressure control with three full-dose drugs, including a diuretic) are scanty and it is not known if PA is associated with a more severe vascular damage.
Aim
To determine the prevalence of PA in patients with resistant hypertension and define the possible association with early cardiovascular damage.
Methods
Seventy two patients with resistant hypertension consecutively recruited from patients referring to a tertiary care center for hypertension.
Results
Diagnosis of PA (aldosterone (pg/ml)/PRA(ng/ml/h) > 400 AND aldosterone >200 basally AND > 100 after NaCl test, after stopping both interfering drugs and low salt diet), was made in 20 subjects (27.8%); the remaining 52 were considered affected by Essential Hypertension (EH, 72.2%). By CT and adrenal venous sampling 4 patients (20%) were diagnosed to have an aldosterone-producing adenoma and underwent surgical treatment. PA and EH did not differ for age, BMI, smoking habit, glycemia, LDL cholesterol, HDL cholesterol and triglyceride levels, renal function, duration of hypertension and family history of hypertension, or diabetes mellitus, or early cardiovascular disease. Blood systolic pressure levels did not differ in the two groups, but diastolic was significantly higher in PA (104.5±10.9 vs 96.6±13.6 mmHg, $P<0.02$). Potassium was significantly lower in PA (3.5±0.5 vs 4.1±0.5 mEq/l, $P<0.01$).
In PA the rate of intima-media thickness > 0.9 mm was higher (41.2 vs 26.7%), though not significantly but microalbuminuria was significantly more frequent (52.6 vs 26.7%, $P<0.05$). Moreover, PA predicted significantly microalbuminuria (OR =6.54, CI 1.02–19.35, $P<0.05$) in a logistic regression model with glycemia, creatinine, IMT, family history of early cardiovascular disease.
Conclusions
PA is very frequent in hypertensive subjects resistant to pharmacological therapy. Patients with PA show early renal vascular damage more frequently than resistant hypertensive patients with normal mineralcorticoid function.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P71

**Enhanced small dense LDL-subfractions, triglycerides and chemerin as early metabolic alterations in young patients with classic CAH due to 21-hydroxylase deficiency**

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Background/Aim
Classic 21-hydroxylase deficiency (21HD) presents some traits of the metabolic syndrome.
Aim
To investigate early metabolic alterations in lipid and adipokine profile together with markers of subclinical inflammation in children and young adults with classic 21HD, which could predict early atherogenesis.
Patients und Methods
Thirty nine patients with classic 21HD (4–30 years); 39 age-, sex- and BMI-matched healthy controls. Clinical parameters, hormonal status and genotype were assessed in patients. Total-cholesterin (CL), LDL-CL, triglycerides (TG), HDL-CL were determined in patients/controls. The relative (%) and absolute (mg/dl) small-dense LDL subfractions (sd-LDL) were measured by density gradient ultracentrifugation. Adipokines (leptin, adiponectin, chemerin) and markers of subclinical inflammation (IL-6, PAI-1) were measured by ELISA.
Results
sd-LDL(%) was significantly higher in patients than controls ($P=0.003$). The same applies for absolute sd-LDL (mg/dl) (44.5+/−10.4 vs 38.3+/−7.5, $P=0.033$). TGs were higher in patients (96.1+/−44.7 vs 76.8+/−32.5 mg/dl, $P=0,031$). No significant difference was found in CL, LDL-CL, HDL-CL, leptin, adiponectin, IL-6 and PAI-1. However, chemerin was significantly higher in patients than controls (136.9+/−40.2 vs 111.2+/−41.9 ng/ml, $P=0.007$).
No obvious differences in sd-LDL or chemerin were seen between clinical forms, genotype groups (built according to the predicted residual enzymatic activity of 21-hydroxylase) or the degree of hormonal control, nor were direct correlations observed between the altered metabolic parameters and the total hydrocortisone dose or the duration of treatment.

Conclusion
Children and young adults with 21-hydroxylase deficiency show significantly higher sd-LDL-subfractions, TG and chemerin concentrations compared to age- and sex-matched healthy controls. As we could earlier show, they also exhibit a trend towards insulin resistance with higher IRI values, correlating with the total hydrocortisone dose and the duration of treatment. These alterations represent a risk constellation for early endothelial dysfunction, with potentially increased cardiovascular risk in later life. Supraphysiological hydrocortisone doses should be consequently avoided.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P72

### ACTH and cortisol responses to the combined DEXA-CRH test in patients with adrenal incidentalomas

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Adrenal incidentalomas (AI) are a common finding in patients studied by abdominal imaging and approximately 9–17% is found bilaterally. So far, the potential role of hypothalomo-pituitary adrenal (HPA) axis dysregulation in the pathogenesis of AI, especially of those found bilaterally, has not been addressed. The DEXA-CRH test has been previously used to detect dysregulation of the HPA axis; herein, it was used to assess ACTH and cortisol responses in a large group of patients presented with unilateral and bilateral adrenal incidentalomas.
We studied 97 patients (51 with unilateral and 46 with bilateral AI) and 26 normal controls. All subjects underwent a formal low dose dexamethasone suppression test followed by a CRH test (100 ug iv). Patients with AI demonstrated statistically higher ACTH and cortisol responses to the DEXA-CRH test compared to the controls ($P<0.05$). Although ACTH responses did not differ between patients with unilateral and bilateral incidentalomas, cortisol responses were significantly higher in patients with bilateral AI ($P<0.05$). According to ACTH responses patients were divided in 3 groups: group A with ACTH max $<$ 10 pg/ml ($n=48$), group B with ACTH max 10–20 pg/ml ($n=34$) and group C ACTH max $>20$ pg/ml ($n=15$). The proportion of patients with bilateral AI in group C was significantly higher than in group A (66.6% vs 37.5%, $P<0.05$). On the contrary more patients with unilareral AI were found in group A compared to group C (62.5 vs 33.3%, $P<0.05$).
In conclusion patients with AI demonstrate higher ACTH and cortisol responses to the DEXA-CRH test compared to normal subjects. This finding appears to be more common in patients with bilateral AI and, provides some ground for the potential involvement of HPA dysregulation in the pathogenesis, in at least a subgroup, of AI of patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P73

### Idiopathic primary hyperaldosteronism frequently undergoes spontaneous remission: frequence and predictors

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Background
Idiopathic primary hyperaldosteronism (IHA) is the subtype of primary aldosteronism (PA) in which lateralization of the mineralocorticoid hypersecretion cannot be demonstrated. Scanty data are available on the clinical history of IHA: a couple of brief reports suggest a remission of aldosterone hypersecretion after years of treatment with mineralocorticoid receptor blocking agents.
Aim of this study was to check the persistence/remission of aldosterone hypersecretion in patients with IHA followed in a tertiary care referral center for endocrine and metabolic diseases, long after the diagnosis, looking for possible associations of persistence/remission with clinical features.
Patients/Methods
Plasma aldosterone to renin acivity ratio (ARR) and plasma aldosterone after saline infusion test, clinical and metabolic features, indices of early target organ damage, adrenal imaging were obtained in 34 patients followed for 8.4 (range 3–15) years after the diagnosis of IHA. All hormonal measurements were carried out after prolonged (30–40 days) withdrawn of both interfering drugs and low salt diet. Criteria for persistence of PA were the same as at the diagnosis: ARR (pg/ml to ng/ml/h) $>400$, plasma aldosterone concentration $>150$ pg/ml basally and $>100$ pg/ml after saline infusion.
Results
In 26/34 patients (76%) PA was not confirmed. At univariate analysis, remission from PA was positively associated with female sex, potassium levels, age over 60, duration of hypertension and duration of follow-up. Absence of unilateral adrenal mass, treatment with mineralocorticoid antagonists and lower aldosterone levels at diagnosis were not associated to remission. At multivariate analysis, age above 60 years was the only independent predictor of remission of PA (OR 64.7, CI 1.1–3758.9, $P=0.044$).
Conclusions
This study suggests that mineralocorticoid function in patients with IHA normalize spontaneously at a high rate. Age is a positive predictor of this evenience.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P74

### Anxiety and depressive symptoms in patients with primary aldosteronism in a longitudinal study

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Context
Recent studies showed a high prevalence of anxiety and depressive symptoms in patients with primary hyperaldosteronism (PA). A cross-sectional analysis suggested only minor improvement following adrenalectomy (ADX) and mineralocorticoidreceptor antagonist (MRA) therapy.
Objective
Our aim was to determine the course of anxiety and depression in untreated patients.
Design
We investigated 15 patients with PA at time of diagnosis and 1 year after initiation of specific treatment (ADX: $n=10$; MRA: $n=5$). Blood pressure, aldosterone and renin were recorded in all patients.
Main outcome measures We used GAD-7 and PHQD questionnaires to assess anxiety and depression.
Results
At time of diagnosis patients showed significantly higher mean values for GAD-7 ($6.3\pm4.5$) and PHQ-D ($7.5\pm6.6$) compared to the general population. One year later mean systolic blood pressure ($153\pm20$ vs $128\pm13$), serum potassium ($3.6\pm0.7$ vs $4.4\pm0.3$) and the aldosterone to renin ratio in ADX patients ($64\pm63$ vs $12\pm15$) had significantly improved. In parallel, psychopathology improved to some degree (GAD-7: $4.7\pm3.8$; PHQ-D $4.4\pm5.2$), but still 11 of 15 patients had scores outside the normal range. Gender differences were found. For depression females showed before (b) and after (a) treatment higher scores by trend (b: f $10.71\pm6.15$; m $4.63\pm5.87$ $P=0.72$; a: f $7.0\pm5.94$; m $2.13\pm3.44$ $P=0.07$). For anxiety females and males showed a significant difference after initiation of treatment. (b: f $7.33\pm3.14$; m $5.5\pm5.63$ p$=0.46$; a: f $7.83\pm3.54$; m $2.63\pm2.2$ $P=0.004$).
Conclusion
Patients with PA show a rapid and sustained improvement following intervention for their somatic parameters, but depressive symptoms and anxiety appear to improve more slowly especially in women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This work was supported, however funding details unavailable.

## P75

### Drug prescription pattern in patients with autoimmune addison's disease in Sweden

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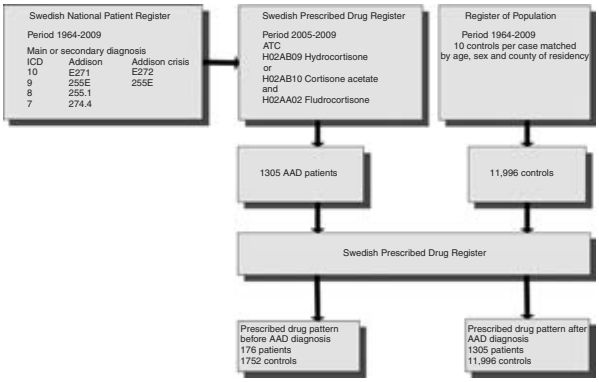
Introduction
There is no published data on dispensed drug pattern in patients with Autoimmune Addison's disease (AAD). Sweden has excellent condition for research in this area, with high-quality population based registers. Among them is the Swedish Prescribed Drug Register (SPDR) that contains information about dispensed prescribed drugs. By linking registry data, we aimed at accurately estimate the prevalence and incidence of AAD, the prevalence of common concomitant autoimmune manifestations and to describe the drug prescription pattern in Swedish patients with AAD.
Methods
Through the Swedish National Patient Register (SNPR) and the SPDR we identified 1305 patients with an AAD diagnosis and combination treatment with hydrocortisone/cortisone acetate and fludrocortisone. Through the Total Population register we identified 11,996 age- and sex- matched controls. Dispensed drug prescription pattern according to the SPDR was analyzed in AAD patients and controls.
Results
The mean age at AAD diagnosis was 42.2 years (standard deviation (S.D.), 16.9) for women and 35.4 years (S.D. 16) for men. 54% were female and 46% male. The prevalence of AAD during the period 2005–2009 was 12.2–13.1 ($P$ for trend 0.062) and the incidence 0.5–0.6 ($P$ for trend 0.131) per 100,000 inhabitants per year. 59.3% of AAD patients had medications indicative of concomitant major autoimmune polyendocrine syndrome manifestations, 46.6% autoimmune thyroid disease, 17.7% B12 deficiency and 14.2% type 1 diabetes mellitus. The mean number of concomitant dispended drugs was 11.6 (S.D. 10.0) in the AAD group and 9.6 (S.D. 8.6) in the control group (Difference in mean 2.1 (95% CI 1.5–2.6), $P<0.0001$). After diagnosis, AAD-patients were dispensed more gastrointestinal medications, vitamins, mineral supplements, osteoporosis drugs, antihypertensive drugs, lipid modifying agents, sex hormones and urological drugs, anabolic steroids, anti-infectives, immunosuppressants, analgesics, hypnotics and sedatives, antidementia drugs, drugs for obstructive airway diseases and opthalmological preparations.
Conclusion
The incidence and prevalence of AAD in Sweden is comparable to the high figures reported from Norway. Patients with AAD have a higher number of dispended drugs compared to controls. The drug prescription pattern is partly expected but also raises concern about the morbidity of patients.




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P76

### Dosing regimens for glucocorticoid replacement therapy - a worldwide patient survey

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There is no consensus on the best dosing regimen for glucocorticoid replacement therapy. The general intention is to individualize according to the clinical response and use the lowest possible maintenance dose without risking adrenal crisis.
Aim
To survey patients with adrenal insufficiency (AI) on their glucocorticoid replacement therapy dosing regimen.
Method
Patients (children and adults) were recruited via patient organizations to respond anonymously to a web-based survey. The survey was open from September 12th to December 19th 2008. Data on age and gender was not collected in order to protect individual identity.
Results
There were 1245 responders. 84% with primary AI, 11% with secondary AI and 5% unsure. 64% were from the USA, 20% from Europe and 15% from the ROW. Hydrocortisone (HC) was used by 75%, Prednisone/Prednisolone by 11%, Cortisone Acetate by 6% and Dexametasone by 4% of respondents. The median dose of hydrocortisone was the same (20 mg) for primary and secondary AI when analyzing the whole dose range (2–80 mg). The most commonly used dose was 20 mg for both primary and secondary AI, followed by 30 mg. There were differences in therapy and dosing regimens between countries.
One third of the respondents had their dose adjusted according to body weight or body surface area. These were more often primary AI patients, treated with HC and received more often a TID regimen.
Conclusion
The most commonly used treatment regimen is hydrocortisone 20 mg, with a median dose of 20 mg, which is the same for patients with primary and secondary AI.There is variation in the dosing regimens among countries. The inter-patient differences in dosing regimens highlights the efforts being made to personalize treatment of patients with adrenal insufficiency.
Declaration of interest
I fully declare a conflict of interest. Details below.
Funding
This work was supported, however funding details unavailable.

## P77

### Significance of ACTH stimulation test in the diagnosis of an aldosterone-producing adenoma

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Primary aldosteronism (PA) is a major cause of secondary hypertension. Among PA, the diagnosis of an aldosterone-producing adenoma (APA) is critical because an APA can be cured surgically. Adrenal venous sampling (AVS) is the golden standard test in the diagnosis of an APA, but is available only in specialized medical centers. Meanwhile, aldosterone secretion of an APA is reported to be more sensitive to ACTH than that of idiopathic hyperaldosteronism (IHA) or essential hypertension. In this study, we evaluated the diagnostic accuracy of ACTH stimulation test with 1 mg dexamethasone suppression (dex-ACTH test) in the diagnosis of an APA among patients with suspected PA. Patients admitted to Kyoto University Hospital on suspicion of PA were included and were classified into three groups; non-PA group ($n=20$), IHA group ($n=16$), and APA group ($n=23$) by captopril challenge test and AVS. In dex-ACTH test, plasma aldosterone concentrations (PACs) were examined every 30 min after ACTH stimulation until 120 min. After ACTH stimulation, PACs increased in all the three groups. PACs in the APA group were significantly higher in patients than those in the non-PA group and the IHA group (Table). Receiver operated characteristics (ROC) curve analysis showed that the diagnostic accuracy of the dex-ACTH test for the diagnosis of the APA group from all the patients included was highest at 90 min after ACTH stimulation, with the optimal cutoff value of PAC $>37.9$ ng/dl, corresponding with sensitivity and specificity of 91.3 and 80.6% (Figure). Our study indicates that the dex-ACTH test is useful in the diagnosis of an APA among patients suspected of PA. Dex-ACTH test, which is available on an outpatient setting and does not require special technique or

devices, can be used to select patients who are highly suspected of an APA and definitely require AVS.

PACs in the three groups before and after ACTH stimulation.

| | **Before** | **30** min | **60** min | **90** min | **120** min |
|---|---|---|---|---|---|
| Non-PA group | 11.1±0.7 | 32.4±2.2 | 34.4±2.4 | 32.2±3.1 | 27.0±2.7 |
| IHA group | 11.6±0.9 | 33.0±2.4 | 34.6±2.8 | 31.9±2.8 | 27.8±2.8 |
| APA group | 17.1±2.9 | 64.7±8.5 | 80.1±13.1 | 75.2±8.8 | 62.6±6.9 |

Data are means±S.E.M. (ng/dl)

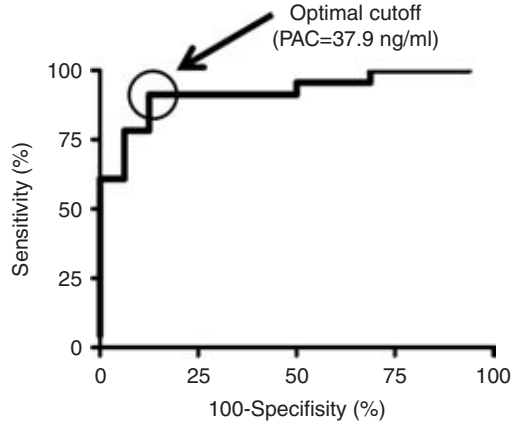


**Figure 1.** The ROC curve of PAC at 90 minutes after ACTH stimulation for the diagnosis of the APA group

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P78

**An open, multi-centre, phase IIIB, long term follow-up study to assess the safety, tolerability and efficacy of once-daily oral dual-release hydrocortisone in patients with adrenal insufficiency**

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Introduction
There are limited prospective safety data on glucocorticoid replacement. The aim of this study was to assess the safety and efficacy during long term treatment using a once-daily oral dual-release hydrocortisone in patients with adrenal insufficiency (AI).
Methods
This is an open, multi-centre study in 71 patients with AI. Most patients were recruited from a previous controlled trial ($n=53$) where they had received the dual-release therapy 6–9 months [1] before entering this trial. Every 6 months, clinical and laboratory assessments were performed.
Results
After 18 months 94.6% of patients remained in the study. The median dose was 30 mg (range 20–40 mg) at entry. Nine patients reduced and 6 patients increased their dose during the 18 months. Total serum cholesterol (0.2 mmol/l; $P=0.03$), LDL-cholesterol (0.2 mmol/l; $P=0.03$) and HDL-cholesterol concentrations increased (0.2 mmol/l; $P<0.0001$) while triglycerides decreased (−0.4 mmol/l; $P<0.0001$). In 13 patients with DM HDL-cholesterol increased (0.3 mmol/l; $P=0.004$) triglycerides decreased (−0.2 mmol/l; $P=0.03$) and HbA1c was unchanged.
Systolic blood pressure (−0.4 mm Hg), diastolic blood pressure (0.7 mm Hg) and body weight (−0.8 kg) did not change significantly.
The average number of AEs per patient was 4.5 from 0 to 18 months. Most AEs were classified as "infections and infestations" (40%). The most commonly reported events were nasopharyngitis (20%), fatigue (7%) and gastroenteritis (6%), which is similar to conventional hydrocortisone replacement [1]. The frequency of AEs were similar in the beginning and at the end of the study.
There were 13 non-fatal serious AEs reported; 2 Addison crisis, 6 gastroenteritis, 1 case each of pyelonephritis, gastritis/esophagitis, virosis, colitis and ectopic pregnancy.
Conclusions
No safety concerns were observed in this open prospective study using a new oral dual-release hydrocortisone replacement therapy. Some continuing beneficial metabolic effects could be seen in particular in patients with concomitant DM.
1. Johannsson, G., et al., J Clin Endocrinol Metab, 2011 Nov 23.
Declaration of interest
I fully declare a conflict of interest. Details below.
Funding
This work was supported, however funding details unavailable.

## P79

**Malignant adrenal incidentalomas: clinical analysis of 2300 patients with adrenal incidentalomas registered at a single endocrinological center**

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This study aimed at searching for malignancy in a group of 2300 patients with incidentally found adrenal tumours (1688 women and 612 men aged 11-87 years) registered at our Department of Endocrinology. The methods of investigation included clinical and imaging studies, hormonal assays and pathomorphological examinations in the patients treated by surgery. In 168 patients, aged 11 to 81 years, adrenocortical cancer was diagnosed ranging in diameter from 2.5 to 25.0 cm. In 7 patients bilateral carcinomas have been observed. 67% of the detected tumours were at III/IV ENS@T stage. All the patients were treated by surgery and in a significant number of them an additional nephrectomy, splenectomy and/or partial hepatectomy were necessary. Following surgery all the patients have been treated with mitotane.
Metastatic infiltrations of adrenals were found in 57 patients aged 35–79 years (out of 228 ones with malignant disease = 25%). The most frequently the tumours originated from renal, pulmonary or colorectal cancer. Additionally, in 14 other patients some malignant adrenal tumours were found, mainly metastasing pheochromocytomas and lymphomas. In bilateral adrenal tumours (a half of metastatic infiltrations and of lymphomas) Addison's disease or pre-Addison's disease has been observed.
Conclusions
Malignant tumours have been diagnosed in 10% of adrenal incidentalomas; adrenal cancer appeared to be about three times more frequent than metastatic laesions.
Supported by a 501-1-08-13/10 & 501-1-08-11/11 CMKP Grant.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P80

**RRM1 Gene expression affects metabolization and activity of mitotane in adrenocortical cancer cell lines.**

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Background
Mitotane (o,p'DDD) is the reference therapy for adrenocortical carcinoma (ACC) and should undergo metabolization in o,p'DDE and o,p'DDA to exert its anti-proliferative activity. We have previously shown that there is a link between

RRM1 gene expression and o,p'DDD activity in an adjuvant setting. Aim of this study was to assess whether RRM1 gene expression is correlated to the bioavailability and cytotoxic activity of o,p'DDD and its metabolites in ACC cell lines.
Methods
The ACC cell lines SW-13 and H295R were incubated with o,p'DDD, o,p'DDE, o,p'DDA at therapeutic concentrations, and cell viability (evaluated using the WST-1 method) was correlated with RRM1 gene expression (evaluated using real time-PCR). The intracellular concentrations of o,p'DDD and o,p'DDE were evaluated in cells lysates using HPLC-UV method in both cell lines.
Results
In H295R cells, o,p'DDD, o,p'DDE and o,p'DDA showed a similar cytotoxic effect (IC50 of 32 μM, <1 μM, 292 μM, respectively) and RRM1 gene expression was not influenced by any drug. In SW-13 cells, o,p'DDD and o,p'DDE were effective at high concentrations only (IC50 of 829 μM, 122 μM, respectively), while o,p'DDA showed a greater cytotoxic activity (IC50 292 μM). An up-modulation of RRM1 mRNA was induced by o,p'DDD and o,p'DDE, whose intracellular concentrations were lower in SW-13 than in H295R cells. The higher sensitivity of SW-13 cells to o,p'DDA was associated with the loss of up-modulation of RRM1 mRNA. Moreover, RRM1 gene silencing in SW13 cells increased the intracellular concentrations of o,p'DDD and o,p'DDE, and their cytotoxic activity as well.
Conclusions
The present data suggest that RRM1 gene expression may affect both pharmacokinetic and anti-neoplastic activity of o,p'DDD in SW-13 cells.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P81

### The routine application of the current clinical recommendations for adrenal incidentalomas the management is highly inefficient

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Introduction
The improvement in imaging techniques has increased the number of incidentally discovered adrenal lesions. Current clinical recommendations are based on the NIH consensus statement (2002) and include: CT at 6,12 and 24 months plus annual hormone evaluation for 4 years.
The aim of this study was to describe the results of the application of the current guidelines in a cohort of consecutive patients whose initial diagnosis was non-functioning adrenal adenoma
Patients and methods
We reviewed the clinical records of the patients who followed the following inclusion criteria: incidental discovered adrenal mass; no extra-adrenal malignancy; no radiological suspicion of malignancy (apart from size). Annual hormone evaluation consisted in: 1 mg DXT, urine metanephrines and catecholamines, K and Renin/Aldo. Regular CT studies were also performed.
Results
Ninty-nine patients were included (54.5% females, mean age 59.8±11.3 years, BMI 28.8±4.9 kg/m$^2$). Hypertension was present in 49.5% of the patients, dyslipidemia in 41.4%, type 2 DM in 25.3%.
Two patients underwent surgery because of sizeand in both cases the final diagnosis was cortical adenoma. Mean initial size was 21.6±9.5 mm and 14.4% of the patients had bilateral lesions. The median follow-up was 45.5 months.
At the baseline evaluation, hormonally active adenomas were diagnosed in 5 patients (2 hypercortisolism, 3 hyperaldosteronism). All the patients with an initial negative evaluation remained without alterations in the folowing hormonal tests. No significant increase in size (1.7±5.2 mm) was observed through the follow-up period.
Conclusion
The routine application of the current guidelines for the follow-up of adrenal incidentalomas whose initial diagnosis corresponds to non-functioning adenoma does not seem justified.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P82

### The Effect of Glucocorticoid Receptor Polymorphisms on the Sensitivity to Cortisol in Addison's disease

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Background
There is uncertainty as to whether glucocorticoid receptor (GCR) polymorphisms play a role in the development of glucocorticoid-related side-effects in individuals receiving hydrocortisone replacement for Addison's disease.
Method
One-hundred-and-forty-seven Addison's patients were age, gender, ethnicity and body mass index (BMI) matched with 147 control subjects. Genotyping was performed using polymerase chain reaction (PCR) for three single nucleotide polymorphisms, two of which are known to be sensitising BclI and N363S and one polymorphism ER22/23EK is known to induce a degree of cortisol resistance. Associations with metabolic derangements were evaluated.
Results
The prevalence of the BclI polymorphism occurred more frequently in whites than in any of the other ethnic groups, but was not associated with any metabolic derangement. The ER22/23EK polymorphism was associated with an increased BMI in both patients (29.4 vs. 24.7 kg/ m$^2$; $P$ <0.02) and control subjects (26.3 vs. 24.2 kg/ m$^2$; $P$ <0.001) compared with the wild-type. The heterozygous polymorphism was associated with lower low-density lipoprotein (LDL) cholesterol in controls vs. wild-type (3.46 mmol/l vs. 3.93 mmol/l; $P$=0.02). The N363S was not associated with any metabolic derangement.
Conclusion
The overall effect of the GCR polymorphisms was to increase the BMI in healthy control subjects and patients harbouring the ER22/23EK polymorphism, which was in contrast to what was expected. No associations between any of the polymorphisms and hydrocortisone doses were found, albeit that doses are prescribed on an empiric basis.
Declaration of interest
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## P83

### Impact of reference values and published thresholds on clinically relevant cut-offs

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The adrenal function of children is investigated similarly to adults' using at rest (basal) and after testing serum cortisol concentration ((F)). di Iorgi et al. (JCEM 95:2132) showed a good diagnostic accuracy of glucagon testing investigating (F) increase. We retrospectively addressed the relevance of using, in children with short stature tested for GH deficiency, 1/ the normal reference range of our assay for basal (F) and 2/ the threshold provided by di Iorgi et al. on (F) response to glucagon-betaxolol testing. This population was not suspect of adrenal insufficiency.
Basal (F)DXi ($N$=140, 10.3 (0.2–15.5) yr (median (2.5th–97.5th percentile)) was 260 (98–604) nmol/l. 26% subjects had lower (F)DXi than the reference range (185–624) nmol/l (Unicel DXi800,Beckman Coulter).
After betaxolol-glucagon testing ($N$=108), GH peak was 34.8 (80–74.4) mUI/l (Liaison analyser, DiaSorin); 29% were low GH responders (peak <20 mUI/l). (F)DXi peak was 502 (117–856) nmol/l. 31% would be considered adrenal insufficient using di Iorgi et al.'s criterion (403 nmol/l: 100% specificity threshold). (F)DXi was compared with results obtained with our prior RIA assay in a subgroup of patients. Basal (F)RIA was 411 (141–1061) nmol/l. 7% subjects had lower (F)RIA than the reference range (200–800) nmol/l. (F)RIA peak was 770 (281–1542) nmol/l. 5% would be considered adrenal insufficient using di Iorgi et al.'s criteria. Passing-Bablock regression showed a significant linear relationship between data from the 2 assays with a significant bias.
This emphasizes again that laboratories have to provide reference values for given populations, (e.g. basal (F) in children with short stature). Clinically relevant thresholds have to be locally defined (e.g. betaxolol-glucagon testing). Data issued from manufacturer's-chosen populations and clinically relevant thresholds established in well-conducted study but with different populations or assays cannot wholly substitute to local references. Failure to do so will elicit false positive or negative results.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P84

**Hormonal and metabolic features in patients with adrenal incidentalomas according to 1 mg overnight dexamethasone suppression test**

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Adrenal masses are among the most prevalent human tumors and are frequently detected unexpectedly by imaging studies performed for reasons unrelated to suspect of adrenal diseases.
Subclinical hypercortisolism (SH) is the most frequent endocrine dysfunction detected in patients with adrenal incidentalomas (AI), accounting from five to 20% of all cases depending on inclusion criteria. one mg overnight dexamethasone suppression test (one mg DST) should be the first screening test; however there is no consensus on the cutoff values to consider the test as positive.
In this study we investigated hormonal and metabolic aspects of patients with AI according to different cortisol suppression. We included 39 patients with AI and divided them into four groups based on cortisol levels after one mg DST: $<50$ nmol/l (group one, normal suppression) between 50 and 83 nmol/l (group two), between 83 and 138 nmol/l (group three) and $>138$ nmol/l (group four).
Since ACTH levels were $<10$ pg/ ml in 100% of patients in group four, these were considered affected by SH according to literature.
ACTH levels $<10$ pg/ ml were observed in 100% of patients in group three, in 67% of group two and in 31% of group one. ACTH and DHEA-S levels were significantly lower in groups three and four than in group one. Moreover, ACTH response to CRF and/or DDAVP was reduced in patients of groups three and four. Prevalence of diabetes was 15% in group one, 25% in group two, 40% in group three and 33% in group four. Glycemia was higher in groups three and four than in group one. Triglycerides were higher in group three than in group one.
In conclusion, group three showed the same hormonal and metabolic alterations of group four, suggesting that in our patients SH was present in patients with cortisol levels after DST $>83$ nmol/l.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P85

**Therapy with steroidogenesis inhibitors in Cushing's syndrome: a reappraisal**

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Objective
To evaluate the outcome of preoperative therapy with ketoconazole (KTZ) and/or metyrapone (MTP), in previously untreated CS patients who later underwent surgery.
Design and methods
A total of 62 CS patients (85% ACTH-dependent), who have been treated with steroidogenesis inhibitors prior to surgery in our centre between 1983 and 2010, were retrospectively studied. T0 and t1 defined visit at baseline and at the end of medical treatment, respectively.
Results
Outcomes were classified into the following three groups based upon clinical and biochemical (normal UFC) control of hypercortisolism at t1: group CO (controlled) included 20 patients (32%) with eucortisolism and significant clinical improvement as compared with t0; group NC (not controlled) included 30 patients (48%) who had persistent hypercortisolism and lack of clinical control of CS symptoms; and group PC (partially controlled) included 12 patients (19%) who presented eucortisolism but no real clinical improvement. Median duration of treatment was 4 months (range: 1–30.7 months), median cumulative dose of KTZ and MTP was 57g (range: 3.6-240 g) and 120g (range: 7.5–1215g), respectively. No differences in baseline characteristics were observed between groups. Systolic blood pressure at t1 was significantly higher in PC than in NC patients ($P<0.05$). Hypertension persisted more frequently in PC patients than in the other groups ($P<0.05$) after a median post-surgery follow-up of 93 months (range: 2–276 months). UFC at t0 ($r=0.458$; $P<0.0001$) and NC status ($r=-0.315$; $P<0.0001$) predicted the decline in UFC during medical treatment, and remained significant after correcting for baseline characteristics, type and dose of the medications (R2, 0.770).
Conclusions
Preoperative administration of KTZ, MTP or both normalised UFC in 52% of CS patients, although concomitant clinical improvement was not reached in all cases. Future studies are needed to categorise the response to medical therapy and to individualise the treatment in CS patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P86

**Ectopic Cushings and Nocardia series**

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Cushing's syndrome results from exposure to excess glucocorticoids. Ectopic Cushings is endogenous ACTH dependant form of Cushing's associated with markedly raised ACTH and cortisol levels. This leads to an impaired immune response, setting the stage for occurrence of opportunistic infections. Nocardia is a gram positive bacterial infection caused by aerobic actinomycetes in genus Nocardia. We report a series of patients diagnosed with ectopic Cushings, with growth of Nocardia asteroides from pulmonary secretions. In one of these cases, the manifestations of Cushing's disappeared with treatment for Nocardia. Two middle aged men presented to the Endocrine clinic in the same year: the first with history of exertional shortness of breath, and weight loss for 1 year, the other with facial swelling, disturbed sleep and lethargy for a month. The third case was a young male who presented with progressive weakness & weight loss for some time. All three had uncontrolled hypertension, high blood sugars & were hypokalemic (K: 2.52, 2.9, 1.5; (normal range 3.6–4.0); 24 hour urine cortisol was elevated at 2000, 27,216 and 9088 (32–243); ACTH 68.5, 159, 255(0–48), respectively. Their MRI pituitary was normal, inferior petrosal sinus sampling revealed no central peripheral gradient. CT chest of these subjects demonstrated cavitatory lung lesions; bronchial washings recovered heavy growth of nocardia. Histopathology revealed no malignancy. Antihypertensives, insulin, potassium replacement, ketoconazole & trimethoprim- sulphamethoxole (TS) were initiated. The patients' symptomatology improved & cavitatory lesions resolved with treatment. The primary source for the ectopic cushings remained unknown. The first case followed a progressively downhill course leading to death. The second case required bilateral adrenalectomy. In the third case, we were able to completely taper off ketoconazole, potassium, insulin & antihypertensives, after starting TS.Opportunistic infections are known to be associated with Cushing's syndrome, as higher levels of glucocorticoid secretion are found in patients with ectopically produced ACTH. Nocardia of lungs is important differential to consider. This series includes the first case reported in which signs and symptoms of cushings subsided after treatment of Nocardia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P87

**Laparoscopic adrenalectomy for large adrenal tumors**

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Background data
Laparoscopic adrenalectomy has became in the last decade "gold standard" for treating of adrenal tumors with diameters smaller than six cm. In addition, one should note than larger tumors or potentially malignant tumors can now also be removed laparoscopically, with virtually no complication.
Objective
The authors evaluate the effectiveness of laparoscopic adrenalectomy for a large adrenal tumors.
Methods
One hundred laparoscopic adrenal procedures over the past five years were reviewed and followed for adequacy of resection.
Results
Indications were: pheochromocytoma ($n=10$), aldosterone-producing adenomas ($n=15$), nonfunctional adenomas ($n=21$), cortisol-producing adenomas ($n=14$), Cushing's disease ($n=32$) and others ($n=8$). Specifically, 32 patients (23 females and nine males) between 20 and 69 (median ages of 46,43 years) had tumors larger than six cm. four of these cases underwent bilateral adrenalectomy by laparoscopic approach; in five cases the surgeons preffered conversion to the open approach. Among the reasons of conversion: bleeding, local invasion (inferior vena cava, liver, diaphragm), unclear landmarks etc. Mean operative time was 114 minutes (from 25 min to 270 min, includind patients with bilateral approach). Mortality among the studied cases was zero and as postoperative complication only a bleeding from spleen (after bilateral adrenalectomy convert to open procedure because of unclear landmarks in a case of Cushing disease) can be mentioned. Patients were discharged anywere between one and 50 days (the complicated case) postoperative (median six days).
Conclusions
Laparoscopic adrenalectomy is a reasonable procedure for selected large adrenal tumors when a complete resection is technically feasible and is no evidence of local invasion.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P88

**Adiponectin gene polymorphisms in primary aldosteronism and their relation to the metabolic syndrome**

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It is well known that primary aldosteronism (PA) is frequently associated with metabolic syndrome. Among possible pathogenic mechanisms, adiponectine gene variants may play a role in the development of metabolic complications especially type two diabetes.
Aim of this study was to assess the presence of two single nucleotide polymorphisms of the adiponectin gene (G276T and T45G) in patients with PA and determine their relation to metabolic parameters.
Subjects and methods
We studied 51 patients with PA and 37 controls with essential hypertension. Twenty three patients had aldosterone producing adenoma (APA) and 28 idiopathic hyperaldosteronism (IHA). Genotypes of the adiponectin gene were determined at positions 45 (exon two) and 276 (intron two) by PCR. BMI, plasma glucose and lipids were measured by routine methods.
Results
The prevalence of G allele of T45G polymorphism in PA was slightly but not significantly higher in comparison to EH (27% vs. 13.7%). Carriers of G allele had significantly higher plasma cholesterol ($P < 0.05$), LDL cholesterol ($P < 0.05$) and slightly but not significantly lower HDL cholesterol. There was no significant difference in the prevalence of G allele between APA and IHA. The prevalence of TT/GT genotypes of the G276T polymorphism was significantly higher in PA as comared with hypertensive controls (54.9% vs. 26%, $P < 0.05$). However patients with T allele did not differ in metabolic parameters from those with G allele only.
Conclusion
Patients with PA have significantly higher prevalence of T allele of the G276T polymorphism than controls with EH. However the presence of T allele was not related to metabolic parameters. On the other side the presence of G allele of the T45G adiponectin gene polymorphism may confer a risk of worse lipid profile in these patients.
Declaration of interest
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## P89

**Primary aldosteronism associated with subclinical Cushing syndrome**

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Introduction
It has been reported that primary aldosteronism(PA) is often associated with subclinical Cushing syndrome (SCS). In the present study, we investigated the frequency of SCS in subjects with PA.
Method
Subjects included consecutive patients ($n=43$) who were diagnosed as PA between 2003 and 2011 in our institute. They were screened by plasma renin activity (PRA (ng/ml/h)) plasma aldosterone concentration (PAC (pg/mL)) ratio (ARR) above 200. The endocrinological examinations such as furosemide posture test, captopril suppression test and low-dose (1 mg) and high-dose (8 mg) dexamethasone suppression test (DST), were performed. The cases of possible diagnosis of PA underwent adrenal vein sampling (AVS) and the laparoscopic adrenalectomy. The diagnosis of PA was confirmed by the pathological findings.
Result
In 43 subjects who were diagnosed as PA, five (11.6%) showed no suppression in low-dose DST, and four of them were also positive in high-dose DST, indicating the presence of SCS, which was confirmed by pathological examination. One case was associated with overt Cushing syndrome (CS). In all of the five cases 24 h urinary cortisol secretion was abnormally high. However, four of five cases showed normal serum cortisol at morning, and two of five cases maintained normal circadian cortisol variations. Postoperatively, three cases needed replacement therapy of hydrocortisone, while no subjects without SCS needed it.
Conclusion
PA is frequently associated with SCS with prevalence of more than 10% in our study. Since it requires postoperative hydrocortisone replacement therapy, our data suggest the importance of routine screening of SCS with DST in PA cases.
Declaration of interest
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## P90

**Primary aldosteronism during 2007–2011 in Iceland**

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Introduction
Recent publications regarding primary aldosteronism (PA) have challenged the concept of PA being a rare disease. In 2007 a standardized protocol for diagnosing and treating PA was introduced in Iceland for patients suspected of having PA. Data from the Icelandic Heart Association suggests that 35–40% of the adult population (age 46–67) have hypertension. The aim of this study was to gather information about PA as a cause of hypertension at the referral center for endocrine hypertension in Iceland.

Methods
A retrospective chart review was performed of all patients (age 18 and older) diagnosed with PA during 2007–2011 at the Landspitali University Hospital in Iceland, a referral center for the whole country (population of 318.000). All patients were diagnosed using the same standardized methods. Patients were taken off interfering medications 4–6 weeks prior. Screening was considered positive if patients had an increased morning -aldosterone and decreased renin levels and/or an increased 24 h urinary aldosterone secretion. Salt loading and positional tests were used to verify the diagnosis. All patients with verified PA were further examined with a CT scan and adrenal venous sampling (AVS). When AVS indicated unilateral disease, patients were offered a laparoscopic adrenalectomy. Patients with bilateral disease were given specified pharmacological treatment options.
Results
Thirty-three patients were diagnosed with PA, 16 patients had bilateral disease and 16 patients unilateral. The results from AVS in one patient is pending. All 16 patients with unilateral disease had an adrenalectomy, 11 of which had a cortical adenoma and four had adrenal hyperplasia. In one patient the PAD was inconclusive.
Conclusion
This study indicates that PA is an important cause of hypertension in Iceland, and emphasizes the importance of detecting curable causes of hypertension. Interestingly, unilateral hyperplasia was ¼ of unilateral PA.
Declaration of interest
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## P91

### Low SLC26A2 expression in adrenal cells is associated with high aldosterone output

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A genome-wide association study indicated a correlation between a locus at 5q32 and high aldosterone to renin ratio in subjects from KORA F4 survey. As we considered the genes in this locus for their potential relevance for the etiology of primary aldosteronism, we observed significantly higher expression of one of them, SLC26A2, in mouse adrenal glands, even though the gene's function in the adrenal was not reported in the literature. Using the human adrenocortical cell line NCI H295r and wt mice, effects of aldosterone regulators on SLC26A2 gene expression were evaluated. Consistently, angiotensin II treatment resulted in a decrease in SLC26A2 gene expression *in vitro* and *in vivo*, while potassium resulted in lower expression levels only in the *in vivo* setting. Interestingly, a shRNA-mediated four-fold knockdown of SLC26A2 in H295r cells yielded a significant increase in aldosterone synthase (CYP11B2) expression and aldosterone output of the cells; this effect of knockdown was sustained after incubation with aldosterone stimulators including potassium, angiotensin or forskolin. An increase in the expression of calcium dependent regulators of CYP11B2 expression including CamK1, NR4A1 and NR4A2 was observed. In order to gain insight into *in vivo* functional characteristics of the gene, we analyzed gene expression profiles in adrenal glands from SLC26A2 knock-out mice. These results suggested a tendency towards higher expression for adrenal enzymes with more specific role in aldosterone synthesis and expression in the zona glomerulosa (CYP11B2, HSD3B6), and a tendency towards lower expression for enzymes with a more unspecified expression pattern (STAR, CYP11A1) or a more fasciculata specific distribution (HSD3B1). Further detailed investigation into the functional link between adrenal SLC26A2 expression and aldosterone production is ongoing.
Declaration of interest
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## P92

### Three-dimensional contrast-enhanced sonography in the diagnosis of incidentally discovered asymptomatic adrenal masses

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Background
Due to improvements in diagnosis capabilities, there has been a recent increase in adrenal tumors, that are incidentally discovered in patients, who underwent examination by sonography (S), computed tomography (CT) and magnetic resonance imaging (MRI) The aim of this study was to define the usefulness of contrast-enhanced three-dimensional sonography in the diagnosis of nonfunctioning adrenal incidentalomas.
Patients and methods
Sixty-one patients with nonfunctioning adrenal adenomas, 51 women and 10 men, aged 42–78 years ($61 \pm 8$) were subjected to analysis. All were earlier diagnosed by sonography and CT or MRI. All received detailed laboratory testing including hormones profile. Examination with ultrasonography were performed by a single physisians throughout the study. The patients were examined by Acuson Antares (Siemens) with convex transducer of 2.5 MHz, including Duplex, Doppler and three-dimensional sonography followed by a three-dimensional sonography with the Sonovue contrast agent. Shape, structure, size and vascularization of adrenals were assessed according to the RECIST protocol.
Results
After administration of contrast, increase of the size, without the visualization of vascularization within the same tumor, was found in $\sim$ 90% of adrenal incidentalomas.
Conclusions
Contrast-enhanced three-dimensional sonography may be a useful method in the diagnosis of adrenal incidentalomas. It may be a valuable procedure in detecting and follow up of the nonfunctioning adrenal tumors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P93

### Evaluation of uni- and bilateral adrenal incidentalomas with (131I) 6β-iodomethyl-norcholesterol scintigraphy.

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The growing incidence of adrenal incidentaloma needs new methods of assessment of their function, especially for cases with subclinical hyperfunction. The bilateral adrenal incidentalomas in patients with subclinical Cushing syndrome (SCS) often need only unilateral surgery, but if their diameters and phenotypes are similar, coming to decision about side of adrenalectomy may be difficult. Another problem generate tumors suspected of subclinical aldosterone hypersecretion: the adrenal venous sampling is an invasive method and patients not always agree to it. Border values of cortisolaemia in dexametasone suppression test may also lead to hesitation about usefulness of adrenalectomy. Iodomethyl-norcholesterol is a highly specific tracer for the functional characterization of the adrenal cortex and scintigraphy with it can be a useful additional tool for the treatment decisions in the cases described above.
We present series of Iodomethyl-norcholesterol scintigraphy performed in 25 patients. The indications for scintigraphy were: subclinical hypercortisolaemia in patients with bilateral adrenal tumors (18 cases), primary hyperaldosteronism (three cases) and uncertainty in assessment of cortex function (border results) (four cases). We used two different protocols, depending of assessed adrenal function (without dexametasone if were no doubt about SCS).
Scintigraphy confirmed unilateral adrenal hyperfunction in 12 of 22 patients with suspicion of SCS. In all operated patients in this subgroup, transient postoperative secondary insufficiency of the second adrenal gland was observed, confirming the

diagnosis of SCS and proper choice of operating side. The scintigraphy in three patients with primary hyperaldosteronism confirmed the source of disorder. Two of these patients have already been operated with clinical and biochemical benefits.
Our observations indicated that adrenal scintigraphy may be the valuable method for adrenal tumor function assessment, which helps in decision about surgery in cases of subclinical adrenal hyperfunction and bilateral adrenal lesions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P94

**The confirmatory Tests of primary aldosteronism should be selected in each countries - the analysis of 83 patients in our hospital**

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It has been well known the primary aldosteronism (PA) is the most common endocrine hypertension, and it is accounted approximately 10% of all hypertensive patients. It is always challenging for physicians which tests we should select for the confirmatory tests for the patients with aldosterone-renin retio (ARR) positive. We analyzed our 83 PA patients for the sensitivity of each confirmatory test.
We screened those PA suspicious patients by ARR and we considered ARR $\geqq 20$ as positive. We, then, performed the confirmatory tests, such as rapid ACTH stimulating, captopril challenge, upright plus furosemide, and saline infusion tests. When any of those confirmatory tests were positive, the patients undergo an adrenal CT scan. When surgical treatment was practicable and desired, the distinction between unilateral (UHA) and bilateral hyper aldosteronism (BHA) was made by the ACTH-loading adrenal venous sampling (AVS).
The mean age was $55.8 \pm 13.4$ years old including 33 males and 50 females. The sensitivity of those four confirmatory tests among 65 AVS performed cases were 91.5%, 52.6%, 78.8%, and 29.6%, respectively, and the rapid ACTH stimulating test showed the highest sensitivity in UHA. Fifty-five out of 65 AVS performed cases showed plasma aldosterone concentration $\geqq 1{,}400$ ng/dl in either (46.2%) or both (46.2%) side, and 18 cases showed the ratio of PAC/cortisol in higher side to one in lower side $>2.6$, which we consider the laterality positive and performed laparoscopic adrenalectomy.
The reason of the sensitivity of saline infusion test is low may be that our daily salt consumption is higher than most countries and this may push down the sensitivity.
In conclusion, our results confirm the usefulness of the confirmatory tests, and revealed the sensitivity of rapid ACTH test was high and saline infusion test was low. This indicate that each country should confirm which confirmatory tests are useful under its' food culture.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P95

**ACTH stimulation improves the sensitivity of aldosterone to rennin ratio (ARR) for detecting inappropriate aldosterone secretion**

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Background
Aldosterone to renin ratio (ARR) is the gold standard for screening of primary aldosteronism (PA). However, the lower value of ARR cannot rule out the possibility of PA. In this study, we evaluated the change of plasma aldosterone concentration (PAC) in ACTH stimulation among hypertensive patients and explored whether it improved the sensitivity of ARR for detecting PA.
Method
Eighty-five patients with hypertension with plasma renin activity (PRA) $<$ 1.1 ng/ml/hr and PAC $>$ 50 pg/ml were enrolled for the ACTH stimulation test. The stimulation was performed by intravenous injection of 250 μg of ACTH to the patients after 30 min rest in the spine position. The blood sample was collected at 0, 30 and 60 min after the injection. Thirty-seven patients were suspected of PA by ARR $>$ 200 or PAC at 30 or 60 min after ACTH stimulation $>$ 240 pg/ml or having adrenal adenomas. The rest of 48 patients were classified into the non-PA group without confirmation (group A). PA suspected group was followed by confirmatory tests according to the Japanese guideline of PA diagnosis in 2009. Eight patients were excluded and re-categorized into the non-PA group with confirmation (group B) and 29 patients were classified into the PA group (group C).
Results
ΔPAC30 and ΔPAC60 were significantly higher in the group C ($5.6 \pm 2.9$, $3.2 \pm 1.6$) than in the group A ($2.2 \pm 1.0$, $1.1 \pm 0.5$) and group B ($3.5 \pm 1.3$, $2.0 \pm 0.9$) ($P < 0.05$). The ROC curve analysis demonstrated ARR0 of 233 (AUC = 0.92, sensitivity = 83%, specificity = 98%), ARR30 of 392 (AUC = 0.94, sensitivity = 93%, specificity = 89%) and ARR60 of 387 (AUC = 0.96, sensitivity = 93%, specificity = 91%) as the optimum cut-off points.
Conclusion
These results indicated that ACTH amplified inappropriate aldosterone secretion. ARR after ACTH stimulation is more sensitive for detecting PA than that without the stimulation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P96

**Diagnostics of primary aldosteronism: is obligatory use of confirmatory tests justified?**

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Introduction
Assessment of the RAAS has been recently granted a much greater role in the evaluation of patients with arterial hypertension (AH). There is no single test efficient in selection of patients for second-step etiological investigation.
Methods
198 consecutive patients- 119 women (60%) and 79 men (40%)- hospitalized in years 2009-2011 at the Clinical Department of Endocrinology UMB as to diagnose PA. In each patient PRA and PAC (basic and after 2l NaCl infusion) were evaluated.
Results
The percentage of patients with PAC $\geq 15$ ng/ml was 53% and percentage of patients with PRA $\leq 0.1$ ng/ml per h was 20%. The percentage of patients screened for PA in which ARR exceeded consecutive cut-offs of 20, 30, 40 and 50 were respectively 57, 45, 34 and 29%. Among 15 patients in which PAC after infusion of two liters of saline was $\geq 6.5$ ng/dl (8.6%) in 13 (6.6%) PA was diagnosed.
Conclusion
obligatory use of tests confirming autonomy of aldosterone secretion in patients screened for PA seems cost-effective in limiting number of patients for further diagnosing.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P97

**Diagnosis of Cushing's syndrome by automatic face classification using frontal and side-view photographs**

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Background
Cushing's syndrome is a disease that presents with clear symptoms and causes considerable harm to the body if left untreated, yet often remains undiagnosed for prolonged periods of time. Face-classification software might recognize typical changes of the face and thus aid in diagnosing the disease early as we have previously shown in the classification of acromegaly.
Methods
Using a regular compact digital camera, we took frontal and side-view pictures of 21 female patients with Cushing's syndrome (14 endogenous, seven iatrogenic) and of 42 age- and sex-matched controls (2:1 matching).
Nodes were then placed on disease-relevant structures of the face to analyze the pictures using computerized similarity analysis based on Gabor-jets and geometry functions. The leave-one-out cross-validation method was employed to classify subjects by the software.
Results
Using the same combination of Gabor-jets and geometry functions as in our previous publication, 80.1% of patients and 97.6% of controls were correctly classified by the software. This resulted in a total classification accuracy of 92.1%. If only frontal views and only one control person for each patient was included, the classification accuracy was 85.7 and 66.7% in patients and controls, respectively.
Conclusions
In this preliminary analysis we found a good classification accuracy of Cushing's syndrome by face-classification software. By employing 2:1 matching and implementing side-view pictures into the classification process, we could substantially improve classification accuracy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P98

**Cushing disease remission is independent of size tumor. twenty years experience in surgical treatment a Mexican Center**

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Background
Cushing disease CD is a rare disorder, caused by an (ACTH) producing tumor. Transsphenoidal pituitary surgery (TSS) remains the treatment of choice for CD. Fewer information is available on the long-term outcome in the group of patients harboring ACTH-secreting adenoma. The aims of this study were to analyze our 20-year experience with surgical treatment in Cushing's disease and to examine whether remission rate is related with size tumors.
Methods
Forty four consecutive patients with proven Cushing's disease who under went to surgical treatment in our center between 1990 and 2010 were analyzed. The diagnosis was made using standard endocrinological criteria. Remission was defined with serum cortisol suppression on oral 1-mg dexamethasone overnight suppression test.
Results
Overall, 41/44 of the patients (80%) were female. The average age was 26.36 ± 7.8. Average urinary cortisol was 566 ± 462.42 μg/24 h and ACTH 61 ± 74 pg/ ml. Microadenoma were present in 32 patients (72%) and macroadenoma in 12 (27%). Total remission was achieved in 34% of the subjects. (11 microadenomas and 3 macro adenomas). In the follow up of the remission patients there were a relapse in three patients. Mean follow up was 43.5 ± 42.28 months in patients with out remission, and 67 ± 59 months in patients with remission. The size of the tumor was not statistically different in the patients with remission (7.3 ± 4.3 mm) compared to the patient with out remission (9.1 ± 7.2 mm) ($P = 0.50$).
Conclusion
The remission confirmed with serum cortisol suppression on oral 1- mg dexamethasone overnight suppression test do not depends on tumor size.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P99

**Cushing's disease – limitations and boundaries**

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Introduction
Cushing's disease is the most frequent cause of hypercortisolism. Although the classical form is easy to diagnose, nonspecific features raise differential diagnosis problems. Severe forms are associated with high mortality, but subclinical hypercortisolism also has significant morbidity.
Patients and methods
Retrospective study of Cushing's disease evolution in 14 patients, diagnosed between 2000 and 2011 (11 women, 3 men, age at diagnostic 22–48 years).
Results
Clinical: 42.86% patients were overweight and 42.86% obese; 78.58% hypertensive; 92.85% had purple striae, 71.42% muscle weakness and 70% of women secondary amenorrhea. All patients had hypercortisolism (384.81 ng/ml ± 140.04), disturbed nictemeral rhythm and high ACTH (311.61 pg/ml ± 422.45). Diagnosis was supported by inhibition and/or stimulation tests. Over 1/3 had metabolic syndrome and three cases had electrolyte impairment. Pituitary adenoma was identified in nine cases (64.28%, two macroadenomas and seven microadenomas). Only four patients had pituitary adenomectomy (one with complete remission), eight bilateral adrenalectomy, followed in four cases by pituitary radiotherapy, one patient, newly diagnosed, had not yet been treated and one was lost of the view. Steroidogenesis inhibitors were administrated in five patients (preoperative or recurrence) and dopamine receptor analogues in three cases. Therefore, half of patients are in remission, 28.58% relapsed and 21.42% in evolution (recent diagnosed).
Discussions
Although the first line therapy in Cushing's disease is pituitary surgery, it is often difficult to perform it. Given the unpredictable evolution of the disease, combined treatment is often necessary, with better outcomes, but many patients relapse after successful initial treatment (10–20% in specialized centers, > 1/4 in our series).
Declaration of interest
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## P100

**Exogenous corticosteroids: guilty or innocent?**

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Introduction
The association between autoimmune diseases, including systemic lupus erythematosus (SLE), and endogenous hypercortisolism is rare. The latter is usually misinterpreted as iatrogenic in the case of patients taking exogenous systemic corticosteroid therapy. The excess endogenous glucocorticoids may play an important role in suppressing autoimmune activity. Similarly, the abrupt resolution of endogenous hypercortisolism may lead to a rebound worsening of autoimmunity.
Case report
Twenty-six year-old female, with SLE with renal involvement, diagnosed at the age of 15, since then on systemic corticosteroid therapy. In the past 2 years on

prednisolone 5 mg on alternate days. Hypertension and osteopenia diagnosed in the previous year. Weight gain (6 kg), striae rubrae on the breast and thighs, moon facies, buffalo hump, hair loss, easy bruising, irritability and insomnia were noticed in the previous 4 months. A month after corticosteroid withdrawal, elevated urinary free cortisol [512.9 μg/day (*n*:36–137)] and serum midnight cortisol [40.9 μ/dl ($n$ <7.5)], positive low-dose dexamethasone suppression test [final 8 am cortisol 23.7 μg/dl ($n$ <1.8)] and suppressed ACTH (<1.0 pg /ml) were found. Diagnosis of ACTH independent Cushing's syndrome was made. Abdominal computed tomography showed a 24×24 mm left adrenal nodule with spontaneous density greater than 35 Hounsfield Units. She underwent left adrenalectomy. Pathological analysis of the surgical specimen confirmed an adrenal cortex adenoma. Patient is currently on prednisolone 10 mg id, with appropriate control of renal disease and regression of signs and symptoms of hypercortisolism.

Discussion

The patient's endogenous hypercortisolism has contributed to control her autoimmune disease with low dose exogenous corticotherapy. Abrupt onset of signs and symptoms of hypercortisolism in patients on stable dose of exogenous corticosteroids for a long period of time should raise the possibility of endogenous hypercortisolism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P101

**Pediatric Cushing disease: case report**

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Cushing disease is defined by signs and simptoms of hypercortisolism due to augmented ACTH secretion from pituitary origin. It is very rare in pediatric age, with general incidence around 13 cases/1 000 000 persons/year.

Case description

S.G.N, male, 14.5 years old, was brought for medical attention due to weight gain and weakness in the last 4 months. At physical examination, he presented with moon face, cervical acanthosis nigricans, hypertrichosis and acne. Blood pression was 155×110 mmHg.

Initial laboratory profile: glicemia 85 mg/dl, insulin 20.1 mUI/l, total cholesterol 242 mg/dl, HDL 93 mg/dl, LDL 121 mg/dl, tryglicerides 138 mg/dl, cortisol 55.02 μg/dl, 17OHP 4.27 ng/dl, androstenedione 61.40 ng/dl, DHEA 3.4 μg/dl, basal cortisol 57.7 μg/dl, after dexamethasone (8 mg) 32.8 μg/dl.

Petrous sinus catheterism; ACTH test with desmopressin - right petrous sinus: 0′: 110 pg/ml 3′: 124 pg/ml 5′: 119 pg/ml 10′:112 pg/ml. Left petrous sinus: 0′: 813 pg/ml, 3′: 1158 pg/ml, 5′: 834 pg/ml, 10′: 1148 pg/ml. Peripheral blood samples: 0′: 98.4 pg/ml 3′: 99.2 pg/ml 5′: 86.4 pg/ml 10′: 92.6 pg/ml.

Image: abdome and pelvic tomography; adrenal bilateral hyperplasia and a small granuloma found in the left lung. Thorax tomography; thickening of pleura, with atelectasic areas in both lungs. Nuclear magnetic ressonance of sela turcica: slightly hyperplastic hypophysis, without adenomas.

Evolution: treated with ketoconazole, presented with diabetes mellitus, hypertension of difficult control and depression. When the surgical procedure was performed (partial hypophysectomy) medications were gradually suspended. Nowadays, under ambulatory follow-up, displays normal glicemic and arterial pressure values. Interestingly, developed diabetes insipidus, and is taking DDAVP and prednisone.

Conclusions

This case illustrates the importance of physical examination, since Cushing disease is a rare condition, with serious complications and potentially fatal If not adequately treated. The diagnostic and treatment procedures, including central ACTH test, image exams and neurosurgical procedures must be performed in tertiary centers, where these patients must be referred to as soon as possible.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P102

**Proteomic approach to human adrenocortical cancer biology**

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Adrenocortical carcinoma (ACC) is a rare tumor with poor prognosis due to its highly malignant phenotype and lack of effective treatment options.

The etiology of ACC is still unknown. To better understand the pathogenesis of this type of tumor and to determine differentially abundant proteins and candidate biomarkers for adrenal cancer diagnosis and prognosis, we used a novel approach based on two-dimensional differential gel electrophoresis (2D-DIGE). Protein samples were isolated from 10 ACC and 11 normal adrenal tissues (NOR) and subjected to 2D-DIGE analysis. Minimal fluorescent dye labeling was applied and electrophoresis performed with triple samples (normal and tumor; internal control) for each gel. After excision from preparative gels, sixty spots were identified by mass spectrometry. Most of the corresponding proteins associated with spots was significantly up-regulated in tumor compared with normal tissue (fold increase: 2–9.7; cut-off: 1.5). Among them, some were associated with glycolysis and the Warburg effect and others were structural proteins already identified as involved in other tumor types. These data suggest that some of the identified proteins could represent valid diagnostic and prognostic biomarkers also for ACC. Our findings support the application of proteomics not only for elucidating ACC biology but also for its potential transferability to clinics.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P103

**GH deficiency in patients with hypoadrenalism**

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Introduction

The role of glucocorticoids in the regulation of GH secretion is not well defined. Hypercortisolism inhibits GH secretion, but also hypocortisolism is correlated with impaired GH response to dynamic tests. This study was aimed to evaluate the GH reserve after GHRH+arginine test in patients with Addison's disease (AD) and in operated patients with Cushing's disease (CD).

Design

Twelve AD patients (6M, 6F, aged 52±3.4 years, BMI 24.8±0.9 kg/m$^2$, mean± S.E.M.) were studied. Ten out of 18 CD patients (14F, 4M; 45±1.7 years, BMI 29.2±1.7; all underwent pituitary adenomectomy 2-18 years before) had central hypoadrenalism (group a) and 8 had normal cortisol (group b). Patients were investigated 18 h after steroid withdrawal.

Serum GH peak responses to GHRH+arginine <16.5 and <9 ng/ml were considered as partial (p-GHD) and severe (s-GHD) GH deficiency, respectively. IGF1 levels were evaluated as SDS.

Results

Four of 12 AD patients had s-GHD (peak 4.2±1.1 ng/ml) and 3 had p-GHD (peak 10.7±0.3 ng/ml); IGF1 SDS was <−2 in 7 patients. In CD group a, impaired responses to GHRH+arginine were found in 9 cases (8 had s-GHD, peak 4.7± 1.2 ng/ml and 1 had p-GHD); IGF1 SDS was <−2 in 7 patients. In CD group b, three patients showed a s-GHD (peak 2.7±1.1 ng/ml) and one patient a partial deficiency; IGF1 score was <−2 in all patients with s-GHD.

In AD patients, BMI was negatively correlated with GH peak ($P$=0.01), and a positive correlation between cortisol and GH peak ($P$=0.01) was observed in males. No correlation was found between ACTH, cortisol and GH responses in groups a and b.

Conclusions

This study demonstrates a higher prevalence of GHD in hypoadrenalism than in CD patients with normalized cortisol levels and suggests the need of careful assessment of GH secretion in cured CD.

Declaration of interest

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## P104

**The functional assessment and growth rates of adrenal incidentalomas: a single center experience**

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Purpose
The aim of this study was to evaluate the functional status and growth rates of adrenal incidentalomas, diagnosed at our center between 2005 through 2010.
Methods
The computer and archive records of 113 patients, diagnosed as adrenal incidentalomas and followed-up at our center, were retrospectively analysed.
Results
A total of 113 patients (133 tumors, 80 women (%70.8) and 33 men (% 29.2), ranged in age between 27 and 77 years old) were included in the study. All patients were evaluated and followed-up by magnetic resonace imaging (MRI) at 6-month intervals. Hormonal evaluation including urinary metanephrine, normetanephrine, free cortisole and serum cortisole (basal and after low dose dexamethasone supression) in all patients, ACTH (in patients with Cushing's sendrome) and plasma renin activity and aldosteron levels (in patients with hypertension) were performed at baseline and then at 6–12-month intervals. According to MRI results, the tumors were located in the right adrenal in 36 (31.8%), in the left adrenal in 57 (50.4%) and in bilateral adrenals in 20 patients (17.7%). A tru-cut biyopsi was performed in 8 patients with suspected tumor appearance on MRI. The clinical diagnosis of patients were presented in Table 1. All patients were followed-up for $15.6 \pm 15.4$ months (min. 6, max. 60 months). During follow up, none of the patients with non-functional adrenal incidentaloma developed hormonal hyperfunction. However, tumor enlargement was determined in ten patients (%7.5), ($<1$ cm in four tumors (%40), between 1 and 2 cm in three tumors (%30) and $>2$ cm in 3 tumors (%30) respectively.
Conclusion
Tumor enlargement may occur in a substantial number of adrenal incidentalomas during follow-up. Since the mass enlargement may be a sign of malignancy, long-term follow-up of these patients with imaging techniques is essential.

**Table 1**

| Diagnoses | Patients ($n=113$) no (%) |
|---|---|
| Non-functional Adenoma Cushing's syndrome Pheochromocytoma Conn's syndrome Congenital Adrenal Hyperplasia Metastasis Malignancy[a] Primary adrenal tuberculosis Adrenomiyelolipoma | 71 (63) 21 (19) 8 (7) 5 (4) 2 (2) 2 (2) 2 (2) 1 (1) 1 (1) |

[a]One non-hodgkin lymphoma and one primary adrenal carcinoma.

Declaration of interest
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## P105

**Adrenal venous sampling: local experience in Asian population**

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Primary hyperaldosteronism is a common cause of secondary hypertension. Distinguishing between the two subtypes (unilateral aldosterone-producing adenoma and bilateral idiopathic hyperaldosteronism) is crucial for patient's management. Adrenal venous sampling is an important method for evaluation of the condition, but selective successful right adrenal venous sampling is often reported to be a difficult procedure. A retrospective review of 51 cases suffering from primary hyperaldosteronism with adrenal venous sampling done in the year of 2007 to 2010 in our local hospital in Hong Kong was performed. Biochemical assay of both cortisol and aldosterone levels were done in each sample. Cortisol gradient of 3 between the adrenal sample and a peripheral sample was set as a criterion to indicate successful sampling. Successful catheterizations of the right adrenal vein were noted in 40 cases, while 11 cases failed to meet the cortisol gradient and were excluded. Of the cases fulfilling the cortisol gradient criterion, it was observed that the first sample taken was most useful in analysis of aldosterone–cortisol ratio. Five different patterns of the right adrenal vein were previously described. The right adrenal vein patterns of our patients were as follows: 42.5% with central vein leading to stellate or spidery branches, 27.5% with delta pattern, 10% with glandlike pattern, 7.5% with no discernable adrenal vessels, 2.5% with triangular pattern, and 10% showed vasculatures not typical of these five patterns. Central vein leading to stellate or spidery branches is the commonest pattern, which may differ from those in Caucasians. Identification of these patterns during the interventional procedure may give radiologists a high confidence level that the cannulation is successful, facilitating efficient and precise sampling.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P106

**Comparison of four immunoassays for measurement of cortisol levels**

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Background
Measurement of serum cortisol levels can be useful in the diagnosis of conditions of both adrenal insufficiency and excess. Several methods are available for the measurement of the cortisol levels and immunoassays are commonly used in routine laboratories. The aim of our study was therefore to compare four different automated immunoassays for measurement of cortisol in serum.
Methods
Serum samples of seventy two healthy volunteers were used for this comparison of methods. Levels of cortisol were measured with the Architect (Abbott diagnostics), Cobas e411 (Roche diagnostics) DxI (Beckman Coulter) and Liaison (DiaSorin) automated immunoassays.
Results
The four immunoassays were significantly correlated among each other ($P<0.0001$). The mean concentrations (range) were: 326 nM (185–811) for the Architect, 422 nM (200–1142) for the Cobas e411, 343 nM (176–777) for the DxI and 251 nM (113–458 nM) for the Liaison. The mean differences observed with the Bland and Altman plots were $-$ 102 nM (Architect - Cobas e411), $-16$ nM (Architect - DxI), 91 nM (Architect - Liaison), 86 nM (Cobas e411 - DxI), 396 nM (Cobas e411 - Liaison) and 113 nM (DxI - Liaison).
Conclusions
Automated immunoassays for the measurement of cortisol levels are significantly correlated. However, the results are not commutable between the assays and more standardization is still required for cortisol measurement.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P107

**Tertiary adrenal insufficiency: case report**

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Introduction
Exogenous Cushing's syndrome is a challenging diagnosis in non-compliant patients or in cases with difficult anamnesis, while the access to self medication is relatively open.
Aim
We present a young female case presenting with tertiary adrenal insufficiency.
Case report
S.C., 40 years old has progressively weight gain of 30 kilos since the last year. The Body Mass Index is 40 kg/m$^2$, with androgen redistribution. The personal and familial medical history is negative. She accuses asthenia, diffuse muscle pain. She presents facial plethora, diffuse hyperemia over the body, multiple red stria at the level of the arm, abdomen, breasts, legs. The menses stopped for several months. She associates high arterial blood pressure, retinal hypertensive angiopathy, dyslipidemia, hyperuricemia, and high levels of the hepatic enzymes. The serum potassium is 3.9 mmol/l. The plasma cortisol is low: 0.11 μg/dl

(normal 11–22). The 24 h free cortisol is 5.04 μg/24 h (normal 30–330). The plasma ACTH is 1 pg/ml (normal 6–65). The computed tomography shows a pituitary microadenoma of 5 by 4.7 mm, that we consider an incidentaloma, and normal adrenals. Central DXA reveales normal bone mineral density for patient's age. An exogenous Cushing's syndrome was suspected, and the anamnesis was resumed. The patient denied any ingestion of some medication, but she did affirm that she was using topic glucocorticoid (Dermovate) for all over her skin for more than a year, recommended at some point by her dermatologist for "dry skin". She no longer used the unguent. Clinical symptoms of adrenal insufficiency were seen. We recommended prednisone 2.5 mg/day for 2 months, then only in case of hypotension or infections, fever. The clinical phenotype persisted for several months with a mild improvement.

Conclusions

In some cases of Cushing's syndrome, the anamnesis is the main clue of diagnosis. The chronic topic use of glucocorticoids is also important in inducing the Cushing's phenotype and the stop of their use might induce tertiary adrenal insufficiency.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P108

### A case of HIV presenting as primary adrenal lymphoma

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Introduction

Non-Hodgkin's lymphoma is an AIDS defining malignancy. However, a primary adrenal lymphoma as a presenting feature of HIV infection has not been previously reported.

Case Report

A 66-year-old male presented with a short history of severe fatigue, weight loss, dizziness and nausea. Baseline blood results revealed normal biochemistry with mild normocytic anaemia, neutropaenia with lymphopaenia and the short synacthen test demonstrated intact HPA axis (baseline cortisol 348 nmol/l, 30 min 607 nmol/l, 60 min 632 nmol/l). Upper and lower GI endoscopies as well as CT abdomen showed no significant pathology.

Three months later he presented acutely with extreme fatigue, vomiting, hypotension and hyponatraemia of 123 mmol/l. His short synacthen test was repeated which demonstrated sub-optimal response with baseline cortisol 298 nmol/l, 30 min 353 nmol/l and 60 min 439 nmol/l. Steroid replacement therapy resulted in significant clinical improvement. The CT abdomen was repeated which revealed bilaterally enlarged, well defined homogenous adrenal masses measuring 6 cm each, which were non-secretory on endocrine testing.

Three weeks later, a pre- biopsy imaging showed 70% reduction in adrenal volume, possibly due to steroid therapy. Laparoscopic right adrenelectomy and biopsy confirmed diffuse large B-cell lymphoma. Further investigations confirmed the patient was HIV positive with the CD4 count of 1 per microlitre and the clinical course was complicated by CMV retinitis. Despite the chemotherapy with incorporated HAART, the course of the disease has been complicated by the subsequent development of cerebral lymphoma.

Conclusion

HIV-associated lymphoma is most commonly diagnosed in patients with advanced HIV, a low CD4 count (often $<100/\mu l$), high HIV viral load, and a prior diagnosis of AIDS.

We present this unusual case of primary adrenal lymphoma presenting with adrenal insufficiency which posed a diagnostic challenge.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P109

### Ketoconazole in surgery preparation and follow-up of Cushing's disease - our experience

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Cushing's disease is the main cause of endogenous hypercortisolism. Comorbidities increase morbidity and mortality and the treatment is fundamentally surgical. Patients may use drugs to lower hypercortisolism as pre-surgical preparation and post-surgical treatment when cure is not reached.

Objective

Evaluate the benefits and collateral effects of ketoconazole in Cushing's disease.

Methods

In 22 patients with Cushing's disease with mean age of 45.36 years, pre and post-surgical, we compared the group that used ketoconazole ($n=15$) with another that did not use it ($n=07$). Diabetes mellitus control, blood pressure, hyperlipemia, body mass index, plasma levels of potassium and basal cortisol were analysed.

Results

Plasma cortisol was the only parameter with significant reduction ($P<0.01$). There were no collateral or adverse effects in 63.6% of the patients.

Conclusion

The use of ketoconazole permits significant reduction of serum cortisol levels. In order to better evaluate the impact on the control of hypercortisolism and its comorbidities, a higher number of patients must be evaluated for a longer period of time.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P110

### Utility of CGM for the determination of optimal glucocorticoid replacement method in patients with adrenal insufficiency

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Introduction

Optimal glucocorticoid (GC) replacement in patients with adrenal insufficiency (AI) is desirable for the prevention of long-term side effects of GC. Both hyperglycemia and hypoglycemia are the risk of atherosclerosis. For the determination of optimal GC replacement method in patients with AI, we tested a utility of continuous glucose monitoring (CGM).

Subjects and methods

Three patients with primary or secondary AI were examined. Case 1, 50 yo female with complete AI because of bilateral adrenalectomy due to bilateral adrenal pheochromocytomas; Case 2, 50 yo male who showed asymptomatic or latent AI due to Rathke's pouch as evidenced by relatively poor response to CRH.; Case 3, 64 yo male of partial panhypopituitarism due to the operation of craniopharyngioma. Under the various regime of hydrocortisone (HC) or dexamethasone (Dex) replacement, the frequency of high (over 110 mg/dl) and low (below 70 mg/dl) blood glucose levels were evaluated by CGM.

Results

In the bilaterally adrenalectomized case 1, regime 1, po HC (20, 0, 10, 0 mg) or regime 2, HC (15, 0, 5, 0 mg) + po Dex 0.25 mg before sleep was tested. While midnight hypoglycemia was more frequent in regime 1, the frequency of daily hyperglycemia and midnight hypoglycemia were dramatically reduced in regime 2. In case 2 of latent AI, even 5 mg HC in the morning increased the frequency of hyperglycemia, leading to the cancellation of the medication. In case 3 of partial AI, regime 1 (15, 5, 10, 0), 2 (15, 10, 5, 0), 3 (15, 0, 10, 0), 4 (10, 5, 5, 0) and 5 (10, 0, 5, 0) were tested. No hypoglycemia was observed in any regimen and the lower the dose of HC, the lower the frequency of hyperglycemia.

Conclusion

CGM is useful for perusing the ideal method of GC replacement in patients with AI.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P111

**A case of subclinical Cushing's syndrome who developed pseudo-aldosteronism caused by green juice**

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Seventy six-year-old man was referred to our hospital for examination of hypertension and hypokalemia. His blood pressure was uncontrolled although he was taking amlodipine besilate, spironolacton and candesartan. In addition, he suffered from atrial fibrillation and chronic heart failure. His serum potassium was low (2.8 mmol/l) and bilateral adrenal glands were found swelling. Hence, we investigated him thoroughly on the suspicion for primary aldosteronism.
Unexpectedly, plasma renin activity (PRA, 0.1 ng/ml per h) and aldosterone concentration (PAC, 10.0 pg/ml) were low. On the other hand, diurnal variation of plasma ACTH and cortisol were lost with suppression of ACTH. At overnight fast, plasma levels of ACTH and cortisol were 4.0 pg/ml (7.2–63.3) and 15.5 μg/dl (4.0–18.2) respectively, and administration of 1 mg nor 8 mg dexamethasone neither suppressed serum cortisol levels. Abdominal CT and MRI revealed bilateral adrenal tumor (right 12×10 mm, left 12×5 mm in diameter). $^{131}$I-adosterol scintigraphy showed bilateral high uptake. On the basis of these findings, we considered possibility of subclinical Cushing's syndrome; however, the causes of hypokalemia and hypertension still remained to be explained.
For differential diagnosis of low-renin, low-aldosterone hypertension, we measured serum DOC and urine THE/THF ratio both of which were within normal range. Liddle syndrome was less likely because of the age of onset.
After admission, his blood pressure returned to be normal without any change in medication. Then, he noticed that he had casually drunk a lot of green juice, which was ceased after admission. Green juice is one of the popular vegetable juices in Japan, and contains licorice. Hence, we concluded that he has pseudo-aldosteronism complicated with subclinical Cushing's syndrome. It could be considered that inhibition of 11βHSD type 2 by licorice in the patient with latent excess of cortisol may develop severe hypokalemia and hypertension through mineralocorticoid receptor.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P112

**Adrenal masses as the first demonstration of non endocrine tumors metastatic disease**

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Introduction
Adrenal lesions occur in more than 10% of the general population. Most are benign, but 2.5% are metastatic disease to the adrenal from tumors such melanoma, renal, breast, colon and lung carcinoma. Adrenal metastatic disease is usually asymptomatic and diagnosed in the extension study or the primary tumor. However it is unusual as a presentation of an endocrine tumor or like the first manifestation of metastatic disease.
We present two patients with adrenal macrolesions like the first manifestation of metastatic disease of non endocrine tumors.
Case study n 1
A 63 year old male with a history of hypertension well controlled and occasional smoker presents with sudden pain in right iliac fosse after physical exertion. Abdominal CT evidence a right adrenal mass (9×11 cm) and two left adrenal nodules (2 and 1.5 cm) reason why he is admitted to our service. Besides the chest radiography and chest CT revealed a nodule of 2 cm in right upper lobe lung.
Urinary catecholamines were slightly elevated and right adrenalectomy was performed with the suspicion of a macropheochromocytoma. The pathological study was consistent with metastatic lung adenocarcinoma. Extension study shown left adrenal and mesentery metastases, and chemotherapy was started.
Case study n 2
A 83 year old male was diagnosed with ampullary due to repeated episodes of cholangitis. Abdominal MRI showed adrenal masses of 4 and 5.7 cm. Six months after the diagnosis he was admitted to hospital due to acute adrenal insufficiency (Addison disease caused by neoplastic adrenal infiltration). Adrenal replacement therapy has clearly improved his quality of life but not his survival.
Conclusions
– The adrenal macrolesions in patients without known malignancy should be considered as metastases because of the rarity of endocrine tumors in this location.
– The metastatic adrenal involvement in patients with disseminated neoplasms may produce hypoadrenalism that must be treated in order to improve the quality of life of patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P113

**Usefulness of FDG–PET for detecting primary aldosteronism in patient who have bilateral adrenal incidentaloma**

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Adrenal masses are common incidental radiological findings. It has been estimated that as many as 10–20% of patients with essential hypertension may suffer from undiagnosed primary aldosteronism (PA). In patients who have both adrenal incidentaloma, which the functioning one must be identified. We report a case where an unilateral PA was detected on $^{18}$F-fluorodexoyglucose (FDG)-positron emission tomography (PET), and comfirmed by adrenal venous sampling.
A 53-year-old man was referred to the clinic due to bilateral adrenal masses (right sized 1 cm, left 2.5 cm) on the abdominal computed tomography. The patient was receiving chemotherapy due to a colon cancer postoperatively. Subsequently, whole body FDG–PET was performed, and showed a focal increase of FDG uptake at the right adrenal adenoma. Serum aldosterone-renin ratio was 104, and not suppressed by captopril. As suspection of PA, adrenal venous sampling was performed. It revealed the aldosterone/cortisol ratio was 33 times higher on right adrenal side than left, which consistent with the result of FDG uptake. He was underwent laparascopic mass excision of right adrenal adenoma.
Therefore, we propose that FDG–PET can be useful diagnostic tool for identifying functional adrenal mass instead of adrenal venous sampling.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Adrenal medulla

## P114

**Next generation sequencing is a cost effective and time saving method in clinical genetic screening of patients with pheochromocytomas**

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Background
Pheochromocytomas are rare tumours arising from adrenal medulla. Recent findings show that about 30–40% of pheochromocytomas are caused by germline mutations in one of the ten hereto known susceptibility genes: SDHA, SDHB, SDHC, SDHD, SDHAF2, RET, VHL, NF1, TMEM127 and MAX. This list of genes is constantly growing. These ten genes together consist of 128 exons and a genetic screening test is both extensive time-consuming and expensive. We introduce utilizing Next generation sequencing as a fast and cost effective method.
Methods
DNA was extracted from pheochromocytoma lesions and were subjected to whole genome sequencing utilizing Illumina Hi seq platform, performed at university core facility. Reads from pair-end fragments were mapped (GRCh37) and variation calling was performed using a commercially available software. Identified mutations were verified by automated Sanger sequencing.
Results
We have identified two point mutations, in RET and SDHC, in two different pheochromocytoma lesions, spending less than two hours hands on, in house,

time. Mutations were verified by traditional Sanger sequencing of a single fragment for each gene.
Conclusions
Utilizing next generation sequencing is a fast and cost effective method for clinical genetic screening in pheochromocytomas. Using this workflow, the analysis can be performed without expert skills in bioinformatics.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P115

**Pheochromocytomas: a single centre experience**

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Introduction
Catecholamine-secreting tumors (pheochromocytomas and paragangliomas) are rare intra and extra-adrenal neoplasms, probably occurring in less than 0.2% of patients with hypertension. Serious morbidity and mortality rates are associated with these tumors which are related to the effects of catecholamines on various organs, especially those of the cardiovascular system.
Methods
We reviewed the cases of pheochromocytomas and paragangliomas that were diagnosed, treated and/or followed in our institution since 1983 till 2011. Statistical analyses was made using SPSS 17. Data is presented in frequencies and mean ± standard deviation.
RESULTS
In this review were included 88 patients (58% women; 42% men). The mean age of diagnosis was 49.2 ± 15.2 years and only 6.8% of the patients presented known familial history of the disease. The diagnosis of pheochromocytoma was made in 59% of the patients.
The most frequent form of presentation was as incidentaloma (36.4%) followed by persistent hypertension (27.3%) and other causes (ex: cervical mass: 26.1%). Urinary metanephrines were determined in 51% of the patients (normetanephrine 3042.7 ± 4702.0 μg/24 h; metanephrine 1991.8 ± 3741.0 μg/24 h). An imaging examination was done in 60% of the cases, being a MIBG scintigraphy in 68% of them.
Surgery preparation was made in 45.5% of the patients (34.1% only α blocking; 11.4% α and β blocking) for a mean time of 25.3 ± 44.9 days. Major surgery complications were observed in 8% of the patients, 43% of them didn't perform preoperative preparation according to clinical standards.
Patients were followed for a mean period of 48.5 ± 52.8 months, 10.5% were not cured after surgery, in 4.7% disease recurrence was observed and 29.1% were lost to following.
Discussion
Pheochromocytoma is a rare entity but with serious consequences if not diagnosed or properly managed. Judicious ad eternum follow up is necessary to ensure early detection of recurrences.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P116

**Removal of the dominantly secreting adrenal lateralized by bilateral adrenal venous sampling (BAVS) with glucagon stimulation significantly alleviated hypertension in patients with bilateral adrenal pheochromocytoma**

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Background
The current recommendation on the use of venous sampling is reserved for difficult cases of small pheochromocytoma. Bilateral adrenal venous sampling (BAVS) with glucagon stimulation has been reported to be safe and useful in the early diagnosis of the disease. For bilateral lesions, localization can be challenging but highly important since the removal of the dominant side can markedly improve fatal cardiovascular outcomes as a consequence of chronic hypertension.
Objective
To demonstrate the usefulness of glucagon-stimulated BAVS in determining the dominant adrenal to be removed.
Methodology
This is a cross-sectional study wherein records of patients who underwent BAVS with glucagon stimulation from 1997–2010 were reviewed.
Results
Of the 46 patients who underwent BAVS with glucagon stimulation, 19 were diagnosed with bilateral pheochromocytoma. The mean age at diagnosis was 33 ± 14 years. The mean duration of hypertension was 5 ± 6 years with an average highest systolic blood pressure (BP) of 186 ± 30 mmHg and diastolic BP of 113 ± 18 mmHg. Headache (68%) is the most common symptom followed by paroxysmal hypertension (58%), palpitation (42%), and flushing (37%).Majority were taking three or more anti-hypertensive drugs. On glucagon-stimulated BAVS, 63% had right adrenal dominance. The mean epinephrine and norepinephrine levels on the dominant side were 24,506 ± 30710 pg/ml and 8,642 ± 13,395 pg/ml, respectively. The mean ratio of the dominant versus nondominant adrenal for epinephrine and norepinephrine were 3.62 and 4.13, respectively. Three patients underwent unilateral adrenalectomy. On follow-up, there was marked improvement in BP and reduction of anti-hypertensive medications.
Conclusion
BAVS with glucagon stimulation is a valuable tool in the identification of the dominant adrenal to be removed in patients with bilateral pheochromocytoma to alleviate chronic hypertension.

Follow-up of patients who underwent adrenalectomy of the dominantly secreting adrenal gland

| Case | Duration of Hyperten-sion (years) | Follow-up Period (months) | Systolic BP (mm Hg) | | Diastolic BP (mm Hg) | | Anti-hypertensive Medications (N) | |
|---|---|---|---|---|---|---|---|---|
| | | | Pre-op | Post-op | Pre-op | Post-op | Pre-op | Post-op |
| 1 | 12 | 24 | 180–240 | 130–140 | 100–110 | 70–90 | 5 | 2 |
| 2 | 6 | 36 | 160–200 | 120–150 | 90–100 | 70–90 | 4 | 2 |
| 3 | 1 | 49 | 160–180 | 120–150 | 90–100 | 80–90 | 3 | 2 |

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## P117

**Neuroendocrine markers in biochemical vs pathology diagnosis of pheochromocytoma**

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Pheochromocytomas, tumors derived from adrenal medulla, are characterized by a polymorphic clinical picture dominated by paroxysmal hypertension. Between the clinical, laboratory and pathology data, discordances are frequently seen.
Patients and Methods
Thirty five cases of pheochromocytoma and paraganglioma (10 men) with adrenal tumors and hypertension were confirmed with pheochromocytoma. They were aged 53.68 ± 12.75 years (36–75), while the tumor diameter ranged from 1.5 to 13 cm. Five cases were oligosymptomatic, with only one or two crises in life suggesting catecholamine excess. The evaluation was done for plasma metanephrines and normetanephrines as well as chromogranin A (CGA) with Elisa commercial assays. Immunohistochemistry was performed on removed tumor tissue on paraffin sections by avidin biotin complex method using antibodies against CGA, neuron specific enolase (NSE), synapthophysin (SYN), protein S100, succinate dehydrogenase, as well as Ki67 index as marker of tumor proliferation. The staining intensity was marked from 0 to 3, considering the number of stained cells.
Results
The initial evaluation confirmed excess of plasma metanephrines (MN) (736.11 ± 886.51 pg/ml), normetanephrines (NMN) (2423.26 ± 1907.6 pg/ml) and CGA (790.44 ± 697.85 ng/ml). Immunostaining for CGA was moderate / intense positive in 30/35 cases, in 3 cases the plasma CGA values being in normal range.

Better pathology markers were NSE and SYN, while S100 protein was nonspecificaly high even in oligosymptomatic tumors. Olygosymptomatic patients were with similar plasma values as compared with pheochromocytoma with frequent crises, but SYN and NSE staining was weaker. Ki67 index, around 2–4%, was not related to plasma or pathology data.
Conclusions
From our data, there is no direct correlation between classical neuroendocrine plasma and pathology markers; still, low expression of NSE and SYN might reflect a lower metabolism rate in oligosymptomatic tumors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P118

**Geranylgeraniols are essential for adrenal catecholamine secretion**

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Introduction
Dolichols are isoprenoids, which are synthesized from acetyl-CoA via mevalonic acid, and they are known to be abundant in endocrine system. Adrenal medulla, which is not only an endocrine organ but also an organ derived from neural crest, secrets catecholamines via sodium and subsequent calcium influxes into the cells through nicotinic ACh receptor-operated cation channels and voltage-sensitive calcium channels, respectively, when the medulla is stimulated by acetylcholine (ACh). In this study, therefore, we investigated the role of dolichols and other isoprenoids in ACh-evoked catecholamine secretion from bovine adrenal chromaffin cells.
Results and Discussion
The treatment of the adrenal chromaffin cells with dolichols did not affect the ACh-evoked secretion of catecholamines. However, fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase which is a key enzyme in the isoprenoids' synthesis, attenuated the ACh-evoked catecholamine secretion from the cells. The statin also reduced both thebACh-induced sodium and calcium influxes into the cells. Under the condition, the production of some isoprenoids in the cells is presumed to be very low. Mevalonate or geranylgeraniol, an alcohol type of geranylgeranyl pyrophosphate (GGPP), overcome the statin inhibition of catecholamine secretion, but not other isoprenoids, isopentenyl pyrophosphate, GGPP, geraniol, farnesol, farnesyl pyrophosphate, cholesterol and dolichols. Fluvastatin affected neither the expression of $\alpha$3 and $\beta$4 nicotinic ACh receptors mRNA nor of voltage-sensitive calcium channel mRNA in the chromaffin cells. Two-dimensional electrophoresis showed that there was no difference between the protein quantity in the control and the fluvastatin-treated cells. These results strongly suggest that geranylgeraniols and/or GGPP in the membranes are essential for calcium-dependent catecholamine secretion in adrenal chromaffin cells.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P119

**Neither classical symptoms of phaeochromocytoma nor elevated urinary catecholamines are always seen in patients with histologically verified phaeochromocytoma**

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Background
The symptom triad of 'headaches, palpitations and sweating' along with elevated urinary catecholamine levels are commonly used to diagnose phaeochromocytoma. We wanted to assess how often patients with histologically verified phaeochromocytoma did not have this triad or had normal urinary catecholamine excretion on one occasion or more.
Methods
Retrospective analysis of clinical presentation and urinary catecholamine levels in 75 patients with phaeochromocytoma in Oxford.
Results
Clinical features: Palpitations were the most commonly reported symptom (67%), followed by sweating (66%), hypertension (65%) and headache (63%). 12% did not have any of the classic triad of headache/palpitations/sweating, 8% did not have the classical triad or hypertension. Of these 8% (six patients), five patients were completely asymptomatic and one presented with isolated abdominal pain.
Urinary catecholamines: Pre-operative biochemical urine analysis was available for 51 patients; 44 had all three of normetadrenaline, metadrenaline and 3-methoxytyramine measured.
90% (4/44) had normal urinary catecholamine excretion on one occasion or more. Of these 4 patients, 1 (2% of the cohort) had multiple normal readings (on 6/7 measurements), 1 patient did not have levels repeated. The remaining 91% had at least one elevated urinary catecholamine on every measurement.
71% had one or more occasions where one of the catecholamines was normal, 3-methoxytyramine was most likely to be normal at some point during investigation (59%).
Conclusions
The triad of headaches, palpitation and sweating misses 12% of cases. The inclusion of hypertension to form a tetrad reduces this to 8%.
Normal urinary catecholamines on one occasion are not sufficient to exclude a phaeochromocytoma. If there is a strong clinical suspicion they should be repeated, only 2% had multiple normal readings.
Declaration of interest
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## P120

**The unique case of adrenal malignant non-functioning oncocytic pheochromocytoma**

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Introduction
Pheochromocytoma is a tumour of chromaffin cells of the sympathetic nervous system and its clinical symptoms are associated with excessive production and release of catecholamines.
Case report
We report a rare case of non-functioning malignant adrenal oncocytic pheochromocytoma incidentally found during yearly repeated ultrasound abdomen examination in a 78-year-old man with history of cardiovascular diseases: aortal abdominal aneurysm, ischemic heart disease and some years ago an episode of hypertension. The patient confirmed a polycyclic high density large left adrenal mass. The preoperative problem in the differential diagnosis of this case was to determine its origin (adrenal or renal). The lack of did not have clinical manifestations of pheochromocytoma. Computed tomography study typical symptoms and signs and the low level of catecholamine metabolites (Metanephrine 53.78 ug/24 h: No. 52-341, Normetanephrine 200.14 ug/24 h: No. 88–440, VMA 11.6 umol/24 h: No. 9–36) preoperatively further eliminated some clues for differential diagnosis. The tumor measured $80\times60\times60$ mm was subsequently excised. It was considered to be a malignant lesion because of the size, extracapsular extension and focal necrosis. Histological examination revealed an oncocytic pheochromocytoma. It consisted of large polygonal tumor cells containing eosinophilic granular cytoplasm. The latter were immunohistochemically positive for chromogranin, vimentin and s-100 protein. During three years follow up patient was in general good condition - free of disease. Three years after adreanlectomy he had a relapse and then died because of dissemination.
Conclusion
There are only few papers on cases of adrenal oncocytic pheochromocytoma.
Because of unusual histology and rarity of this type of tumor, we report a case of adrenal oncocytic pheochromocytoma including its' visual, histologic and immunohistochemical features.
The presented case appears to be the first adrenal oncocytic malignant and non-functioning pheochromocytoma. It confirms the complexity of biology and difficulties in diagnosing this type of neoplasm.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P121

**A rare case of adrenocorticotropic hormone-producing Pheochromocytoma**

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Introduction
Cushing's syndrome caused by adrenocorticotropic hormone-producing pheochromocytoma has been rarely reported. In such cases, the high production of ectopic adrenocorticotropic hormone produced by pheochromocytoma results in bilateral adrenocortical hyperplasia and thus in consecutive Cushing's syndrome. We report a case of a 43-year-old patient with a 6 cm right-sided adrenal tumor producing both elevated levels of catecholamines and adrenocorticotropic hormone.
Case
The 43-year-old patient presented with a hypertensive crisis with blood pressure up to 230/160 mmHg, sinus tachycardia and pulmonary oedema. An arterial hypertension was 1 by the patient until the moment of admission. The abdominal ultrasonography and the later computerized tomography showed a 6 cm adrenal tumor with necrotic areas, typical for pheochromocytoma. Urinary catecholamines war extremely increased. The MIBG scan revealed a substantial uptake of the right adrenal tumor without any other pathological findings. The serum and urine cortisol concentrations were normal, but the cortisol was not at all suppressed on the overnight 2 mg and later 8 mg oral dexamethasone suppression test. After proper premedication the right adrenalectomy was performed. By means of histological examination the clinical, laboratory and imaging diagnosis of pheochromocytoma was confirmed. Furthermore, the cytoplasmatic staining revealed a positive reactivity for adrenocorticotropic hormone in about 5% of the cells. The laboratory test results concerning the preclinical Cushing's syndrome normalized after performing the right adrenalectomy. The genetic analysis showed no genetic mutations.
Discussion
Adrenocorticotropic hormone-producing pheochromocytomas are rare entities, which should not be overlooked. When investigating an adrenal adenoma, an overproduction of all specific hormones, including of adrenocorticotropic hormone, should therefore be excluded.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P122

**A Novel SDHC mutation associated with Head-Neck Paraganglioma**

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Germline mutations in the succinate-dehydrogenase subunits A, B, C, and D (SDHA/B/C/D) predispose to development of the phaeochromocytoma-paraganglioma (FPGL) syndromes. SDHC and SDHD are anchoring proteins located in the inner mitochondrial membrane. The SDH complex has two major roles in energy production it is part of the tricarboxic acid cycle catalysing the oxidative dehydrogenation of succinate coupled to the reduction of ubiquinone. It is also the complex II component of the electron transport system. Mutations in SDHC are present in ~4% of Head-Neck paraganglioma (HNPGL) patients. In a paraganglioma cohort more than 80% of patients with SDHC mutations had HNPGL and two had a thoracic FPGL. We now report a 64 years old woman with a cerebral paraganglioma with metastases to the neck. Thirty years ago, the patient was diagnosed with a glomus tumor in the middle ear and received radiotherapy. In 2009, a MR scan revealed a large tumor in the posterior fossa. Histological examination showed an atypical neuroendocrine carcinoma, positive for chromogranin A and synaptophysin. The Ki67 index was 30%. Urine catecholamines, plasma metanephrines and plasma chromogranin A were 5 to 7 times upper normal limits. The patient was treated with neurosurgery and chemotherapy. Due to neck lymph node metastasis, radionuclide therapy is currently under consideration. Analysis of the SDHC gene revealed a 17 basepair germline deletion (c.191_207del17) in exon 4. The deletion causes a frameshift and has not been described before. The patient's son and two brothers were not interested in genetic testing.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P123

**Features at presentation of adrenal pheochromocytomas in castilla la mancha (spain). Is the clinical spectrum changing ?**

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Background
Typical presentation of adrenal pheochromocytomas (PHEOs) is a combination of variable hypertension with paroxysmal symptoms. However, recent improvements in diagnostic imaging techniques and increasing availability of genetic testing have facilitated presymptomatic diagnosis of PHEOs.
Objetive
To analyse the clinical features at presentation of PHEOs in a multicentric study population.
Design and patients
Seven Spanish endocrine centres participated in this study. Medical records of 69 patients who were diagnosed of PHEO between 1991 and 2011 were reviewed.
Results
34 were male and 35 were female. Mean age at diagnosis was 52.11 years (17–78). Typical triad of symptoms was found only in 18.6% of cases. Nine patients (12.9%) were diagnosed during hypertension study. Hypertensive crises during diagnostic or surgical procedures occurred in 10.1% of cases. Thirty two patients (45.7%) were incidentally discovered. 5 cases were diagnosed before 2000, and 27 after this year. These patients were significantly older than patients in whom the diagnosis was suspected on clinical grounds. ($57.6 \pm 12.2$ vs $46.8 \pm 17.3$ years; $P < 0.01$) The mean size of incidental PHEOs were $4.79 \pm 2$ cm, smaller than the mean size of the whole study group PHEOs ($5.35 \pm 3.29$ cm). Seventeen genetic testing were done (seven negative and ten positive: nine MEN2 and one Von Hippel Lindau). Three patients (4,3%) was discovered by this way. Patients with positive genetic study were significantly younger ($32.1 \pm 11.4$ vs $52.1 \pm 16$ years; $P < 0.001$). Six patients (8.7%) had bilateral tumors; four of these were found to have MEN2. Only one patient had metastasic disease at the time of diagnosis.
Conclusions
In our study group, presymptomatic diagnosis were done in a half of the patients with PHEOs. Frecuency of incidentally discovered PHEOs seemed to be increasing over time and familial PHEO was found in a significant proportion of the patients. Every adrenal incidentaloma should be investigated for the presence of PHEO and genetic testing should be considered in patients with a family history, young age, or multifocal, bilateral, extra-adrenal, or malignant tumors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P124

**Incidentally discovered pheochromocytoma (PH)**

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The main concern of endocrinologist exploring an adrenal incidentaloma (AI) is the identification of malignant and/or secretant AI leading to rapid cure by removal of the tumor. PH remains one of the most difficult lesions to diagnose. In this study we analyze PH clinic, morphologic and secretory profile. Among a series of 91 AI recruited between 1987 and 2007 having benefited from an adrenal exploration, 56 have been removed and 18/56 were diagnosed as tumors of medullar origin.

Results
This group is composed of 15 benign and 2 malignant PH and 1 neuroendocrine tumor. Benign PH are found in 7F/8H, 44.2+/−14y. Initial presentation includes HTA in 40%, paroxystic in 30%, adrenergic signs in 20%, DS in 20%, weight loss in 13.3%. Average axis PH=64.4+/−29.7 mm, heterogeneous spontaneous density in 84.6%. Rise of urinary metanephrines (uMN) is noted in 47.6% of PH, 23.8% of cortical AI and 28.6% of extra adrenal AI (sensitivity 52.9% specificity 61.7%) threshold 4times normal rates is more contributory to PH diagnosis. Within this group, PH are sécrétant in 60% with concentration of uMN =3.1+/−3.2 mg/24 h. Iodine 131 MIBG Adrenomedullary Scintigraphy showed a hormonal/isotopic match in 71% (sensitivity 62.5% specificity100%) Malignant PH have been diagnosed in one hypertensive male, AI 200 mm, uMN↑3,02 mg/24 h, no fixation and one female, AI 100 mm, ↑Dopamine.
Conclusion
In this study PH represented 32% of the operated AI and are in more than 80% benign, recruited among large AI with malignant radiological phenotype. 3/5 patients are normotensive. The current uMN assay is not very sensitive, secretory activity is noted in about half cases. Scintigraphy revealed excellent specificity and a hormone-isotopic match in about 3/4. The discordant aspects are related to the relative small size of AI, the significance of intratumoral metabolism or the scarcity of storage granules.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P125

**Pheochromocytoma incidentally discovered after thyroid surgery manifesting dramatic hypertensive crisis induced by metoclopramide**

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Introduction
Metoclopramide is known to exert stimulatory effects on catecholamine secretion, having been used in the past as provocative test in the diagnosis of pheochromocytoma. We describe a patient with an incidentally discovered pheochromocytoma after metoclopramide administration.
Case report
A 64 years-old woman underwent total thyroidectomy for multinodular goiter with 'follicular proliferation Thy 3' at FNAB of the target nodule. The patient was affected by mild not paroxysmal hypertension adequately treated with doxazosine 2 mg/day. About 12 h after surgery she has postoperative nausea treated with 10 mg i.v. metoclopramide. After few minutes she developed a severe hypertensive crisis non-responsive to i.v. furosemide and i.m. clonidine but responsive to 10 mg i.v. phentolamine. Blood samples showed a severe hyperglycemia (>700 mg/dl). The patient was transferred in intensive care unit for pulmonary edema and acute respiratory distress. Hypertensive crisis and hyperglycemia arisen after the administration of metoclopramide were strongly suggestive for pheochromocytoma. The diagnosis was confirmed by urinary catecholamines and metanephrines which resulted exceptionally high (epinephrines 95.8 mcg/day [n.v. 2–22]; norepinephrines 154.3 mcg/day [n.v. 12–86]; metanephrine 4424 mcg/day [n.v. 50–340]; normetanephrine 4288 mcg/day [n.v. 90–445]) while abdominal CT scan showed a 66 mm right adrenal mass. Therefore, patient underwent right adrenalectomy after one week treatment with doxazosine by nasogastric tube and i.v. phentolamine. Adrenal histology confirmed pheochromocytoma, while thyroid histology showed a papillary microcarcinoma. The patient was carefully followed after adrenal surgery by copious hydration: blood pressure normalized in absence of anti-hypertensive drugs. Urinary catecholamines and metanephrines normalized.
Conclusion
The incidentally diagnosis of pheochromocytoma after an hypertensive crisis induces by metoclopramide has been described in Literature. Thus, it is crucial to consider this possibility in presence of a severe hypertensive crisis after the administration of this drug particularly in the post-operative course.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P126

**A case of adrenoleukodystrophy presenting as progressive cerebellar dysfunction**

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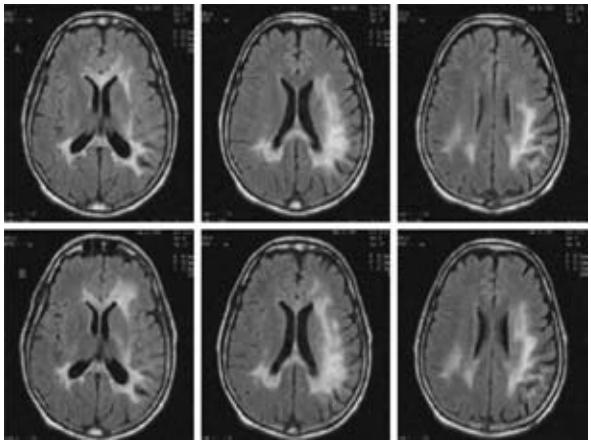
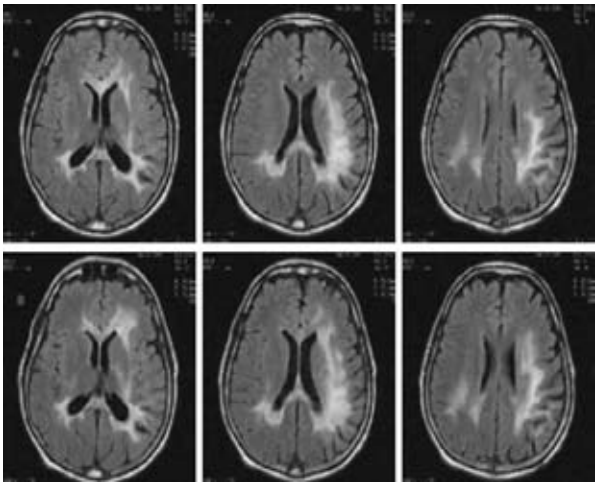
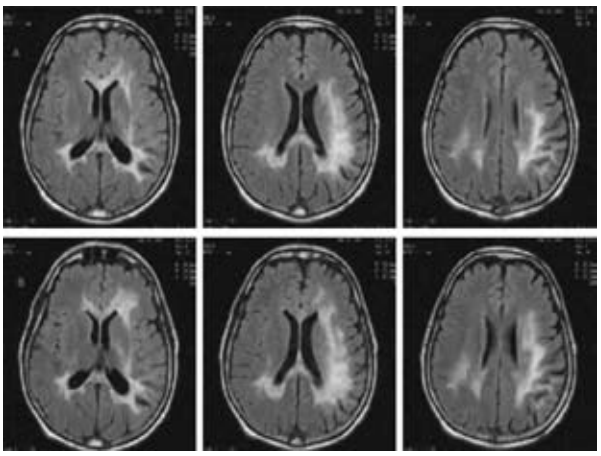
Previously asymptomatic 29-year-old Saudi male patient, presented to with a history of dizziness, abdominal pain and diarrhea for three days.
His morning 800 h serum cortisol was 6.4 μg/dl (normal: 5–25 μg/dl); plasma ACTH was 7579 pg/ml (normal: <46.00). The diagnosis of acute adrenal crisis was made which confirmed later by a defective rise in the cortisol level with ACTH stimulation test.
The patient was admitted to ICU at Medical City in Jeddah, and made a full recovery within 24 h with fluids, hydrocortisone f. Six months post-treatment, the patient presented with complaints f calf muscle pain and weakness, exacerbated by exercise. These symptoms progressively worsened and neurological assessment a spastic diplegia associated with reduced sensation to vibration, light touch and pinprick. The neuropathic pain increased and the patient developed myoclonus. He became wheelchair-bound with an indwelling urinary catheter and subsequently developed features of a dementia. The diagnosis of AMN was confirmed by raised circulating concentrations of very-long-chain fatty acids (VLCFA). Molecular genetic analysis was performed and SSCP analysis of each of the exons of the adrenoleukodystrophy (ALD) gene suggested the presence of a gene mutation at Xq28 in our patient. The treatment of our patient included a comprehensive physiotherapy program for the neurological disturbances and hormone replacement therapy for the adrenal insufficiency. Dietary restriction of VLCFA o Lorenzo's oil were applied in our patient.
Adrenomyeloneuropathy is a rare X-linked inherited disorder of peroxisomes characterized by accumulation of very-long-chain fatty acids.
(VLCFA) in the central and peripheral nervous system, adrenal glands and testes, leading to dysfunction of these organs and systems.







Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding

## P127

**Clinical peculiarities of pheochromocytoma in a MEN2A family**

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Introduction
Pheochromocytoma occurs in 50% of patients with multiple endocrine neoplasia syndrome type 2A (MEN2A). It is characterized by bilateral location, production of large amounts of adrenalin and a benign course, extraadrenal location being rare. Diagnosis is achieved by measuring catecholamines and metanephrines in serum or urine and/or through imaging techniques, including CT, MRI and 123I-MIBG scintigraphy.
Objective
To describe clinical features of pheocromocytomas found in a MEN2A family.
Subjects and methods
Descriptive observational study. Eight MEN2A patients belonging to the same family who carry C634Y mutation at proto-oncogene RET.
Results
Three out of eight subjects developed pheochromocytomas. All of them of bilateral location. Case 1 (index) was diagnosed because of typical spells after the surgery of a thyroid nodule that proved to be a medullary thyroid cancer (MTC). CT evidenced bilateral adrenal masses. Case 2 (case 1's son) was diagnosed in a familial study through a MRI which showed an image suggestive of pheochromocytoma on the right adrenal gland, although metanephrines levels were normal. Five years later, another mass appeared at the left adrenal gland, suggestive of adenoma and with a non-conclusive 123-MIBG image. Epinephrine and metanephrine rose slightly although they did not reach a diagnostic value. Adrenalectomy was performed and anatomic pathology confirmed the diagnosis. Case 3 (case 1's daughter) was also diagnosed in a familial study. MRI evidenced a left adrenal nodule suggestive of adenoma but having a high uptake at 123I-MIBG. Adrenalectomy was executed and anatomic pathology confirmed diagnosis. Three years later MRI showed a right adrenal nodule suggestive of pheochromocytoma. Catecholamines and metanephrines were always moving among the normal limits.
Conclusion
In our family with MEN2A by C634Y mutation, pheochromocytoma showed peculiarities such as absence of catecholamines hyperproduction and bilateral but asynchronous presentation. It requires a continuous and combined follow-up with imaging and laboratory techniques.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P128

**Pheochromocytoma in neurofibromatosis type 1**

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Introduction
Neurofibromatosis type 1 (NF-1) is a relatively frequent syndrome, with an estimated incidence of 1/3000 per year. Patients with NF-1 are at an approximately fourfold higher risk of developing tumors than the general population, most frequently gastrointestinal stromal tumors, central nervous system tumors and endocrine tumors. Pheochromocytoma may occur in about 1% of these patients.
Case report
We report a 56-year-old male presenting with fatigue and chronic headaches, attended at an endocrinology consult for adrenal gland incidentaloma detected on a chest-CT. Previous diseases: hypertension and class I obesity. Family medical history: two sisters with NF-1.
Abdominal-CT showed a 6 cm nodule on the right adrenal gland, with 72 Hounsfield unit density pre-contrast and delayed contrast washout. Laboratory findings were consistent with pheocromocytoma: 24-h urinary fractionated metanephrines 1389 μg/24 h (25–312), 24-h urinary serotonin and 5-hydroxitriptophan 10.9 mg/24 h (0–5), 24-h urinary 5-hydroxyindoleacetic acid 9.74 mg/24 h (2–6). The other biochemical and hormonal tests as well as tumor markers were unremarkable. Several clinical diagnostic criteria of NF-1 were identified: $>6$ café-au-lait macules $>15$ mm in longest diameter, $>2$ neurofibromas, two first-degree relatives with NF-1; genetic testing was also preformed. The screening for additional endocrine and tumor disorders related with NF-1 was negative. The treatment with β-blockers (propranolol, 10 mg, b.i.d.) and alpha-blockers (phenoxybenzamine, 10 mg, b.i.d.) kept the patient asymptomatic. Right suprarenalectomy was successfully preformed and the anatomopathological examination of the surgical sample confirmed the diagnosis of pheochromocitoma.
Conclusion
Pheochromocytoma in genetic disorders is more commonly diagnosed at a younger age in asymptomatic patients, due to premature screening of related tumors. In this case, the lack of clinical follow-up allowed the development of a large tumor, however with benign characteristics and few symptoms, with successful medical and surgical treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Bone & Osteoporosis

## P129

**Unilateral and bilateral adrenal incidentaloma: comparing clinical and biochemical features**

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Introduction
Subclinical hypercortisolism (SH), affecting about 30% of patients with unilateral adrenal incidentaloma (UAI), has been associated to hypertension (HT), type 2 diabetes mellitus (T2DM), dyslipidemia (DL), osteoporosis and vertebral fractures (FX). The prevalence of SH in patients with bilateral adrenal incidentaloma (BAI) seems to be even higher. Data on metabolic and bone complications in these patients are lacking.
Methods/design
We enrolled 175 patients with UAI (116 F; 59 M) and 35 patients with BAI (24 F; 11 M). In all patients we evaluated: BMI, bone mineral density (BMD) by DEXA at spine, total and femoral neck (expressed as *Z*-values: Z-LS, Z-FT, Z-FN respectively), the presence of HT, T2DM, DL and FX (diagnosed using a

semi-quantitative visual assessment). We diagnosed SH in the presence of at least two out of: urinary free cortisol levels (UFC) >70 μg/24 h, serum cortisol levels after 1 mg-dexamethasone test (1 mg-DST) >3.0 μg/dl or ACTH levels <10 pg/ml.
Results
Age, BMI, diameter of adenoma, UFC, 1 mg-DST, ACTH e Z-LS were comparable between the two groups. Total and femoral BMD were significantly lower in patients with BAI when compared to those of UAI (−0.28±0.99 vs Z-FT: 0.25±1.1, $P=0.008$; Z-FN: −0.40±0.88 vs 0.09±1.1, $P=0.01$). The prevalence of SH, HT, T2DM, DL was comparable between groups, while the prevalence of FX was higher in BAI than in UAI (51% vs 28%, $P=0.007$). Logistic regression analysis showed that the presence of FX was independently associated to the presence of BAI (OR 2.7, 95% CI 1.2–6.0; $P=0.02$), age (OR1.07, 95% CI 1.0–1.1; $P=0.001$), and Z-LS (OR 0.7, 95% CI 0.6–0.9; $P=0.01$) regardless for the presence of SH (OR 2.0, 95% CI 0.9–4.1; $P=0.06$).
Conclusion
Patients with BAI show a lower BMD and a higher prevalence of FX than patients with UAI, independently of SH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P130

### Bone mineral density is associated with BsmI vitamin D receptor's polymorphism in adult patients with epilepsy

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Introduction
The BsmI restriction fragment polymorphism of the vitamin D receptor (VDR) has been related with the development of bone disease in postmenopause, homozygous β thalassemia and hyperthyroidism. The present study aimed to evaluate the association between bone metabolism in patients with epilepsy and the BsmI VDR's polymorphism.
Methods/design
This cross-sectional study evaluated 73 adult patients with epilepsy, under antiepileptic drug monotherapy for at least 1 year and with regular menses for women. Bone mineral density of the lumbar spine was measured by dual energy X-ray absorptiometry. Fasting blood samples were obtained for biochemical assessment and genotyping.
Results
Significant differences were observed in levels of BMD according to the VDR's genotype (BMD: Bb genotype 1.056±0.126 g/cm$^2$; BB genotype 1.059 ±0.113 g/cm$^2$; bb genotype 1.179±0.120 g/cm$^2$; $P$-value <0.05). Presence of at least one B allele associated with lower serum levels of 25 hydroxyvitamin D when compared with absence of the B allele (22.61 vs 33.27 ng/ml; $P$-value <0.05). Bone density below the expected range for age (4.1% of subjects) correlated with the presence of the BB genotype ($P$-value =0.031), even after adjusting for the daily drug dosage, duration of therapy, sex, age and BMI. Regression analysis revealed that the presence of at least one unfavorable B allele was a significant predictor of BMD, independently of age, sex, BMI, daily dosage and duration of therapy ($\beta=-0.149$; $P$-value=0.001)
Conclusion
BsmI VDR's polymorphism is significantly associated with BMD in patients with epilepsy. The potential role of this polymorphism in the development of bone disease in patients with epilepsy should be evaluated by larger studies, as it might be helpful to ordinate patients as high risk and low risk, regarding the development of osteoporosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P131

### Vitamin D and estrogen receptors' polymorphisms in postmenopausal women

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Osteoporosis is a polygenic disease. The intensive search for genetic markers has led to the identification of several genetic polymorphisms, associated with the decrease of bone tissue and therefore a higher risk of osteoporosis.
The study aimed to evaluate estrogen receptor alpha (ESR1) and vitamin D receptor (VDR) polymorphisms in postmenopausal women with or without osteoporosis and their correlations with bone markers and adipocytokines.
Subjects and methods
We enrolled 56 postmenopausal women, aged over 60, without treated osteoporosis and no secondary osteoporosis. Subjects were divided into two groups: group 1 – postmenopausal women with osteoporosis (19 subjects), group 2 – postmenopausal women without osteoporosis (37 controls).
Hematological, biochemical profile, bone turnover markers and adipocytokines were assayed. DXA of the spine and the left hip was performed. ESR1 (XbaI and PvuII) and VDR (Bsm I, ApaI, TaqI and FokI) polymorphisms were determined by RFLP method on genomic DNA.
Results were statistically analyzed using SPSS program.
Results
Genotype distribution differed significantly in the osteoporosis group compared to controls for ESR1 PvuII ($P=0.03$) and XbaI ($P=0.04$) polymorphisms. VDR polymorphisms genotype distribution did not significantly differ between the two groups.
We found higher TNFα mean values in bb genotype (4.506±0.92) compared to Bb genotype (1.472±0.53, $P=0.036$) and TT with Tt genotype ($P=0.03$) in group 1.
Higher CRP mean values were present for PP genotype compared to Pp ($P=0.046$) in controls. In controls, VDR BsmI, Bb genotype associated lower $T$ hip scores ($P=0.035$) and lower BMD compared to BB genotype and lower $T$ hip scores ($P=0.035$) where present associated with VDR Taq1, Tt genotype compared to tt genotype.
Conclusion
XbaI and PvuII estrogen receptor α polymorphisms may be involved in the pathogenesis of osteoporosis, while Bsm I and TaqI VDR polymorphisms may influence BMD, bone turnover and the inflammatory state.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P132

### Osteoporosis and vitamin D deficiency in patients with lipid storage disease

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Introduction
Bone metabolism is impaired in several lipid storage diseases. At now no data are available in Fabry disease (FD), a genetic lipid storage disease characterized by systemic accumulation of glicolipids. The aim of this study was to evaluate bone metabolism and calcium/vitamin-D pathway in patients with FD.
Methods/design
Study population included 15 FD patients (8 M and 7 F, age 28–59 years) and 15 sex-, age- and BMI-matched normal subjects. Measurements of serum concentrations of calcium, phosphorus, PTH, 25-hydroxy-vitamin-D and assessment of $T$-score by using MOC-DXA were obtained in all subjects. Vitamin D receptor (VDR) polymorphisms Fok1 and Taq1 were also investigated by using PCR.
Results
Osteopenia/osteoporosis was found in 80% patients and 33% controls ($P<0.05$). In FD patients, osteopenia/osteoporosis was found at lumbar spine in 10/15 and femur neck in 9/15 (5 M and 7 F, three of whom in premenopausal age). Serum concentrations of 25-hydroxy-vitamin-D were 11.2±1.40 ng/ml in patients and 30.7±2.55 ng/ml in controls ($P<0.01$). Vitamin-D deficiency was found in 60% and insufficiency in 40% of patients without significant difference between summer and autumn assessment. There was a significant correlation between 25-hydroxy-vitamin-D and $T$-score values ($P<0.05$). Fok1 FF and ff polymorphisms were found in 93 and 7% of patients and 15 and 25% of controls,

respectively, while Taq1 TT and tt polymorphisms were found in 13 and 67% of patients and 50% and 8% of controls, respectively ($P<0.05$).
Conclusion
Lumbar and femur osteoporosis is common in FD patients, regardless from age and gender and is associated with vitamin-D deficiency/insufficiency in all cases. There is also a different distribution of Fok1 and Taq1 polymorphisms between FD and controls which needs to be functionally characterized. To include bone evaluation in the work-up of patients with FD and to prevent bone damage by replacing vitamin-D deficiency in these subjects are suggested.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P133

### *De novo* autoimmune hepatitis associated with PTH(1–34) and PTH(1–84) administration for severe osteoporosis in a liver transplant patient

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Introduction
Recombinant parathyroid hormone 1–34 (PTH(1–34)) and 1–84 (PTH(1–84)) stimulate new bone formation and are associated with significant decrease in the risk of vertebral and non-vertebral fractures. *De novo* autoimmune hepatitis (AIH) is a rare graft dysfunction occurring in patients having undergone liver transplantation (LT) for causes other than AIH.
Case report
A 61-year old woman was referred to our metabolic bone clinic due to severe osteoporosis (*T*-score of −2.7 in lumbar spine and −3.2 in femoral neck), 3 years after LT for primary biliary cirrhosis. The investigation for other conditions compromising her bone health revealed only vitamin D deficiency.
Taking into consideration the patient's severe osteoporosis in need of treatment, along with the impaired renal function that made the use of bisphosphonates rather problematic in addition to the prior use of glucocorticoids, the decision to proceed to teriparatide (PTH(1–34)) administration along with calcium carbonate 1000 mg and cholecalciferol 800 IU/day was made. At 3 month-assessment, asymptomatic hypertransaminasemia (two-fold the upper limit of normal) developed, which normalized after drug discontinuation.
A new flare of transaminases (three-fold the upper limit of normal), along with elevated alkaline phosphatase, was observed, after administration of PTH(1–84), which did not resolve after PTH(1-84) withdrawal. After exclusion of common causes of liver enzyme elevation, a liver biopsy was performed. Histological findings showed *de novo* AIH, which responded rapidly to treatment with methylprednisolone.
Conclusions
To our knowledge, this is the first case described in the literature involving development of post-LT *de novo* AIH, following PTH administration for severe osteoporosis. The exact mechanisms linking PTH with AIH are not clarified. However, it can be hypothesized that, since Kuppfer cells in the liver are implicated in PTH degradation, expressing the PTH/PTH-related protein type 1 receptor, they produce interleukin-6 which plays an important role in the pathogenesis of AIH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P134

### Osteogenesis imperfecta: phenotypic characteristics and response to treatment in an Irish cohort

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Background
Osteogenesis imperfecta is a group of heterogenous disorders affecting connective tissue. The disorders are inherited by autosomal dominant or recessive patterns. The phenotypes vary both between and within the subgroups of which there are eight described by the modified Sillence criteria. The common features are reduced bone mineralization, predisposition to fracture and resulting bony deformities. In addition blue sclerae and joint hyperlaxity can be present.
Methods
Charts of 17 patients with OI attending a metabolic bone disease clinic in Dublin were reviewed. The subtype of OI was determined by clinical characteristics in most cases. The treatment given in the form of calcium, vitamin D and bisphosphonates was identified. The response to treatment was assessed by DXA and bone turnover markers: osteocalcin, bone alkaline phosphatase, procollagen I amino propeptide, carboxy telopeptide, all in serum, and urine amino telopeptide.
Results
The response to treatment varied between patient and OI subtype. In general there was an increase in bone mineral density in response to bisphosphonates. The response in terms of frequency of fracture was not possible to determine from our data.
Conclusion
OI is debilitating but the course of the disease can be improved by treatment that increases bone mineral density. The response to treatment in our cohort is shown here. The optimum time course for bisphosphonate treatment is unclear and further study is needed to determine this and outcomes in terms of fracture incidence.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P135

### Bone mineral density in diabetic postmenopausal women with bone fracture

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Introduction
There are contradicting data about bone mineral density (BMD) in patients with type 2 diabetes mellitus (DM), although most studies describe increased BMD values in them. Due to this finding it is not clear how tightly bone fragility is bound with BMD in type 2 diabetic people.
Design
We provided dual-energy X-ray absorptiometry of lumbar spine and proximal femur to 46 postmenopausal women with type 2 diabetes mellitus (DM), 14 of them had bone fracture within last month. The control group included 40 non-diabetic women, 11 within a group were recently fractured. Groups were comparable in body mass index, age, duration of menopause and calcium intake. Diabetic subgroups were comparable in HbA1c level. We analysed lifestyle factors, calcium homeostasis indexes and bone turnover markers in all patients.
Results
There was a trend toward elevation of BMD in diabetic women (statistically significant for total hip). The frequency of osteoporosis and ostepenia in non-diabetic group was twice higher compared to diabetic group (correspondingly up to 45.2% vs up to 14.3% for osteoporosis and 24.1–81.8% vs 12.5–42.8% for osteopenia). In both groups with fracture (diabetic and non-diabetic) BMD was significantly lower than in groups without fracture ($P<0.01$ for most regions). Direct correlation with BMD were found for body mass index, lifetime weight gain and phosphorus excretion. There was inverse correlation BMD with C-terminal telopeptide of type I collagen ($r=-0.24$; $-0.55$). In diabetic group we found inverse relationships of BMD with target organs lesion (glomerular filtration rate decrease, presence of retinopathy and macroangiopathy).
Conclusion
Our data indicate that bone fragility in diabetic women is defined by BMD just the same as in non-diabetic ones despite elevation of BMD associated with type 2 DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P136

**Evidence for vitamin D deficiency and increased fractures' prevalence in autoimmune bullous skin diseases**

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Background
Vitamin D deficiency plays a role in autoimmune diseases and risk of fractures. No data are available on vitamin D levels and vertebral fractures in autoimmune bullous skin diseases.
Objectives
To assess vitamin D serum levels and vertebral fractures' prevalence in patients with pemphigus vulgaris (PV) and bullous pemphigoid (BP), potentially deadly autoimmune bullous disorders.
Methods
We studied 13 consecutive active untreated inpatients with PV (six males, seven females, age 53.5 ± 14.3 years), 15 with BP (seven males, eight females, age 76.9 ± 12.4 years) and 28 age- body mass index- and sex- matched controls. In all subjects, the 25(OH)Vitamin D (25OHD) levels and presence of vertebral fractures by spinal radiographs were assessed.
Results
In PV patients, 25OHD levels were lower (12 ± 4.4 ng/ml) and prevalence of severe hypovitaminosis D higher (61.5%) than in controls (22.2 ± 11.7 ng/ml, $P=0.012$; 23.1%, $P=0.0047$, respectively). The prevalences of fractures were 53.8 and 30.8% in PV patients and controls, respectively. Patients with BP showed lower 25OHD levels (9.6 ± 7.2 ng/ml) and higher prevalence of severe hypovitaminosis D (73.3%) than controls (22.6 ± 18.7 ng/ml, $P=0.022$; 26.7%, $P=0.01$, respectively). The prevalences of fractures tended to be higher in BP patients than in controls (66.7 vs 33.3%, respectively, $P=0.068$).
Conclusions
The low 25OHD levels found in PV and BP may suggest a role for this agent in their pathogenesis. The increased prevalence of fractures should be taken into consideration in patients who must be given corticosteroid.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P137

**A correlation between bone mass density and body mass index and the lipid profile?**

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Bone mass density and beta cross laps have been recognized as valid tests for the diagnostic and treatment control, as well as fracture prediction for osteoporotic patients. Statin treatment has been reported to be associated with a reduced risk fracture in these patients also. However, no studies, as of our knowledge, have tried to see if adding the lipid profile to the analysis of body mass density would improve the statistical power of prediction for any of the afore mentioned events. In our study 613 osteoporotic (*T*-score mean −3.16, S.D. 0.81) postmenopausal (mean 15.79 years, S.D. 8.88 years.) women were enrolled and they were grouped according to age (under 50 years, 51–60 years, 61–70 years, over 70 years) and the presence/absence of a history of bone fracture. Bone mass density was not correlated with body mass index ($P>0.05$, $r^2<0.02$). However, when bone mass density was correlated with both body mass index and the lipid profile (represented by triglycerides, cholesterol, LDLc, HDLc,) we obtained very good correlations in nine of the 12 groups, with five of them having a great statistical power: under 51 years ($n=72$, $r^2=0.27$, $P=0.11$) with fractures ($n=56$, $r^2=0.38$, $P=0.37$), 51–60 years with fractures ($n=61$, $r^2=0.14$, $P<0.01$), 61–70 years ($n=201$, $r^2=0.09$, $P<0.01$) with fractures ($n=88$, $r^2=0.14$, $P<0.01$) or without fractures ($n=113$, $r^2=0.24$, $P=0.02$) and over 70 years ($n=247$, $r^2=0.11$, $P<0.01$) with fractures ($n=67$, $r^2=0.35$, $P=0.11$) or without fractures ($n=51$, $r^2=0.35$, $P=0.08$). Similar results were obtained when bone mass density was correlated with alkaline phosphatase or 25OH Vitamin D or beta cross laps and the lipid profile.
Although we recognize the limitations of this study (the lack of complete data for some our patients and of statistical power in a part of the correlations), we consider that this pilot study opens a new perspective on the management of osteoporosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P138

**Vertebral fracture risk and bone metabolism in patients with addison's disease**

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Forty seven patients suffering from Addison's disease (AD: 12 males and 35 females, 44.5 ± 12.7 year old) were compared with 63 normal age-, sex- and BMI-matched subjects.
The duration of disease ranged from 0.6 to 44 years (10.23 ± 9.07 years). AD had received a cumulative dose of cortisone acetate of 290.5 ± 326.8 g, equivalent to a daily dose of 44.5 ± 15.5 mg, and to 26.34 ± 10.57 mg/m$^2$ of body surface. AD had average values of both daily urinary cortisol excretion and salivary cortisol higher than controls (583 ± 348 vs 374 ± 184 nmol/24 h, and 15.5 ± 11.2 vs 8.1 ± 4.2 ng/ml, respectively; $P<0.001$ in both cases), suggesting that cortisone replacement was higher than the physiological dose.
Vertebral fracture were assessed by DXA morphometric analysis in 25 AD patients and 43 controls. For this purpose a Hologic Discovery W device was used with a specific software for the vertebral height measurement. Eight patients showed at least one vertebral morphometric fracture, while only three subjects showed morphometric fractures in the control group (odds ratio = 6.27; 95% CI = 1.48–26.57; $P=0.013$).
Despite the higher number of vertebral fractures, AD showed densitometric variables similar to those of the control group in any evaluated area, including lumbar spine, femoral neck and whole body. Body composition (lean and fat body mass, expressed both in grams and in percent of total body mass) is similar in the two groups. Concerning laboratory parameters, daily calcium excretion is lower in AD patients than in controls (3.35 ± 2.32 vs 5.21 ± 2.38 mmol/24 h; $P=0.0001$). The other biochemical variables, including serum calcium and phosphate, bone alkaline phosphatase, serum CTx, 25OH vitamin D, 1,25(OH)2 vitamin D and PTH were similar in the two groups. No correlations were found between urinary cortisol excretion, salivary cortisol, duration of disease, cumulative and daily dose of replacement therapy and either laboratory or densitometric parameters in AD patients.
In conclusion, AD patients showed a risk of vertebral morphometric fractures higher than normals. Such risk does not seem to be related to a lower bone density or to common alterations of mineral metabolism, suggesting that fractures could be related to qualitative alterations of bone structure and the concomitant endocrine deficiency.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P139

**Bone mineral density and muscle mass in women with multiple sclerosis**

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Aim
The aim of this study was to compare the bone mineral density (BMD) and muscle mass between ambulatory women with multiple sclerosis (MS) and control subjects and to examine the influence of motor disability, muscle mass and/or glucocorticoids (GC) use on BMD.
Methods
Body composition and BMD were measured by dual-energy X-ray absorptiometry in 250 ambulatory MS women (153 premenopausal and 97 postmenopausal women) with Expanded Disability Status Scale (EDSS) $\leq$6.5 and in 193 controls.
Results
Compared to controls, patients had significantly lower values for total body bone mineral content and BMD at all measured sites except for the distal radius.

Patients with MS had significantly lower amount of total muscle mass as well as total leg muscle mass when compared to the control group. The EDSS score was negatively associated with BMD at the proximal femur in both premenopausal and postmenopausal women, while the deficit of total body muscle mass was significantly associated with a loss of BMD at the lumbar spine and whole body BMD only in premenopausal women. GC treatment was negatively associated with BMD at the lumbar spine in premenopausal women.
Conclusion
The total body muscle mass was an important predictive factor for the total body BMD and the lumbar spine BMD in premenopausal women with MS. Further prospective studies are required to verify the protective influence of muscle mass on BMD in patients with MS and to assess the role of systemic factors modulating the bone-muscle relationship (e.g. estrogen deficiency).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P140

**Teriparatide: effects on cortical bone microstructure**

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Teriparatide (TPTD) reduces the vertebral and nonvertebral fracture risk. TPTD increases bone formation and improves the cancellous bone microstructure which is an important determinant of the mechanical integrity of vertebrae. The aim of our study was to evaluate first time the effect of TPTD on cortical microstructure and dynamic histomorphometric indices in patients with osteoporosis with or without prior therapy with ALN. Sixty-six postmenopausal women with osteoporosis, mean age (S.D.) of 68.0 (7.0) years and mean BMD *T*-score of $-1.7$ (0.9) at total hip and $-2.8$ (0.8) at lumbar spine; 62% with prevalent fractures, have been treated with 20 μg/day subcutaneous TPTD for 24 months; 28 were osteoporosis pretreatment naive (TN), 38 stopped previous ALN treatment (70 mg/week, mean duration of 63.6 months) and switched to TPTD. 45 paired iliac crest biopsies were collected and analyzed for three-dimensional structural changes by micro-computer tomography and for two dimensional structural and dynamic changes by histomorphometry at baseline and after 24-month. At baseline, mineralizing surface/bone surface (MS/BS, %) values were lower in the ALN pretreated group than in the TN, at both the periosteal ($0.61 \pm 1.29$ vs $1.39 \pm 0.96$; $P=0.04$) and endocortical surfaces (baseline: $3.16 \pm 5.05$ vs $6.19 \pm 5.07$; $P=0.06$). After 24 months TPTD treatment, the MS/BS (%) increased in the ALN and the TN patients at the periosteal surfaces ($1.34 \pm 1.05$ vs $3.94 \pm 2.7$; $P<0.0001$), and endocortical surfaces ($4.95 \pm 4.03$ vs $11 \pm 9.57$; $P=0.005$). The cortical porosity was not different between the two groups of patients indicating that alendronate treatment does not reduce cortical porosity. After 24 months TPTD treatment, a significant increase was observed in cortical area and thickness and in double tetracycline labeling of cortical osteons. In summary, TPTD therapy increased bone formation and improved the cancellous and cortical bone quality in postmenopausal women with osteoporosis irrespective of whether they had received prior ALN antiresorptive therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P141

**Serum soluble RANKL levels are related to breast arterial calcification and hypertension in Japanese osteoporotic postmenopausal women**

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It has been suggested that the association between osteoporosis and cardiovascular diseases is not just due to the aging process. The osteoprotegerin (OPG)/RANKL system has been identified as a possible mediator of arterial calcification suggesting common links between osteoporosis and vascular diseases. Moreover, breast arterial calcification (BAC) is reported to be associated with an increased prevalence of both cardiovascular risk factors and cardiovascular morbidity. In contrast, it has known that hypertension, diabetes or hyperlipidemia is one of risk factors for progressing arteriosclerosis and vascular calcification. The purpose of this study is to investigate the relationship between serum soluble RANKL (sRANKL) levels and breast arterial calcification in Japanese osteoporotic postmenopausal women. This study was carried out in 51 postmenopausal women aged 46–82 years who underwent screening mammography. Participants were divided into four groups of the number of calcified vessels including, 0BAC: no calcification, 1BAC: one calcified vessel, 2BAC: two calcified vessels and >3BAC: more than three calcified vessels. Serum sRANKL, OPG, alkaline phosphatase (Alp) and urinary type-I collagen cross-linked-N-telopeptide (uNTX) were measured. The mean age of these 51 women was $64.5+1.1$ years-old, 0BAC; $n=25$, 1BAC; $n=11$, 2BAC; $n=8$ and >3BAC; $n=7$. The prevalence of BAC was clearly increased in osteoporosis group as compared with normal BMD group. In the subgroups of BAC, BMD in >3BAC group was clearly decreased, and serum sRANKL levels were significantly increased in >3BAC group compared with those in 0BAC group. In addition, sRANKL/OPG ratio was pivotally higher in 2BAC and >3BAC groups compared with that in 0BAC group. Moreover, serum sRANKL levels are increased in postmenopausal women with hypertension, but not diabetes and hyperlipidemia. In contrast, serum Alp and uNTX were not significantly changed, increasing the number of BAC in postmenopausal women. In conclusion, the prevalence of BAC in Japanese postmenopausal women was remarkedly high in osteoporosis. Especially, that sRANKL and sRANKL/OPG ratio were increased in postmenopausal women with severe calcification of breast artery and hypertension, suggesting that serum sRANKL might play a role in common links between osteoporosis and cardio-vascular diseases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P142

**BMP-3b inhibits osteoblast differentiation via Smad2/3 pathway by counteracting Smad1/5/8 signaling**

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Involvement of BMP-3b (also called GDF-10) in osteogenesis, embryogenesis and adipogenesis has been reported to date. However, the functional receptors and intracellular signaling of BMP-3b have yet to be determined. In the present study, we studied the cellular mechanism of BMP-3b in osteoblast differentiation using mouse myoblastic C2C12 cells, which show osteoblasitc differentiation in response to osteogenetic BMP ligands. BMP-3b stimulated activin/TGF-β-responsive promoter activities. In contrast, the stimulatory actions of BMP-3b on activin/TGF-β-responsive activities were suppressed by co-treatment with BMP-2. BMP-responsive promoter activities stimulated by BMP-2 were significantly inhibited by treatment with BMP-3b. BMP-3b suppressed the expression of osteoblastic markers including Runx2, osteocalcin and type-1 collagen induced by BMP-2, -4, -6 and -7. BMP-2-induced Smad1/5/8 phosphorylation and mRNA levels of the BMP target gene Id-1 were also suppressed by co-treatment with BMP-3b, although BMP-3b failed to activate Smad1/5/8 signaling. Of interest, the BMP-3b suppression of BMP-2-induced Id-1 expression was not observed in cells overexpressing co-Smad4 molecules. On the other hand, BMP-3b directly activated Smad2/3 phosphorylation and activin/TGF-β target gene PAI-1 mRNA expression, while BMP-2 suppressed BMP-3b-induced Smad2/3 signal activation. BMP-2 inhibition of BMP-3b-induced PAI-1 expression was also reversed by overexpression of Smad4. Experiments using inhibitors (dorsomorphin and LDN-193189) for BMP-Smad1/5/8 pathways demonstrated that these BMP-3b effects were mediated via receptors other than ALK-2, -3 and -6. Furthermore, results of inhibitory studies using extracellular domains for BMP receptor constructs showed that the activity of BMP-3b was functionally facilitated by a combination of ALK-4 and ActRIIA. Collectively, BMP-3b plays an inhibitory role in the process of osteoblast differentiation, in which BMP-3b and BMP-2 are mutually antagonistic possibly by competing with the availability of Smad4.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P143

**Glucocorticoid - treatment in the adrogenital syndrome due to deficit of 21-hydroxylase: long-term bone side effects**

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The purpose of this study was to compare bone mineral density (BMD) and bone metabolism in patients suffering from adrogenital syndrome due to deficit of 21-hydroxylase with those of a group of healthy subjects. A longitudinal follow-up was also carried out in a subgroup of patients.
Thirty-eight patients 19-47 years old were compared with a group of healthy, age- and sex-matched controls. Sodium, potassium, calcium, phosphorus, parathyroid hormone (PTH), bone alkaline phosphatase (bALP), serum CrossLaps (CTx), 25-hydroxy vitamin D, creatinine, daily urinary calcium excretion (uCa), 17-hydroxy progesterone, testosterone and androstenedione were dosed. Bone mineral density (BMD) was evaluated at lumbar spine L1-L4, femur (neck and total) and total body by a Dual X-Ray densitometry (Hologic-Discovery). A densitometric follow up was performed in 15 patients, 10 years after the first observation, with the same device.
A significant increase of CTx was observed only in female patients compared to normal women ($369 \pm 171$ vs $237.7 \pm 152$ pg/ml; $P = 0.04$); no difference was found in bALP serum levels. BMD was lower in patients at any site, however, adjusting BMD values for height, the difference remained significant only at the femoral neck ($0.422 \pm 0.16$ vs $0.498 \pm 0.05$ g/cm$^2$; $P = 0.0085$). In the 10-years follow-up lumbar spine BMD showed a slight increase, while the femoral parameters showed a slight decrease (L1L4 BMD basal $= 0.982 \pm 0.10$ vs 10-years BMD $= 1.007 \pm 0.10$ g/cm$^2$, $P = 0.04$; femoral neck BMD basal $= 0.813 \pm 0.12$ vs 10-years BMD $= 0.752 \pm 0.11$ g/cm$^2$, $P = 0.0004$; total femur BMD basal $= 0.911 \pm 0.11$ vs 10-years BMD $= 0.870 \pm 0.10$ g/cm$^2$, $P = 0.004$). The same trend is observed in the healthy population. No correlation was found between the densitometric and biochemical variables and the cumulative GCs dose assumed in the last 1-3 years or the time from diagnosis. The lower BMD in AS patients does not seem to be related to the GCs replacement therapy. It could be a consequence of the pathology itself, which causes a precocious bone development, thus leading to an earlier achievement of the peak of bone mass, that is consequently reduced.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P144

**Polymorphism of the vitamin D3 receptor gene and bone mineral density in girls with functional hypothalamic amenorrhea subjected to estroprogestagen treatment**

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Background
We analyzed in the vitamin D3 receptor gene (VDR) polymorphism can modulate therapeutic response of functional hypothalamic amenorrhea (FHA) patients to the estroprogestagen (EP) treatment.
Material and methods
The study included 84 FHA girls and 50 controls. FHA patients underwent a 4-year sequential EP therapy with 17-β estradiol (2 mg from the 2nd to 25th day of the menstrual cycle) and didrogesterone (10 mg from the 16th to the 25th day). Their hormonal parameters were monitored along with bone turnover marker levels and bone mineral density (BMD). Additionally, the VDR gene BsmI polymorphism was determined.
Results
Hormonal therapy was reflected by a substantial improvement of BMD. However, the values of BMD observed after 4 years of treatment in FHA patients were still significantly lower than baseline bone mineral density determined in the control group ($1.007 \pm 0.100$ vs $1.141 \pm 0.093$ g/cm$^2$, respectively; $P < 0.001$). No significant effects of the VDR genotype were observed on the dynamics of BMD during consecutive years of hormonal treatment and mean bone mineral density determined after completing the therapy ($1.006 \pm 0.101$ vs $1.013 \pm 0.114$ vs $1.006 \pm 0.094$ g/cm$^2$ for BB, bb and Bb genotypes, respectively; $P = 0.973$).
Conclusions
This study did not confirm that VDR polymorphism can modulate therapeutic outcome of FHA girls subjected to the hormonal treatment. Nonetheless, this study confirmed the effectiveness of EP therapy in the simultaneous treatment of menstrual disorders and the normalization of bone mineral density in FHA patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P145

**Altered bone mineral density in unilaterally orchiectomized subjects**

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Introduction
Testicular cancer (TC) is the most common cancer in white males aged 20–40 years, with a worldwide incidence of 7.5/100.000. Recently, we demonstrated an association between testiculopathy and alteration of the bone status, despite conserved bone-sparing effects of androgens and estrogens. Furthermore, recent published data from our group documented a strong reduction in 25(OH)D plasma levels in bilaterally orchiectomized patients compensated by testosterone-replacement therapy. These data suggested a potential role of testis in vitamin D activation. In fact, we demonstrated that the Leydig cell represents a main actor in this process expressing a high amount of CYP2R1, a key enzyme involved in vitamin D 25-hydroxylation. Few reports have investigated the incidence and pathogenesis of altered bone status in subjects with TC and the available literature shows contrasting data.
Materials and methods
One hundred and twenty five patients orchiectomized for unilateral TC, followed up over a 2-year period, and 41 age-matched healthy male controls were enrolled. Serum levels of total testosterone, oestradiol, LH, FSH, PTH, 25(OH)D, 1,25-(OH)2D, bone-specific alkaline phosphatase (BAP), and carboxyl-terminal telopeptide of collagen type I (ICPT) were measured in all subjects. Eighty-four patients and 41 controls underwent bone densitometry analysis by DEXA.
Results
25-(OH)D levels were significantly lower and PTH, BAP and ICTP levels higher in TC patients compared to controls ($P < 0.05$). Femoral neck and/or lumbar spine $T$-score was $< -1$ S.D. (osteopenia) in 30/84 TC subjects (35.7%) and $< -2.5$ S.D. (osteoporosis) in 8/84 TC subjects (9.5%). None of 41 control subjects showed alterations of BMD.
Conclusions
Our data show an association in patients orchiectomized for unilateral TC and alteration of the bone status, despite unvaried androgen and oestrogen levels and no other evident cause of vitamin D reduction.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P146

**Effects of aminobisphosphonates and thiazides in patients with osteopenia/osteoporosis, hypercalciuria and recurring renal calcium lithiasis**

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Introduction
A relationship between osteopenia/osteoporosis and calcium nephrolithiasis has been recognized in several studies. An association has also been observed between bone mineral density loss, hypercalciuria, hypocitraturia and nephrolithiasis.

The aim of this study was to analyze the effects of aminobisphosphonates and thiazides on renal lithogenic activity and bone mineral density in patients with recurring renal calcium lithiasis.
Methods/design
A prospective cohort study with a 3-year clinical follow-up was performed in two groups of patients with recurring calcium lithiasis, hypercalciuria and bone mineral density loss. Group 1 included 35 patients undergoing treatment with 70 mg/week alendronate, and Group 2 included 35 patients undergoing treatment with 50 mg/day alendronate and 70 mg/week hydrochlorothiazide. Biochemical analysis was performed at baseline, 6 months and 2 years, bone densitometry at baseline and at 2 years and clinical follow-up during 3 years of treatment. Biochemical variables in blood and urine, recurrent lithiasis and bone mineral density were analyzed.
Results
Age, sex, baseline biochemical markers and bone density showed no differences between treatment groups at the onset of treatment. After 2 years of treatment, Group 1 showed a significant decrease in bone turnover markers and calciuria as well as a significant improvement in bone mineral density. After 2 years of treatment, Group 2 showed a decrease in calciuria and in bone markers. Bone densitometry improved significantly. At 2 years, the decrease in calciuria and the improvement in bone mineral density were greater in Group 2 in than Group 1, and the difference was statistically significant.
Conclusion
Aminobisphosphonates improve bone mineral density and slow lithogenic activity; however, administration of aminobisphosphonates in association with thiazides produces the same clinical effects and also reduces calciuria and improves bone mineral density. Bisphosphonate-thiazide coadministration is most appropriate in patients with bone mineral density loss, hypercalciuria and relapsing calcium lithiasis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P147

**Bone mineral density in patients with thalassaemia major in the UK**

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Introduction
Osteoporosis is a major cause of morbidity in β thalassaemia major patients. Our institution serves one of UK's largest populations of thalassaemia patients. This study aimed to examine the prevalence of osteopenia and osteoporosis in thalassaemia major patients and identify risk factors for low bone mineral density (BMD).
Methods
BMD of lumbar spine and neck of femur were measured using dual-emission X-ray absorptiometry (DEXA). Osteopenia was defined as $Z$ score between $-1.5$ and $-2.5$ and osteoporosis as $< -2.5$.
Age, gender, smoking, hepatitis C infection, compliance with chelation therapy, mean ferritin over 10 years, highest Ferriscan liver iron concentration from 2008 to 2010, highest liver iron concentration as estimated by T2* and lowest cardiac MR T2* value from 1999 to 2010, endocrinopathies, glycaemic status and vitamin D status were tested for associations with osteopenia and osteoporosis, using univariate analysis.
Results
Ninety-nine patients, 49 males and 50 females, with mean age $36 \pm 9$ years were included. 68.7% of patients had hypogonadism, 14.7% hypothyroidism and 13.7% hypoparathyroidism. 40.9% of patients had diabetes and 16.1% impaired glucose regulation. Lumbar spine BMD suggested that 22.4% had osteoporosis, 48.0% osteopenia and 29.6% normal BMD. Median $Z$ score was $-1.9$ (range 0.9 to $-5.0$). Neck of femur BMD demonstrated that 13.3% had osteoporosis, 40.8% osteopenia and 45.9% normal BMD. Median $Z$ score was $-1.5$ (range 0.8 to $-3.7$).
Parameters with $P$-values $<0.05$ on univariate analyses for associations with osteoporosis/osteopenia are presented in the table. On multivariate analysis, the only statistically significant association was between hypogonadism and spine low BMD ($P$ value $=0.048$).
Conclusions
Most β thalassaemia major patients have low BMD. Hypogonadism was an independent risk factor for reduced BMD in spine. Prevention of hypogonadism by effective iron chelation and hormone replacement therapy may help to prevent osteopenia/osteoporosis.

**Table 1**

| Type of abnormality in BMD | Parameter | *P*-value |
|---|---|---|
| Spine low BMD | Low cardiac T2* (1999–2010) | 0.038 |
| | Hypogonadism | 0.009 |
| | Diabetes mellitus/ impaired glucose regulation | 0.047 |
| Hip low BMD | Female gender | 0.005 |
| | Low Ferriscan® LIC (2008–2010) | 0.019 |
| | Hypothyroidism | 0.018 |
| | Diabetes mellitus/ impaired glucose regulation | 0.040 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P148

Abstract withdrawn.

## P149

**Both high- and low levels of procollagen type 1 amino-terminal propeptide are associated with increased risk of hip fracture in elderly women. Norwegian Epidemiologic Osteoporosis Studies**

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Aim
The study aimed to investigate the relation between the levels of the bone formation marker P1NP and the risk of subsequent hip fractures in elderly women.
Materials and methods
Women born 1924–27 who participated in two population-based health studies in Bergen (The Hordaland Health Study (HUSK) 1997–2000) and in Oslo (The Oslo Health Study (HUBRO) 2000–2001), were followed up regarding hip fractures from date of inclusion until end of 2007 in HUBRO and until end of 2008 in HUSK. Serum P1NP was determined by Multigamma RIA kit (Orion Diagnostica, Espoo, Finland) at the Hormone Laboratory at Oslo University Hospital, in serum samples collected at baseline and stored at $-80$ °C. The study was designed as a case-cohort study, including identified hip fracture patients ($n=306$) and a random sample of 9% of women in the same cohorts ($n=311$). Cox proportional hazards regression with inverse probability weighting and robust variance with serum P1NP as explanatory variable was performed.
Results
P1NP levels ranged from 7 to 127 ng/ml. A U-shaped relation between P1NP and hip fracture was suggested. Compared to the lowest quartile of P1NP, the hazard ratio (HR) in the 2nd quartile was: 0.68 (95% CI: 0.42–1.09), in the 3rd quartile:

0.77 (95% CI: 0.49–1.22), and in the 4th quartile: 1.04 (95% CI: 0.66–1.63). Adjusting for covariates or excluding participants using medication known to lower P1NP did not substantially alter the results. A confirmatory spline analysis showed a significant U-shaped relation.
Conclusion
A U-shaped relation between P1NP and hip fractures was found, suggesting both low and high levels of P1NP confer increased risk of osteoporotic fractures. Whether the fracture risk among those with the lowest P1NP levels is mediated through generally low bone turnover, or isolated impaired osteoblast collagen synthesis, remains to be elucidated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P150

### Alendronate inhibits adipocyte differentiation in 3T3-L1 through a Vitamin D receptor mediated effect

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Background
Adipocyte and osteoblast derive from the same mesenchimal progenitor. Age-related decrease in bone mass is accompanied by an increase in marrow adipose tissue. Vitamin D3 (VD) inhibits adipogenesis in 3T3-L1 preadipocytes. Recently it has been demonstrated that alendronate (ALN) inhibits adipogenesis while promoting osteoblast differentiation of mesenchimal stem cells.
Aim of the study
To evaluate the *in vitro* role of ALN on 3T3-L1 adipose differentiation and the potential sinergic role of VD cotreatment.
Procedures
Murine 3T3-L1 and 3T3-F442A preadipocytes were routinely differentiated for 7 days adding ALN and VD 10-9-10-7M, then stained with Red Oil. We analyzed through RT-PCR the effect of such treatments upon mRNA expression of main molecular markers of differentiation, PPARγ and C/EBPα, and VD receptor (VDR).
Results
ALN displayed a marked anti-adipogenic effect on 3T3-L1 cells. VD showed a clear dose-dependent anti-adipogenic effect. Interestingly co-incubation of ALN 10-8M and VD 10-9M did not show synergic effect in inhibition of adipogenesis. PPARγ mRNA expression was significantly reduced by ALN and VD. mRNA expression of C/EBPα was reduced only by the highest doses of VD. Interestingly a concomitant increase in VDR mRNA expression was observed in the presence of ALN and VD, suggesting that VDR may represent the molecular target of the anti-adipogenic effect of ALN.
To confirm this hypothesis, we explored the effects of ALN and VD on 3T3-F44 cells that are in a more advanced differentiation stage in adipogenesis; the results were compared to those obtained in 3T3-L1 cells; we found that expression of VDR mRNA was much lower than in 3T3-L1 cells. Interestingly adipose differentiation in this cell model was not affected by ALN nor VD.
Conclusion
These data represent an indirect evidence of the role of VDR in mediating the anti-adipogenic effect of ALN. Further studies are required to clarify this mechanism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P151

### Genetic predispositions do not explain short- and long-term effects of hormonal therapy on bone mineral density in girls with functional hypothalamic amenorrhea

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The aim of this study was to verify if genetic factors influence the short- and long-term therapeutic response to estroprogestagen (EP) therapy implemented in girls with functional hypothalamic amenorrhea (FHA) in order to improve their bone mineral density (BMD). The study included 78 FHA girls who underwent a 4-year sequential EP therapy with 17β-estradiol and didrogesterone. Changes in the lumbar spine BMD were determined at the end of the therapy and 6 years after its discontinuation, and analyzed in regards to PvuII i XbaI polymorphisms of estrogen receptor-α gene, BsmI polymorphism of vitamin D3 receptor gene, and Sp1 polymorphism of the type-one collagen gene. After 4 years of EP therapy, significant increase of BMD was documented in the studied group. Follow-up densitometry performed 6 years after completing the therapy revealed significant decrease in BMD level; nonetheless, the values of this parameter were still significantly higher compared to pretreatment level. Neither single polymorphisms or their combinations did not influence the relative change in BMD at the end of the therapy and after 6-year follow-up.
Conclusion
Variability of genes involved in estrogen, vitamin D3 and collagen metabolism does not influence the short- and long-term results of EP therapy in girls with FHA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P152

### Improved assessment of bone turnover by the PTH-(1–84)/ large C-PTH fragments ratio in dialysis patients

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Background
The 'intact' parathyroid hormone (PTH) assays recognizes PTH (1–84) as well as amino terminally truncated fragments (C-PTH fragments). The present study investigated whether the use of plasma PTH-(1–84)/C-PTH fragment ratio enhances the noninvasive assessment of bone metabolism in patients on dialysis.
Methods
Blood samples of 45 healthy subjects with normal 'intact'-PTH values and 135 samples of dialysis patients were collected from laboratory routine. The samples of the dialysis patients were classified in order of their 'intact'-PTH levels: 45 with a 'intact'-PTH value $<100$ pg/ ml, 45 with a 'intact'-PTH value between 100 and 500 pg/ ml, and a third group with 'intact'-PTH levels above 500 pg/ ml. The determination of the 'intact'-PTH was performed on a ROCHE E170 analyzer with the Elecsys PTH assay. The testing of PTH-(1–84) and of OSTASE (BAP) as a parameter of osteoblast activity was perfomed on a DiaSorin LIAISON analyzer with the LIAISON PTH3 assay and the LIAISON BAP Ostase assay respectively. Blood levels of large C-PTH fragments were calculated by subtracting PTH-(1–84) from 'intact'-PTH.
Results
Both plasma intact- and PTH-(1–84) were significantly higher in patients with high and normal Ostase levels as a parameter of bone turnover, whereas the calculated C-PTH fragments were similar. Patients with low Ostase levels had significantly more C-PTH fragments than PTH-(1–84) ($P<0.001$), whereas patients with high or normal bone turnover had significantly more PTH-(1–84) than C-PTH fragments ($P< 0.001$), and the PTH-(1–84)/C-PTH fragment ratio was significantly higher than in patients with high or normal (range of 0.66–12.2) than low bone turnover (range of 0.1–0.88).

Bone turnover is reflected by the balance between the relative amount of circulating PTH-(1–84) and the large C-PTH fragments. The data lend support to the notion of an antagonistic effect of the C-PTH fragments on the PTH-(1–84) action on bone. The wide range of the obtained resuts in the PTH-(1–84) / C-PTH fragments ratio demonstrates that a simple fixed percentage of intact PTH cannot be identified for prediction of the large C-PTH fragments.
Conclusions
The PTH-(1–84) / C-PTH fragment ratio predicts bone turnover with acceptable precision for biological measurements.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P153

**Comparaison of serum PTH concentrations obtained with a second and a third generation assay in a cohort of hemodialysis patients according to the KDIGO guidelines**

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Introduction
PTH concentrations obtained with the second generation (2G) assays may vary widely according cross-reactivities against 7–84 PTH fragments. 1–84 bio-intact PTH is only measured with a third generation (3G) assay.
Objective
To evaluate whether the results found with a 3G method improve the classification of dialysis patients according to the KDIGO recommendations.
Patients and methods
We studied the PTH values measured in 84 hemodialysis patients'sera with a 2G immunoradiometric assay (Total intact PTH IRMA, Scantibodies) and with an automated 3G PTH (Liaison, DiaSorin). The decrease of PTH 3G levels was calculated as ((PTH 2G–PTH 3G)/PTH 2G)×100. Bone alkaline phosphatase (bAP) levels were measured using an IRMA (Beckman-Coulter; reference values: 9–14 ng/ ml).
Results
PTH results obtained with both methods were significantly correlated ($r=0.97$ (IC95%=0.96–0.98); $P<0.000001$). Patients were classified according to the KDIGOs obtained with the PTH 2G: group one: <two×upper normal limit; group two: between two×and nine×upper normal limit; group three:> nine×upper normal limit) (Table 1).
A significant correlation was seen between bAP and PTH 2G ($r=0.49$ (0.31–0.64); $P<0.00001$ or PTH 3G ($r=0.46$ (0.27–0.61); $P<0.00001$). Among the patients, 93% were classified in the same group according to the PTH 2G or 3G values.
Conclusion
The new automated PTH 3G gives the same classification in 93% of patients. Further investigations including bone biopsy would be necessary in 7% of misclassified patients to evaluate the performance of PTH 2G or PTH 3G according to the KDIGO guidelines.

**Table 1**

| Group | BAP ( ng/ ml) | PTH 2G ( pg/ ml) | PTH 3G ( pg/ ml) | Decrease of PTH 3G (%) |
|---|---|---|---|---|
| Group 1 | 11.9±6.4 | 58.3±18 | 38±10 | <33±6 |
| Group 2 | 19.3±6.4 | 231±97 | 154±75 | <34±15 |
| Group 3 | 37±24 | 716±374 | 486±250 | <32±1.8 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P154

**Endoscopic comparison of esophageal and gastroduodenal mucosal effects of enterocoating alendronate with calcitriol combined drug and alendronate in Korean postmenopausal women**

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Introduction
The study was a single-center, open label, randomized, head-to-head clinical study to compare the mucosal findings after perform esophagogastroduodenoscopy between two groups before and after use of alendronate only and enterocoating alendronate (5 mg) with calcitriol (0.5 μg) combined drug (Maxmarvil).
Methods
The 33 postmenopausal healthy volunteers, aged 50–70 years (mean age: 58±5 years) without gastrointestinal symptoms and normal baseline endoscopic findings were participated. Esophagogastroduodenoscopy was taken at the time of the baseline and repeated at 2 weeks later after daily intake of Maxmarvil ($n=17$ subjects) or alendronate ($n=16$ subjects). Mucosal injury scores were reported by a endoscopist after 2 weeks of each medication.
Results
Esophageal mucosal injuries were developed in two out of 16 subjects with alendronate group only. Gastric mucosal injuries were developed in eight subjects in alendronate group and in four out of 17 subjects with Maxmarvil group, which showed statistically significant differences.
Conclusions
Our study showed that the mucosal damage scores for the alendronate group (total score; 24) are significantly higher than those of scores for the Maxmarvil group (total score; nine) in the esophagus and stomach. Therefore, this study suggests that enterocoating Maxmarvil is more safe on gastrointestinal mucosa than alendronate and may improve the tolerability of osteoporosis medication in clinical practice.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P155

**Functional hypoparathyroidism is a risk factor for bone fragility of postmenopausal women with vitamin D insufficiency**

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Aim
Vitamin D insufficiency is associated with an increase in PTH, which might be critical for an increase in bone fragility. However, the role of endogenous PTH in vitamin D insufficiency-induced increase in fracture risk still remains unclear. The present study was performed to examine the relationships among vitamin D insufficiency, bone fragility, PTH and sclerostin, which is produced by osteocytes and inhibits bone formation by inhibiting the Wnt pathway.
Methods
Subjects were 190 healthy postmenopausal women who had undergone osteoporosis screening. Bone mineral density (BMD) was measured using the DXA method at the lumbar vertebrae and femoral neck (FN). The presence of vertebral fractures was confirmed on X-ray and nonvertebral fractures were assessed by the clinical interview. Serum levels of Ca, P, Cr, C-terminal cross-linked telopeptide of type I collagen (CTX), intact PTH, 25-hydoroxyvitamin D (25(OH)D) and sclerostin were measured.
Results
Mean values of age and BMI were 63±8 years and 22.9±3.1 kg/ $m^2$, respectively. Levels of intact PTH, 25(OH)D, CTX and sclerostin were 46±15 pg/ ml, 16.3±4.4 ng/ ml, 0.4±0.2 ng/ ml and 1.3±0.4 ng/ ml,

respectively. The percentages of subjects with 25(OH)D levels below 20 ng/ ml were 80.7%. Serum 25(OH)D levels were negatively related to age, Cr, CTX and PTH, although it was positively related to BMD. Multiple logistic regression analysis showed that lower 25(OH)D levels were significantly related to prevalent fracture risk, when adjusted for age, BMI, Ca, P, Cr, CTX, PTH, FN BMD, and sclerostin. Logistic regression analysis revealed that the group with lower PTH and lower 25(OH)D was a significant risk factor for prevalent fracture (odds ratio, 2.33 (95% CI: 1.04–5.25), $P < 0.05$) even after adjustments for these indices.
Conclusions
Vitamin D insufficiency was related to prevalent fracture risk. In vitamin D insufficiency, functional hypoparathyroidism rather than functional hyperparathyroidism might be a risk factor for bone fragility.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P156

**Platinum nanoparticle reduces ovariectomy-induced bone loss by decreasing osteoclastogenesis**

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Platinum nanoparticles have shown to have a remarkable antioxidant activity. Growing evidence between oxidative stress and bone loss suggests that platinum nanoparticle could protect bone loss via modulating oxidative stress. Intragastrical administration of platinum nanoparticle reduced ovariectomy (OVX)-induced bone loss with lowered level of *in vivo* bone resorption. Platinum nanoparticle inhibited osteoclast (OC) formation via an impaired receptor activator of nuclear factor-κB ligand (RANKL) signaling due to reduced reactive oxygen species. Our data clearly highlights the potential of platinum nanoparticle for amelioration of bone loss after estrogen deficiency via delayed OC formation.
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Declaration of interest
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## P157

**The level of Osterix expression in osteoblasts is important to prevent IL6-induced inflammatory-prone state**

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Osterix (Osx) is an essential transcription factor for osteoblast differentiation and bone formation. Osx null mutants died perinatally with a complete absence of bone formation and Osx conditional knockouts in osteoblasts showed the osteopenic phenotype after birth. Even in Osx heterozygous mice with normal bone morphology, the reduced cortical thickness and osteoblast differentiation were observed by QCT and *in vitro* cell culture, respectively. This result exhibited that the level of Osx expression was still important for *in vivo* and *in vitro* bone formation. Here, we investigated a potential role of Osx to regulate physiological homeostasis in adult tissue. In Osx heterozygous mice with a low expression of Osx in bones, the expression levels of pro-inflammatory cytokines were significantly increased, indicating that their body due to the reduced Osx expression may remain an inflammatory-prone state. Especially, the expression of interleukin (IL)-6, a key mediator of chronic inflammation, was increased in Osx heterozygotes and decreased in Osx overexpressed osteoblastic cells. Kidney as well as bone is one of the most important organs in order to maintain a physiological balance for mineral ions controlled by numerous endocrine factors. The cross-talk between the bone and kidney for physiological homeostasis has been studied for a long time. In Osx heterozygous mice compared to wild-type, no significant difference was observed in renal morphology and urine calcium and phosphate levels. However, the recovery of kidney after ischemic damage was remarkably delayed in Osx heterozygous mice, as indicated by elevated blood urea nitrogen and creatinine levels, and morphological alterations consistent with acute tubular necrosis. Eventually, the low level of Osx expression caused an inflammatory-prone state in the body, resulting in the enhanced susceptibility to renal injury after ischemia/reperfusion. This study suggests that the maintenance of Osx expression in bone is important to prevent inflammatory-prone state.
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Declaration of interest
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## P158

**Osteopenia/osteoporosis in patients with calcium nephrolithiasis**

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Introduction
Calcium nephrolithiasis is the most frequently diagnosed type of nephrolithiasis. It is related to metabolic alterations, inhibitor deficiencies, anatomical factors, alterations in urinary pH, and bone alterations.
The objective of this study is to analyze the alterations in bone mineral density and bone and calcium-phosphorus metabolism in patients with calcium nephrolithiasis.
Methods/design
We designed a study with 182 patients who were distributed among three groups: Group O included 56 patients without nephrolithiasis; group A included 67 patients with calcium nephrolithiasis and mild lithogenic activity; and group B included 59 patients with calcium nephrolithiasis and severe lithogenic activity. Metabolic parameters of blood and urine that were related to calcium-phosphorous and bone metabolism and bone densitometry were assessed in all patients. A comparative study was performed on the variables of bone and calcium-phosphorus metabolism and bone densitometry as well as the presence or absence of osteopenia/osteoporosis among the groups.
Results
The patients in group B had a greater loss of bone mineral density, measured by the *T*-score, than the patients in groups O and A. Moreover, the proportion of patients in group B with osteopenia/osteoporosis was statistically significantly higher than the proportion of patients in groups O and A. We observed higher values of calciuria, fasting calcium/creatinine ratio, and 24-h calcium/creatinine among the patients in group B compared to the other two groups. Calciuria, citraturia, and fasting calcium/creatinine were independent factors that showed a relationship with severe lithogenic activity compared to the control group, and -crosslaps is an independent factor that has a relationship with severe lithogenic activity as compared to mild lithogenic activity.
Conclusions
Patients with calcium lithiasis and severe lithogenic activity have a greater loss in bone mineral density and therefore a greater risk of osteopenia/osteoporosis compared to patients without lithiasis or with calcium lithiasis and mild lithogenic activity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P159

**No effect of long-term valproate monotherapy on bone mineral density in adults with epilepsy**

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Introduction
Anti-epileptic drugs, usually defined according to their effect on the cytochrome P450 system, might lead to bone mineral density (BMD) decrease and fractures. The anticonvulsant valproate is a hepatic enzyme inhibitor, effective in many types of epilepsy. The present study aimed to assess the impact of long-term valproate monotherapy on BMD in a sample of consecutive ambulatory adult patients with epilepsy.
Methods/design
This cross-sectional study recruited 41 consecutive adult epileptic patients receiving valproate monotherapy for at least two years. BMD at the level of the lumbar spine was assessed by dual energy X-ray absorptiometry (DXA). All subjects underwent an evaluation that included serum levels of total calcium, phosphorus, magnesium, 25-hydroxyvitamin D3 and parathormone.
Results
No case of osteoporosis was documented in the sample, while osteopenia was present in 24% of subjects. Duration and dosage of valproate monotherapy did not correlate with any of the *T*- and *Z*-scores at the lumbar spine. Dividing patients according to the duration of valproate monotherapy into long- and short-term group resulted in similar findings. In addition, no association was documented between duration or dosage of valproate monotherapy and biochemical parameters.
Conclusion
The present study could not identify any correlation between duration or dosage of valproate monotherapy with BMD measurements in adult patients with epilepsy. No case of osteoporosis was identified in patients treated with valproate for a mean period of more than ten years. These findings provide indirect evidence against a potential detrimental effect of long-term valproate monotherapy on bone metabolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding

## P160

**Bone cross-sectional geometry and strength parameters at the radius by pQCT in male obese adolescents**

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Introduction
Male gender, adolescence and obesity are known risk factors for upper limb fractures, although the mechanism by which obesity confers the increased fracture risk is unknown. In addition, most studies investigating the influence of body fat mass on bone in adolescents have been performed in school based populations using the DXA technique, which does not take into account the material and geometric bone properties.
Methods
We investigated volumetric bone density and cross-sectional bone geometry at the non-dominant forearm using peripheral quantitative computed tomography (pQCT) in 51 obese (BMI *z*-score $> +2$) male adolescents (aged 10–19 years) at entry of a residential weight loss program in comparison with 51 non-obese age, pubertal stage and height matched controls.
Results
Obese adolescents (OA) had a higher trabecular density ($215 \pm 33$ vs $197 \pm 32$ mg/cm$^3$; $P=0.007$) at the distal 4% end, but comparable cortical density ($996 \pm 60$ vs $992 \pm 54$ mg/cm$^3$; $P=0.720$) at the proximal 66% site. Trabecular area ($158 \pm 26$ vs $143 \pm 33$ mm$^2$; $P=0.02$) as well as cortical area ($72 \pm 21$ vs $65 \pm 17$ mm$^2$; $P=0.08$) and periosteal circumference ($46.8 \pm 4.7$ vs $44.8 \pm 4.8$ mm; $P=0.04$) were slightly larger in OA. While absolute bone strength index (BSI) assessed at the distal radius ($37.1 \pm 14.04$ vs $31.2 \pm 12.27$ mg/mm$^4$; $P=0.03$), was higher in OA, the ratio BSI/ forearm load was lower.
Conclusion
Male OA have greater periosteal circumference, trabecular bone area and density and absolute bone strength at the distal radius. Hence, the higher forearm fracture risk in obese adolescents is not due to a lower bone accumulation, but bone strength is probably not adapted to the excess body weight in case of unusual loading as a fall on outstretched hands.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P161

**Osteonecrosis of the jaw (ONJ) with superimposed osteomyelitis related to actinomyces in a patient with multiple myeloma receiving thalidomide and intravenous zoledronic acid**

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Patient, methods and case report
A 50-years-old AAF with a PMH of multiple myeloma (MM), hypertension, and schizophrenia presented with jaw pain for 2 months. The patient had been diagnosed with MM for 10 years and had been treated with thalidomide and IV zoledronic acid for 1 year. CT scan of facial bone was consistent with osteonecrosis of the jaw with possible, superimposed osteomyelitis. Jaw biopsy revealed necrosis with a fistulous tract and filamentous organism consistent with actinomycetes.
Discussion
Numerous reports and meta-analyses have linked bisphosphonate use with a rare, but significantly increased incidence of osteonecrosis of the jaw, especially in cancer patients treated with IV bisphosphonates. This is believed to be due to an anti-angiogenic effect resulting in inhibition and apoptosis of osteoclasts. Decrease in bone circulation and cellularity may impair bone remodeling in response to skeletal injury. Through inhibition of endothelial cell proliferation, bisphosphonates may compromise intraosseous blood flow, contributing to the development of bisphosphonate-related ONJ (BRONJ). Thalidomide also exerts an anti-angiogenic effect by inhibiting vascular endothelial growth factor (VEGF) and basic fibroblast growth factor. BRONJ was initially believed to be a direct, non-infectious complication of bisphosphonate therapy, however, recent histological/microbiological data strongly suggest that actinomyces play a pivotal role in the development of BRONJ.
Conclusions
Bisphosphonates and thalidomide have anti-angiogenic effects on bone predisposing to avascular necrosis and ONJ. Actinomyces are an underrecognized agent in the pathogenesis of ONJ, which can result in superimposed osteomyelitis. Timely actinomyces specific therapy in BRONJ may improve patients outcome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P162

**Effect of alcohol consumption on bone mineral density in healthy elderly Spanish males**

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Background
There are no data concerning a relationship between alcohol and bone status of elderly Spanish men. Previous studies in other countries on the influence of alcohol intake on bone mineral density (BMD) in men are inconsistent and the effect of these variables on BMD is yet to be explored.
Objective
The aim of this study was to describe the associations between total and beverage-specific alcohol intake and both BMD and bone ultrasound in elderly Spanish men.
Research methods and procedures
A cross-sectional study of 250 healthy elderly men ($>65$ years) was undertaken. BMD was measured by DXA. Bone ultrasound was also performed and

amplitude-dependent speed of sound (Ad-SoS) in the phalanges and broadband ultrasound attenuation (BUA) in the calcaneus were also determined. Lifestyle variables were collected through a structured questionnaire.
Results
Participants with a moderate intake of alcohol (20 g/day) had both a significantly higher BMD at femur ($P=0.0098$) and Ad-SoS ($P=0.0016$) compared to non-alcohol consumers. The intake of alcohol (g/day) significantly and positively correlated with BMD ($r=0.199$; $P=0.0051$) and Ad-SoS ($r=0.193$; $P=0.0096$) but not with BUA ($P>0.05$). The intake of beer (g/day) also positively correlated with BMD ($r=0.148$; $P=0.0392$) and BUA ($r=0.153$; $P=0.0428$) but not with Ad-SoS parameter ($P>0.05$). Wine intake (g/day) significantly correlated with BMD ($r=0.164$; $P=0.0209$) and Ad-SoS ($r=0.176$; $P=0.0178$) but not with BUA ($P>0.05$).
Conclusions
In elderly healthy Spanish men with well-defined lifestyle conditions, alcohol consumption was associated with higher femoral BMD, and bone ultrasound. The effect of alcohol is complex and will need further studies to clarify its role in bone metabolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P163

**Effect of alendronate on bone mineral density in primary hyperparathyroidism**

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Background
Bisphosphonates are capable of suppressing parathormone (PTH)-mediated bone resorption and can be used as an adjunct treatment in acute hypercalcemia. We investigated the effects of oral alendronate on bone mineral density (BMD) and biochemical markers of calcium and bone metabolism in patients with primary hyperparathyroidism (PHPT).
Methods
Twenty-six patients aged $58.5\pm13.9$ years with confirmed PHPT were treated with alendronate (70 mg once a week) for 1 year. The patients were low symptomatic or symptomatic and were unwilling to have parathyroid surgery. Five patients underwent previously unsuccessful parathyreoidectomy.
Results
After 1 year of treatment, mean BMD increased significantly at lumbar spine (2.5%), femoral neck (1.8%), ultradistal part of the forearm (3.2%) and total body (2.2%), but not at the distal part of the forearm. The highest increases in BMD were in the lumbar spine (13.4%) and ultradistal part of the forearm (17%). In some patients BMD decreased despite taking alendronate. Changes in BMD were not correlated with serum PTH, 25-OH vitamin D, baseline BMD values or age of the patients. PTH did not change with alendronate treatment (mean$\pm$S.E.M.: $164\pm13$ vs $144\pm10.5$ pg/ml of baseline value, reference range: 11–67 pg/ml). Serum calcium, and urinary calcium excretion did not change significantly from the baseline values. Mean alkaline phosphatase concentration decreased significantly.
Conclusions
Our results suggest that alendronate may be effective in decreasing bone resorption in some patients with PHPT and does not affect PTH or serum and urine calcium. The greatest increase in bone density occurs at ultradistal part of the forearm and lumbar spine: sites with a high content of trabecular bone which is more metabolically active than cortical bone.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P164

**Association of monocyte chemoatractant protein-1 and adiponectin with bone mineral density and osteocalcin in postmenopausal women**

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Recent findings suggested two-way interaction between bone and adipose tissue. Osteocalcin, a hormone of osteoblasts, was shown to stimulate insulin sensitivity by direct interaction with insulin. Adipose tissue has an important effect on insulin sensitivity by secretion of anti-inflammatory and proinflammatory adipocytokines like adiponectin and monocyte chemoattractant protein-1 (MCP-1). It is unknown whether they are related to bone metabolism.
Aim of this study was to examine the association of MCP-1 and HMW-adiponectin with bone mineral density (BMD) and osteocalcin in postmenopausal women.
In 32 postmenopausal women we determined serum levels of HMW-adoponectin, MCP-1, osteocalcin, insulin, glucose, triglycerides, HDL-cholesterol and C-reactive protein. Anthropometric measurements, body composition analysis and bone densitometry were performed. Participants did not have any metabolic or inflammatory diseases.
Positive correlation was found between femoral BMD and measures of obesity ($P<0.05$). Negative correlation of HMW-adiponectin with femoral BMD was noted, but it didn't remain significant after adjusting for body weight. HMW-adiponectin was negatively associated with measures of obesity and insulin ($P<0.05$), but MCP-1 showed no relationship with these parameters. Osteocalcin was inversely correlated with weight circumference, insulin and MCP-1 ($P<0.05$). Association between MCP-1 and osteocalcin remained significant even after adjusting for insulin and body weight.
In our results adiponectin did not appear to be among adipocytokines related with bone metabolism but the close association between osteocalcin and insulin was confirmed. Serum levels of MCP-1 in our study did not reflect its presumed role of chemokine secreted in obesity by macrophages in adipose tissue. In fact, the link between MCP-1 and osteocalcin seems to result from a mechanism that is independent from obesity and insulin sensitivity, for instance the previously demonstrated MCP-1 secretion by osteoblasts during administration of parathyroid hormone. This suggests that close links of bone and adipose tissue involve additional mediators with probably simultaneous impact on bone remodeling and energy metabolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P165

**Age-dependent and sexual features of bone mineral density changes in rats with hyperthyroidism**

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The aim of the research was studying the influence of age and sex on the changes of bone mineral density detected on rats with experimental hyperthyroidism.
Research object
Research is conducted on 180 white females and males rats of 'Wistar' breed. Animals were arranged in the 12 groups: six controls groups (CG) of pre-pubertal, reproductive and old males and females; six age-dependent groups of males and females rats getting L-thyroxin injection in a dose of 25 μg/100 g of body weight/day i.m., during 30 days.
Research methods
Bone mineral density (BMD) was measured using dual energy X-ray densitometry (DXA). The index was calculated according to the formula: $\Delta$BMD (%)=($\Delta$BMD/BMD ref.)$\times$100, BMD ref.-initial indexes of bone mineral density of the entire body.
Research results
It was revealed that high doses of L-thyroxine injections in during 30 days caused the different changes of BMD of rats. Only of the pre-pubertal females increased the $\Delta$BMD30 index with $15.83\pm1.99\%$ in CG to $44.73\pm5.41\%$, Student's t-criterion=4.49, $P<0.0001$, and does not change of $\Delta$BMD30 index in the pre-pubertal male group. The maximum loss of $\Delta$BMD30 index occurred in reproductive age, as for females rats (with $15.16\pm3.43\%$ in CG to $-10.04\pm2.32\%$, Student's t-criterion$=-6.22$, $P<0.0001$), so for males (with $0.63\pm0.97\%$ in CG to $-4.31\pm2.63\%$, Student's t-criterion$=-4.17$, $P<0.0001$). Not much was detected $\Delta$BMD30 index at old males rats (with $1.43\pm1.20\%$ in CG to $6.91\pm1.49\%$, Student's t-criterion=2.73, $P=0.012$).
Conclusions
It is revealed that high doses of L-thyroxine injections during 30 days caused the different changes of BMD depending on sex and age rats.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P166

**Sinomaxillary myopericytoma associated with oncogenic osteomalacia**

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Oncogenic osteomalacia (tumor induced osteomalacia-TIO) is a rare disease that can lead to severe physical handicap and pains. Its pathogenesis involves the secretion of fibroblast growth factor-23 inducing urinary phosphate loss and consequent hypophosphataemia. We report a case of a TIO caused by a tumor in the nasal cavity. In 2010, a 61-years-old woman was examined because of muscle weakness and arthralgia in the extremities. Her symptomes started ~3 years before her presentation. He denied any family history of metabolic bone disease. At presentation, she was unable to walk without support. Upon physical examination diminished muscle strength of upper and lower limbs was found without any neurologic alteration. Radiological images, osteodensitometry and technetium-99 m bone scan were characteristic for osteomalacia. Initial laboratory testing revealed a normal serum calcium level, very low phosphate level of 0.46 mmol/l (normal range: 0.84–1.45 mmol/l), an elevated alkaline phosphatase, a normal parathyroid hormone level and slightly decreased 25-hydroxivitamin D. Based on the low serum phosphate levels and her symptoms, the possibility of oncogenic osteomalacia was raised. Chest and abdominal CT was negative. Since the patient reported some episodes of nasal bleeding, an otorhinolaryngological examination was performed. This was revealed a mass in the left nasal cavity. The cranial MRI showed a large soft tissue mass in the left nasal cavity, the sphenoethmoidal recess and the frontal sinus. Given the possibility that this tissue mass was associated with the osteomalacia, it was removed by endoscopic surgery. The histological examination revealed a mesenchymal tumor, myopericytoma. One week later, levels of serum phosphate returned to the normal range, confirming the diagnosis of TIO. At 6-month follow-up, she felt well, she can walk without support and her serum biochemistry is normal.

Conclusion

Tumor induced osteomalacia is mostly associated with mesenchymal tumors that are often occurred in nasal sinuses. Tumor removal - as in our case- may lead to complete recovery.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P167

**Body composition in postmenopausal women with vertebral fractures**

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The aim of this study is evaluating body composition in postmenopausal women depending on presence of vertebral fractures in their anamnesis.

Materials and methods

We've examined 144 postmenopausal women aged 50–83 years (mean age – 62.9 ± 0.7 years; mean height – 160.6 ± 0.5 cm; mean weight – 71.9 ± 1.2 kg). The patients were divided into two groups: group A – women without vertebral fractures ($n=82$), group B – women with vertebral fractures ($n=62$) in their anamnesis. We also divided the patients into the following age-dependent groups: 50–59 years, 60–69 years, 70 years and older. Total body, lumbar spine, femoral neck bone mineral density (BMD), lean and fat masses were measured by DXA using a densitometer Prodigy, GE.

Results

Fat mass significantly decreased with age (50–59 years – 31 906.9 ± 1508.0 g; 60–69 years – 30 876 ± 1186.5 g; 70 years and older – 25 257.4 ± 1398.6 g; $F=5.0$; $P=0.008$). Lean mass didn't show significant differences. Fat and lean masses in postmenopausal women with vertebral fractures were significantly lower compared with the data of women without vertebral fractures (fat mass: group A – 42 089 ± 583.4 g, group B – 38 421.4 ± 652.4 g, $F=17.4$, $P=0.00005$; lean mass: group A – 31 783.7 ± 1212.1 g, group B – 27 831.4 ± 1048.5 g, $F=5.6$, $P=0.02$). We have founded the positive significant correlation between fat mass and BMD of spine and femoral neck depending on the presence of vertebral fractures in patient's anamnesis:

- Group A: total fat (g) = 1.06 + 0.26 × BMD (L1–L4); $r=0.07$; $t=2.40$; $P=0.02$.
Total fat (g) = 0.87 + 0.34 × BMD (femoral neck); $r=0.12$; $t=3.23$; $P=0.002$.
Group B: total fat (g) = 0.84 + 0.45 × BMD (L1–L4); $r=0.20$; $t=3.91$; $P=0.0002$.
Total fat (g) = 0.70 + 0.32 × BMD (femoral neck); $r=0.10$; $t=2.54$; $P=0.01$.

Conclusion

Fat mass was significantly decreased with age. Fat and lean masses significantly differ in postmenopausal women depending on the presence of vertebral fractures in their anamnesis. The positive significant correlation was found between fat mass and BMD of spine and femoral neck.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P168

**Bone mineral density in relation to metabolic syndrome in postmenopausal women with diabetes type 2**

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Diabetes type two is associated with greater bone mineral density (BMD) due to obesity although observed rapid bone loss over time could be explained with elevated chronic inflammation. The objective of this study was to investigate the relationship between central adiposity and hyperinsulinaemia as well as inflammation markers with vertebral and femoral BMD and bone turnover markers in postmenopausal women with type two diabetes. Femoral and vertebral BMD, osteocalcin, pyrilinks D, β-CrossLaps (B-CTx), insulin, CRP, fibrinogen and plasminogen activator inhibitor-1 (PAI-1) were measured in 114 female postmenopausal patients with diabetes type two. The patients similar in age, HbA1c levels and diabetes duration were divided in two groups based on their BMI values: lower or equal to 27 (31 patients) and higher than 27 kg/m$^2$ (83 patients). The sample means, S.D. and medians were calculated for all variables in each group, whereas *t*-test as parameter and Mann–Whitney as non-parameter test were used to calculate distribution differences for continuous variables. Spearman's correlation coefficients were computed to estimate the magnitude of the association between variables of interest. Lower levels of osteocalcin ($P=0.001$) and B-CTx ($P=0.000007$) compared to higher femoral BMD ($P=0.00006$) as well as insulin ($P=0.0002$), PAI-1 ($P=0.000000$) and CRP ($P=0.002$) were found in the overweight group. There were no significant differences in the vertebral BMD, pyrilinks D and fibrinogen. Osteocalcin and B-CTx were inversely correlated, while femoral BMD positively correlated with waist circumference, insulin levels and PAI-1 (Table 1). This suggests that components of the metabolic syndrome, abdominal obesity and hyperinsulinaemia could increase femoral BMD by lowering bone rate. In addition, the only inflammation marker linked with femoral BMD was PAI-1, which is associated with increased mineralization of cortical bone in mouse models.

Spearman's correlation coefficients for femoral BMD and bone turnover markers versus components of metabolic syndrome.

**Table 1**

| Femoral BMD<br>osteocalcin<br>B-CTx | Waist circumference | Insulin | PAI-1 |
|---|---|---|---|
| | $R=0.3616$<br>$P=0.001$ | $R=0.2981$<br>$P=0.005$ | $R=0.345$ $P=0.001$ |
| | $R=-0.2693$<br>$P=0.011$ | $R=-0.2439$<br>$P=0.021$ | $R=-0.2809$ $P=0.007$ |
| | $R=-0.3359$<br>$P=0.002$ | $R=-0.2239$<br>$P=0.037$ | $R=-0.2653$ $P=0.012$ |

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P169

**Assessment of bone turnover and vitamin D levels in women with postmenopausal osteoporosis and type 2 diabetes mellitus**

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Background
Past research suggests that diabetes mellitus, although with still insufficiently known mechanisms, has an effect on bone turnover.
Objective of this study was to determine the level of bone metabolism parameters and vitamin D in women with postmenopausal osteoporosis and type 2 diabetes mellitus.
Material and methods
The prospective study included 30 women with postmenopausal osteoporosis and type 2 diabetes mellitus and a controls – 30 women with postmenopausal osteoporosis. Osteoporosis is verified by lumbar spine and hip DXA. Levels of osteocalcin, β-crosslaps and 25(OH)D3 were measured.
Results
Diabetes mellitus duration was $15.37 \pm 11.08$ years. HbA1c was $7.52 \pm 1.62\%$. Women with diabetes mellitus was $61.6 \pm 8.07$years old and controls $61.9 \pm 7.23$ years. Women with diabetes mellitus BMI was $26.61 \pm 5.45$ kg/m$^2$ and $24.77 \pm 3.57$ kg/m$^2$ for controls. There was no statistically significant difference in age or BMI. Women with diabetes mellitus L1-L4BMD was $0.846 \pm 0.075$ g/cm$^2$, total hip $0.746 \pm 0.108$ g/cm$^2$ and femoral neck $0.704 \pm 0.078$ g/cm$^2$. Controls L1-L4BMD was $0.799 \pm 0.058$ g/cm$^2$, total hip $0.787 \pm 0.085$ g/cm$^2$ and femoral neck $0.749 \pm 0.084$ g/cm$^2$. BMD of the lumbar spine in women with diabetes mellitus were higher, without statistical significance, while the hip BMD was significantly lower on the femur neck ($P < 0.05$). Women with diabetes mellitus osteocalcin was $29.68 \pm 11.67$ ng/ml, crosslaps $530.73 \pm 239.96$ pg/ml and 25(OH)D3 $37.9 \pm 20.22$ nmol/l. Controls: osteocalcin $32.83 \pm 7.76$ ng/ml, crosslaps $525.03 \pm 167.94$ pg/ml, and 25(OH)D3 $40.85 \pm 17.79$ nmol/l. Among these parameters there was no statistically significant difference. All women with diabetes mellitus had a lack of vitamin D, and 14(46.7%) of them had vitamin D deficiency. Controls: sufficient levels of vitamin D had 2 (6.7%) women; vitamin D deficiency had 9(30%) women.
Conclusion
In women with type two diabetes mellitus bone density at the femur and the femoral neck is lower. There is no statistically significant difference in bone turnover in women with type two diabetes mellitus compared to healthy women with osteoporosis. Inadequacy of vitamin D is more often present in women with type two diabetes mellitus than in healthy women with osteoporosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P170

**Osteoblast and adipocyte differentiation from human adipose tissue-derived cells**

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Human adipose tissue provides abundant source of mesenchymal stem cells, which can be easily and safely harvested as compared with human bone marrow. The aim of this study was to differentiate a viable osteoblast and adipocyte cell culture from mesenchymal stem cells, an experimental model to study the interrelation between adipocytes and bone forming cells, osteoblasts.
Materials and methods
Subcutaneous adipose tissue liposuction aspirate was obtained from a female subject, aged 27 years undergoing plastic surgical procedure in a specialized clinic, after she gave the informed consent.
The adipose tissue sample was extensively washed with sterile PBS containing antibiotic/antimycotic (AA) and let 30 min to decant. Blood fraction was collected into 50 ml conical tubes. Cells were cultured in DMEM, 10% FBS, AA. After 3 weeks, cells were splitted and seeded in osteogenic (DMEM, 10% FBS, 100 nm dexametasone, 10 mm beta glycerolphosphate, 0.05 mm ascorbic acid, 1% AA) and adipogenic medium (DMEM, 10% FBS, 1 μm dexametasone, 10 μm insulin, 200 μm indomethacin, 0.5 μm IBMX, 1% AA) respectively. Specific phenotype was assessed by von Kossa specific staining for osteoblasts and Oil red staining for adipocytes.
Results
The specific phenotype occurred in the second passage. Cell growth rate was higher in osteoblasts than adipocytes (4.5 vs 3.2 in passage 7). Osteoblasts were fully diferentiated (mineralized) in passage six. The log phase of the cellular growth curve was 10 passages long, that offers a reasonable period of time for experiments that require adipocyte-osteoblast co/culture.
Conclusion
Differentiation of osteoblasts and adipocytes starting from the same cellular sample from human adipose-tissue stromal derived cells confers a great advantage for co-culture studies because immunological rejection is overcome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P171

**Denosumab increases BMD in primary hyperparathyroidism**

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Primary hyperparathyroidism (PHPT) is often associated with reduced bone mineral density (BMD). An open-labeled, prospective trial was conducted to determine whether denosumab 60 mg subcutaneously given every 6 months, maintains or improves BMD in patients with PHPT.
Six patients had symptomatic PHPT and met surgical guidelines however refused surgery, and twelve patients had mild PHPT, asymptomatic except low BMD. The primary outcome index, BMD, was measured at the lumbar spine (LS) and femoral neck (FN) after six and 12 months by dual-energy X-ray absorptiometry. Serum calcium, phosphorous and PTH, and urinary calcium excretion were monitored every 3 months.
Treatment with denosumab was associated with a significant ($5.8 \pm 0.4\%$; $P < 0.01$) increase in LS BMD after 12 months in comparison with baseline. FN BMD increased significantly at 12 months with denosumab by $2.3\% \pm 0.7$ ($P < 0.01$) from baseline. Serum calcium, phosphorus and PTH, and urine calcium excretion did not change significantly with denosumab therapy. In PHPT, denosumab given subcutaneously twice yearly, significantly increases BMD at the LS and FN at 12 months from baseline values with stable serum calcium and PTH levels. Denosumab may be a useful alternative to parathyroidectomy in PHPT among those with low BMD, who are candidates for surgery but either decline or for whom surgery is contraindicated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P172

**Relationship between bone metabolism markers, bone mineral density and insulinresistance in healthy and diabetic postmenopausal women**

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Introduction
Some metabolic diseases, including type 1 (T1DM) and type 2 (T2DM) diabetes mellitus have an influence on bone homeostasis. These facts are of great socio-medical impact, since currently the DM2 and osteoporosis are considered public health problems.
Objectives
(1) Determine the relationship between markers of bone metabolism and insulin resistance in postmenopausal women with and without T2DM.
(2) Compare the bone mineral density between the two groups.
Methodology
Prospective and case–control study. Postmenopausal women attending in outpatient Endocrinology from January to December 2010 were included consecutively. T2DM patients treated with oral hypoglycemic agents (except glitazones) and patients with other diseases that not interfere with bone

metabolism were included. The study included 18 women with diabetes and 58 without diabetes. The average age of the sample was 54.6±1.8 years. In the diabetic group the duration of diabetes was 4.6±2.4 years. Measurements included anthropometric (BMI), biochemical (insulin, C-peptide, HOMA-IR, P1NP, β-crosslap, osteocalcin, 25-OH-vitamin D, PTH, calcium, phosphorus, renal function) and radiographic (bone densitometry: *T*-score) variables.
Results
BMI was similar between groups. The HOMA-IR was 7.9±0.6 in the DM2 group and 3.0±0.7 in the control group ($P<0.05$). Osteocalcin was lower in diabetics (14.6±3.8 ng/ml, $P<0.01$) than in healthy patients. The same trend was observed at concentrations of P1NP. In densitometry, 20% of diabetics had osteoporosis and 40% osteopenia. In the healthy group, 35% had osteoporosis and 52% osteopenia. In the diabetic group HOMA-IR was associated directly with BMD ($r=0.568$, $P<0.05$) and HOMA-IR inversely with osteocalcin ($r=-0.624$, $P<0.05$). Also in the group of healthy patients with P1NP was related directly with BMD and HOMA-IR inversely with BMD.
Conclusions
Insulin resistance is a predictor of BMD. Osteocalcin is related to insulin resistance, as it is clearly diminished in diabetics. P1NP concentration is related directly with BMD in the healthy group. Osteoporosis and osteopenia were higher in the healthy patients than in diabetic patients. More studies are needed to explain the fact why despite having higher BMD, diabetics are more likely to fracture than healthy women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P173

**The relation between quantitative characteristics of smoking and level of female skeleton damage**

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Objective
Skeletal disability pathogenesis is multifactorial. Many studies have proved a negative relation of smoking on calcium-phosphate metabolisms, bone density and quality; however in some other papers these results have not been confirmed. The aim of the study was to monitor the influence of the length of smoking, a number of cigarettes smoked and the age of the first use of cigarettes on the whole-body bone density.
Methods
Whole-body densitometry determining bone and soft tissues using dual-emission X-ray absorptiometry (DXA) was measured with 40 women, where 22 women were premenopausal (average age 35±8) and 18 women postmenopausal (average age 57±5). All probands had been smoking in the long term continuously until the day of examination. Partial correlations adjusted to constant age and physical activity were used to evaluate relations between the smoking intensity indicators on one side and anthropometric characteristics and bone quality indicators on the other side.
Results
Monitoring the effect of smoking upon whole-body bone density and the volume of muscle mass with women, we have not proved statistically significant relation of the whole-body mass to the number of cigarettes smoked, or to the length of smoking, or to the age of the first use of cigarettes.
Conclusions
Though the study is not fully comparable with those monitoring the relation of smoking to bone density of the selected high-risk skeleton localities, fundamentally it comes up with the identical results when compared with other studies.
Acknowledgement
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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P174

**The assessment of the behavior of the interleukins IL1β and IL6 and bone mineral density (BMD) and bone metabolism (IBM) in women of postmenopausal age**

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Women's bone metabolism is regulated by estrogens and interleukins. During the menopausal period the estrogens' protective activity is decreased and the production of IL1β and IL6 is increased.
The aim of the study
The assessment of the dependency between IL1β i IL6 and estradiol values and BMD and IBM in postmenopausal women.
Material
Forty-eight women, age 49–61, without menstruation and not edocrinologically treated. Group I – 19 women with normal BMD, Group II – 29 women with osteoporosis.
Methods
Densitometric (BMD, *T*-score). Radioisotopic dynamic and static scintigraphy Tc 99 m MDP and IBM designation. Biochemical interleukins IL1β and IL6 and E2, P, ALP, Ca+i TSH.
Results
In Group I - IBM within the normal values range $86.2<IBM<98.9$, in Group II, the IBM within $75.1<IBM<90.3$ (in 40% of women the IBM was below the normal values).
Group I – a strong positive correlation between BMD and IBM
Group II – a medium positive correlation between BMD and IBM
Group I – normal biochemical and hormonal values
Group II – elevated concentrations of phosphatase and phosphates.
The estradiole value in Group I was higher than in Group II. The difference was statistically significant.
The interleukins concentrations in Group II were significantly higher than in Group I (statically significant $P=0.000$).
In Group I there was no correlation and in Group II a negative correlation between BMD and interleukins.
Conclusions
During the menopausal period the estradiol's decrease is accompanied by the increase of interleukins IL1β and IL6 which results with the loss of bone mass and slower bone metabolism and degradation of bone quality parameters.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P175

**Correction of bone mineral density losing in female rats with exogenous thyrotoxicosis with calcium and alendronate preparations**

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Numerous hormonal and metabolic disorders caused by thyrotoxicosis lead to serious complications, so early diagnosis and adequate treatment becomes very important in warning of functional and organic changes in several organ systems, particularly in the structure of bone tissue.
The aim was study of the effectiveness of calcium and alendronate for prevention and treatment of BMD losing in experimental models of thyreotoxicosis.
Material and methods
Used 47 female Wistar rats 8-10 month age with body mass 207.81±3.27 g. Depending on the medication, animals are divided into five groups: isotonic solution of sodium chloride (control), L-thyroxine, L-thyroxine and calcium, L-thyroxine, calcium and alendronate, L-thyroxine for two weeks before and during the experiment, calcium and alendronate. L-thyroxine (25 mcg/100 g) and solution of sodium chloride injected sucutaneously daily, calcium (2.1 mg/100 g) daily and alendronate (1.75 mg/100 g) once at week entered through the esophageal probe. BMD of whole skeleton, spine and hind limbs measured at

the beginning and after five weeks of experiment using a densitometer Prodigy (GE Medical systems). Data were analyzed using ANOVA and Student's *t*-test to examine differences among the groups.
Results
In the control group of animals BMD increases in all parts of the skeleton (1.25 ±3.2% to 15.76±1,05%). The greatest loss of BMD was observed in L-thyroxine medicated rats (−5.95±2.36 to −14.13±1.46%). In the group of animals with thyrotoxicosis that received medication calcium, observed retardation of sceleton growth and a moderate loss of BMD from (0.49±2.13% to −5,11±2.52%), that has significant differences with group with thyreotoxicosis at hind limbs and whole sceleton. In groups of animals with thyrotoxicosis and calcium and alendronate medication observed increasing BMD that has not significant differences with control group.
Conclusion
In the experiment, exogenous hyperthyroidism resulted in reliable loss of BMD, mainly peripheral skeleton (by 14.13% at the hind limbs and 5.95% at the spine). Use of calcium during exogenous hyperthyroidism in female rats significantly reduces bone loss. The combination of calcium and alendronate prevents losing BMD, regardless of the duration of hyperthyroidism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P176

**Reference intervals for serum N-MID osteocalcin concentration measured with the IDS-iSYS automated system**

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Background
Osteocalcin (OC) is a bone-specific protein produced primarily by osteoblasts during bone formation. The OC concentration is used to assess fracture risk and monitor treatment of osteoporosis and other disorders of bone metabolism. To adequately interpret the OC concentration, it is necessary to calculate reference ranges from a healthy reference population, adapted to a specific laboratory method.
Methods
We established a healthy reference population from the participants of the first follow-up of the Study of Health in Pomerania. Serum OC concentrations were measured from frozen aliquots with the IDS-iSYS N-Mid Osteocalcin assay on the IDS-iSYS Automated System (Immunodiagnostic Systems, Frankfurt am Main, Germany). The coefficients of variation were 6.98% at low, 6.44% at medium, and 5.44% at high levels of control material. The reference interval was defined as the central 95% range between the 2.5th and the 97.5th percentile. Age-specific reference intervals were calculated for men aged 25–80 years and for pre-menopausal women aged 25–54 years by means of quantile regression. As in post-menopausal women aged 50–80 years OC was not influenced by age, we calculated an age-independent reference interval.
Results
Median (1st–3rd quartile) OC concentrations were 15.4 ng/ml (12.0–19.5 ng/ml) in 1119 men, 14.4 ng/ml (11.3–18.5 ng/ml) in 545 pre-menopausal women, and 18.7 ng/ml (13.7–25.6 ng/ml) in 502 post-menopausal women. Median OC concentrations were highest in 25–29-year-old men and women, were stable during the middle ages, and rose again after 65 years of age in men, and at transition to post-menopause in women. We observed that subjects with type 2 diabetes, intake of oral contraceptives or hormone replacement therapy had lower OC concentrations than subjects without these conditions.
Conclusion
We present sex-specific reference intervals for the serum OC concentration over a broad range of age groups to assess bone metabolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P177

**Prevalence of morphometric vertebral fractures and bone quality measured by spinal deformity index in type 1 diabetic patients**

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Background and objectives
There are a lot of studies demonstrating low bone mineral density (BMD) and elevated fracture risk in patients with type 1 diabetes. But there are scarce data about the prevalence of morphometric vertebral fractures (MorfoFx) and spinal deformity index (SDI) that is considered as a surrogate marker of bone quality. Therefore, the objective of this study was to evaluate BMD, the prevalence of MorfoFx and bone quality by SDI in type 1 diabetics.
Methods
There were evaluated 82 patients type 1 diabetes (26 males, 56 females, age 31.1±8.3 years, BMI 23.2±3.3 kg/m$^2$, disease duration 12.9±7.8 years) and age-, sex-, BMI-matched 48 controls (12 males, 36 females, age 33.8±7.3 years, BMI 23.0±4.7 kg/m$^2$). BMD and MorfoFx were measured with DXA. After then SDI was calculated in all patients.
Results
Type 1 diabetic patients, comparing with controls, had lower BMD either at spine (−0.5±1.2 vs 0.03±1.0, $P=0.012$ respectively) and at femoral neck (−0.7 ±1.1 vs 0.02±0.8, $P<0.001$ respectively), higher prevalence of MorfoFx (26.8%, $n=22$ vs 10.4%, $n=5$, $P=0.027$ respectively) and higher SDI (0.5±1.1 vs 0.15±0.5, $P=0.031$ respectively). Age, diabetes duration, age of diabetes manifestation, BMD and the prevalence of chronic complications were not different between type 1 diabetics with and without MorfoFx. In the multiply regression analysis MorfoFx/SDI were not associated with BMD, diabetes duration, age of diabetes manifestation, the presence of complications.
In conclusion, this study demonstrates that type 1 diabetic patients have low BMD, elevated prevalence of MorfoFx and poor bone quality evaluated by SDI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P178

**Clinical evaluation of a new specific 1–84 PTH automated platform**

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Introduction
2nd generation 'Intact' PTH assays currently available, measure 1–84 PTH and additional fragments. Recently a third generation kit developed which detects only active.
1–84 PTH molecule, based on an immuno-radiometric sandwich assay.
Design
Evaluation took place in an HMO central lab. 240 plasma samples collected. One hundred and sixty-one randomly out patients and 78 hospitalized nephrologic patients. Patients samples, controls and NEQAS samples tested by CLIA.
Method
DiaSorin LIAISON 1–84 PTH assay, DiaSorin LIAISON N-TACT PTH II, SIEMENS Centaur iPTH and DiaSorin IRMA N-tact PTH SP ('Gold Standard'). Results were compared with laboratory parameters and patients clinical status.
Results
Correlation between LIAISON 1–84 PTH and LIAISON N-TACT PTH II showed $R=0.9164$, slope=0.9076. Correlation between LIAISON 1–84 PTH and Centaur iPTH showed $R=0.98$, slope=2.7622. Correlation between LIAISON 1–84 PTH and IRMA N-tact PTH SP showed $R=0.97$, slope=1.0893. The following LIAISON 1–84 PTH parameters obtained: samples and controls within run precision showed 2.58 and 5.34% CV. Samples and controls between run precision showed 4.03 and 4.65% CV. Functional sensitivity matched manufacturer claim at 4 pg/ml. Recovery between 77 and 101%. Clinical agreement between LIAISON 1–84 PTH and IRMA N-tact PTH SP on nephrologic patients was 97.5%. Three discrepant patients were clinical matched with LIAISON 1–84 PTH results. From the healthy out patients tested samples

54% were found high with SIEMENS Centaur iPTH and 16% were high with LIAISON 1–84 PTH. The same behavior observed with NEQAS data.
PTH levels were influenced similarly by low Ca levels.
Conclusions
High diagnostic correlation was observed between IRMA N-tact PTH SP and LIAISON 1–84 PTH together with high clinical correlation of patients status. It is concluded that the LIAISON 1–84 PTH can be used as a reliable and accurate kit for setting up a clinical and high throughput laboratory.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P179

### Successful treatment of thyrotoxicosis is accompanied by a decrease in serum sclerostin levels

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Sclerostin, a product of a SOST gene, is a protein expressed by osteocytes that inhibits osteoblastic bone formation. Several hormones including PTH and glucocorticosteroids and have been suggested as possible regulators of sclerostin production. The influence of thyroid hormones on sclerostin synthesis has not been investigated so far. The aim of the study was to evaluate sclerostin concentrations in patients before and after treatment of thyrotoxicosis.
Patients and methods
The study involved 15 patients (four men), mean age 51.8 ± 15.3, mean BMI 24.7 ± 3.5, with thyrotoxicosis due to Graves' disease or toxic multinodular goitre. Serum sclerostin was measured by immunoassays at diagnosis of thyrotoxicosis and after 6–10 weeks of treatment with thiamazole. The data were analysed by means of simple descriptive statistics of location and dispersion and Mann–Whitney *U* test for pairs of results 'before' and 'after' therapy. Association between variables was evaluated with use of Spearman correlation coefficient.
Results
Here was a significant decrease in free $T_3$ and free $T_4$ concentrations (from 8.74 ± 4.79 to 3.54 ± 2.40 pg/ml, and from 4.48 ± 2.21 to 1.02 ± 1.07 ng/ml, $P < 0.001$), for free $T_3$ and free $T_4$ respectively. This was accompanied by a marked decrease of serum sclerostin levels from 55.46 ± 20.90 to 35.73 ± 15.70 pmol/l, $P < 0.0015$). Interestingly, however, sclerostin levels did not correlate with serum free $T_3$ or free $T_4$ concentrations.
Conclusions
Restoration of a euthyroid state in patients with thyrotoxicosis results in a significant decrease in serum sclerostin concentrations. The above mentioned phenomenon may reflect lowering of bone metabolism, but possible direct influence of thyroid hormones on SOST gene needs to be investigated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P180

### Investigating recurrent hypophostaemia

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Oncogenic osteomalacia is a rare paraneoplastic phenomenon characterised by abnormal phosphate metabolism typically caused by discrete benign tumours. Due to the indolent presentation and slow progression, diagnosis is often delayed and localisation of the tumour can prove difficult. We present the case of a 64-years-old gentleman who was investigated by the endocrinology department for hypophosphataemia. Three years prior to this, he had started to experience exertional fatigue and myalgia. Within the same time frame, he sustained a minor injury to his ankle and later developed severe pain in the joint lasting several months. This was attributed to Looser zones on plain X-ray. He had no further significant medical history. Development and growth had been normal. Relevant results are tabulated. The low Tmp/GFR value confirmed renal phosphate wasting. In the context of a mildly elevated PTH level and inappropriately normal 1,25 (OH) Vitmain D/ FGF 23 levels, the hypophosphataemia was deemed consistent with oncogenic osteomalacia. Though CT images demonstrated low attenuation hepatic lesions, these were characterised as insignificant on MRI. No potential source was identified on functional imaging modalitites (octreotide scintigraphy, FDG-PET imaging or Ga68 DOTATATE PET). Symptomatic relief was gained with Sando Phosphate and alfcalcidol. Biochemical parameters normalised (fasting phosphate 0.77 mmol/l, ALP 117 μ/l) and a repeat bone scan showed full resolution in the areas of increased activity. Our case illustrates the difficulties in identifying tumours associated with this paraneoplastic phenomenon and how, despite unsuccessful localisation, medical therapy can be effective in the absence of definitive treatment.
Results

**Table 1**

| Variable | Values | Reference range |
|---|---|---|
| Fasting Phosphate Level | 0.53 mmol/l | 0.8–11.4 mmol/l |
| Corrected Calcium | 2.24 mmol/l | 2.15–2.60 mmol/l |
| Creatinine | 100 mmol/l | 75–114 mmol/l |
| Parathyroid Hormone Levels | 14.6 pmol/l | 1.1–6.8 pmol/l |
| Alkaline Phosphatase | 255 μ/l | 30–130 μ/l |
| 25-OH Vitamin D | 51.1 nmol/l | 25–100 nmol/l |
| 1–25(OH) Vit D | 73 pmol/l | 40–150 pmol/l |
| Fibroblast Growth Factor-23 | 63 RU/ml | < 100 RU/ml |
| 24 hour urinary phosphate excretion | 20.73 mmol | 16–48mmol |
| Fractional excretion of phosphate | 14% | |
| Tubular maximum for phosphate corrected for GFR (TmP/GFR) | 0.35 mmol/l | 0.89–1.34 mmol/l (corrected for age and gender) |
| Bone Scintigraphy | Multiple sites increased activity throughout skeleton with few small hot spots | |
| Bone Mineral Density (DEXA) Scan | T4-L2: (T score) −2.7: Femoral neck: (T score) −1.7 | |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P181

### Ovariectomy modulates the effect of phytoestrogens on creatine kinase specific activity in skeletal organs in female rats

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Ovariectomy of immature female rats, results in significant decreased parameters in different organs. Previously we found that estradiol-17b ($E_2$) restored these parameters in the different organs of ovariectomized female rats (Ovx) to values obtained in intact immature female rats. $E_2$ also stimulated creatine kinase specific activity (CK) a hormonal-responsive marker in organs containing estrogen receptors. In the present study, we compared the effects of $E_2$ to those of the phytoestrogens: quercertin (Qu), daidzein (D), genistein (G), biochainin A (BA) and their carboxy-derivatives cD, cG and cBA in immature and Ovx female rats, on CK in diaphyseal bone (Di) and epiphyseal cartilage (Ep) as well as uterus (Ut) and pituitary (Pi), when injected for 24 h with and without the SERM raloxifene (Ral), or with and without pre-treatment for three days with the less-calcemic vitamin D analog JK 1624F2-2 (JKF). Ovariectomy resulted in significantly reduced CK levels in all organs tested. All estrogenic compounds tested in both age groups stimulated CK. Ral stimulated CK in all organs except Ut but inhibited enzymatic stimulation by $E_2$ and some of the estrogenic compounds in an age- and organ-specific patterns. Pre-treatment with JKF increased CK response to $E_2$ and some of the estrogenic compounds in age- and organ-specific patterns. In summary, estrogenic target organs of female rats are

modulated differently in an age- and organ-dependent manner in a yet unknown mechanism which might be connected to estrogen receptors in the organs.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P182

**Bone turnover markers in medicamentous and physiological hyperprolactinemia in female rats**

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Background
The aim of this study was to compare the influence of medicamentous and physiological hyperprolactinemia on bone turnover in female rats.
Methods
Experimental animals (18 weeks old, Wistar female rats) were divided in the following groups: Group I: 9 rats, three week pregnant; Group II: ten female rats that were i.m. administrated Sulpirid (10 mg/kg) twice daily for three weeks; Group III: ten female rats that were i.m. administrated Sulpirid (10 mg/kg) twice daily for six weeks; Group IV: ten female rats, control group. Laboratory investigations included serum and urinary calcium, inorganic phosphorus, alkaline phosphatase and serum procollagen type 1 N-terminal propeptide (P1NP).
Results
Experimental animals in Group I (physiological hyperprolactinemia) compared to control group, displayed lower mean serum calcium ($0.5 \pm 0.2$ vs $1.12 \pm 0.04$ mmol/l; $P<0.001$); higher mean serum phosphorus ($2.42 \pm 0.46$ vs $2.05 \pm 0.2$ mmol/l; $P<0.05$); decreased urinary calcium ($0.13 \pm 0.06$ vs $0.39 \pm 0.18$ mmol/mmol creatinine;$P<0.001$) and significantly increased P1NP ($489.22 \pm 46.77$ vs $361.9 \pm 53.01$ pg/ml; $P<0.001$). Experimental animals in Group II (medicamentous hyperprolactinemia) had significantly decreased P1NP, compared to control group ($309.6 \pm 36.74$ vs $361.9 \pm 53.01$ pg/ml; $P<0.05$). Prolongated medicamentous hyperprolactinemia (Group III) induced increased serum calcium ($1.21 \pm 0.025$ vs $1.12 \pm 0.04$ mmol/l; $P<0.05$); decreased serum phosphorus ($1.69 \pm 0.13$ vs $2.05 \pm 0.2$ mmol/l; $P<0.001$); decreased alkaline phosphatase ($49.61 \pm 14.39$ vs $77.07 \pm 22.6$ U/l; $P<0.01$) and significantly decreased P1NP ($291.7 \pm 71.03$ vs $361.9 \pm 53.01$ pg/ml; $P<0.05$).
Conclusions
Physiological hyperprolactinemia does not determine such harmful effect on bone metabolism as medicamentous hyperprolactinemia. Chronic medicamentous hyperprolactinemia displayes lower serum levels of P1NP, which reflects poor bone formation. Assessment of bone turnover markers in prolongated medicamentous hyperprolactinemia, provides an opportunity for earlier diagnosis of bone metabolism disturbances and should be considered as mandatory.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P183

**Adiponectin and bone mineral density in obese postmenopausal women**

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Introduction
Adiponectin and its receptors are expressed in human osteoblasts, suggesting that adiponectin may be a hormone linking bone and fat metabolism. In contrast to adiponectin anabolic effect on bone *in vitro* studies, clinical and animal studies suggest that adiponectin may have negative effects on bone by stimulating the receptor activator of nuclear factor-κB ligand (RANKL) pathway and inhibiting the production of the decoy receptor for RANKL and osteoprotegerin. Several clinical studies have shown a negative correlation between adiponectin and bone mineral density (BMD) in females independently of confounding factors.
Aim
The aim of this study was to investigate the relationship between adiponectin and bone mineral density in obese postmenopausal women.
Methods
Eleven obese postmenopausal women were recruited in our study (mean age $= 51.8 \pm 4.21$ years, mean BMI $= 30.5 \pm 2.29$ kg/m$^2$). Levels of adiponectin (ng/ml) were measured using ELISA test (Mercodia, Sweden). Bone mineral density (BMD) was assessed by dual energy X-ray absorptiometry (DXA) at the level of lumbar spine L2–L4 (BMD L2–L4) and left hip (Hologic). Statistical analysis was performed by SPSS 19. Pearson's correlation coefficients were calculated to evaluate the relationship between BMD and adiponectin.
Results
Adiponectin was inversely associated with BMD of left hip, ($r = -0.910$, $P<0.05$), *T*-score of left hip ($r = -0.992$, $P<0.01$). There was no statistically significant correlation between BMD of lumbar spine (L2–L4), *T*-score of lumbar spine and adiponectin.
Conclusion
Our result confirmed negative correlation between adiponectin and BMD of left hip but not with BMD of lumbar spine in obese women which suggest need for further investigation and elucidation of this interrelationship between adipokines and BMD.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P184

**The body mass index and heel quantitative ultrasound analyze in 300 women**

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Introduction
The heel Quantitative Ultrasound (QUS) evaluates the bone health, with good results if the clinical risk factors are also evaluated for each patient. Among these, the body mass index (BMI) may be protective or risk factor for a fragility fracture, depending on the values.
Aim
We present a study in postmenpausal women evaluated by QUS and BMI.
Patients & method
This is a study in 330 patients (women in menopause who were drug free for osteoporosis). To all of them we evaluated the BMI and heel QUS (GE Lunar Achilles device). The QUS analyze was performed using the Stiffness Index (SI), in Units (U) a combined parameter, useful in evaluation of the fracture risk.
Results
Based on BMI, we formed three groups based on BMI: group 1 BMI $\leq 24.9$ kg/m$^2$, group 2 with BMI between 25 and 29.9 kg/m$^2$, and group 3 with BMI $\geq 30$ kg/m$^2$. The av. values for BMI in the three groups were: $22.45 \pm 1.99$, $27 \pm 1.64$, $34.5 \pm 4.09$ kg/m$^2$. The av. age for the three groups was: 56.3 +/7.99 years, $58.26 \pm 8.44$ years, $55.45 \pm 7.6$ years. The av. SI was for the three groups: $70.35 \pm 16.44$ U, $79.16 \pm 17.54$ U, $83.39 \pm 18.32$ U. The SI increases with BMI. The student *t*-test between the normal weight and overweight group was $P=0$, between obese and normal was $P=0$, and between overweighed and obese was $P=0.06$.
Conclusions
We found statistically significant results regarding the QUS-SI, between the BMI groups, meaning that a higher SI, so a lower fragility fracture risk associates a higher BMI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P185

**Cimetidine (histamine H2-receptor antagonist and antiandrogenic drug) exerts a beneficial effect in the injured tissues by periodontal disease in male rat molars**

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Studies have demonstrated the presence of androgen receptors in osteoblasts and osteocytes, confirming that androgens are important for the maintenance of bone in men. Additionally to steroid hormones, histamine stimulates osteoclast differentiation and survival and plays a role in the inflammatory response via H2 receptors. We purposed to investigate whether cimetidine, an histamine H2 receptors antagonist and antiandrogenic drug, interferes in the periodontal tissue damages induced by periodontal disease (PD). PD was induced in the upper left first molars of male rats. Two PD groups ($n=8$ per group) were treated with 100 mg/kg bw of cimetidine (PD-Cim) or saline (PD-S), for 50 days. The right molars were used as controls: Cim and S groups, respectively. Blood samples were collected for detection of testosterone levels and the fragments of maxilla were removed and fixed in formaldehyde, decalcified in EDTA, and embedded in paraffin. Some fragments were processed for transmission electron microscopy (TEM). In HE-stained sections, the distances between cemento-enamel junction (CEJ) and the AB crest, as well as CEJ and junctional epithelium insertion level (JE) were measured; the number of inflammatory cells was also computed. TRAP method (osteoclast marker) was used for quantification of osteoclasts in the AB surface. Statistical analyses were performed ($P \leq 0{,}05$). Testosterone levels decreased significantly in cimetidine-treated rats. The significant increase in both CEJ-AB crest and CEJ-JE distances, observed in PD groups, were significantly softened in PD-Cim group. In Cim and PD-Cim groups, osteoclasts number decreased significantly in comparison to S and PD-S, respectively. Moreover, these cells exhibited apoptotic features under TEM. The inflammatory process reduced significantly in PD-Cim group. Although reduced testosterone levels promote osteoporosis, cimetidine treatment was able to reduce PD-induced periodontal tissue injuries. The possible histamine H2 receptors antagonist effect should be considered.

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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P186

**Age-dependent and sexual features of bone mineral density changes in rats in remote terms after hyperthyroidism abolition**

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The aim
To study age-dependent changes of bone mineral density of females and males rats in remote terms after hyperthyroidism abolition.
Research Object
Research is conducted on 180 white females and males rats of 'Wistar' breed. Animals were arranged in the 18 groups: six controls groups (CG) of pre-pubertal, reproductive and old males and females; six groups of rats with the experimental model hyperthyroidism (HTG); six groups of rats 30 days after stopping L-thyroxine injections (STI).
Research methods
Bone mineral density (BMD) was measured using dual energy X-ray densitometry (DEXA) and 'Experimental animals' software.
Research results
Through 30 days after STI, was revealed decline the Δ BMD of pubertal females rats at 4.3 time (with $44.73 \pm 5.41\%$ in HTG to $10.47 \pm 6.32\%$, Student's $t$-criterion $t = -4.03$, $P < 0.0001$) and of males at 5,7 time (with $22.83 \pm 2.84\%$ in HTG to $44.73 \pm 5.41\%$, Student's $t$-criterion $= 4.49$, $P < 0.0001$). Only reproductive females cans recover a total D BMD to the level of age control group (with $-10.04 \pm 2.32\%$ in HTG to $22.63 \pm 6.87\%$, Student's $t$-criterion $= 5.68$, $P < 0.0001$.). The reproductive males showed only 70 percent recover of DBMD (with $-4.31 \pm 2.63\%$ in HTG to $6.69 \pm 3.04\%$, Student's $t$-criterion $= -2.64$, $P < 0.015$). In old males rats D BMD was declined (with $6.91 \pm 1.49\%$ in HTG to $-3.75 \pm 1.93\%$, Student's $t$-criterion $= 4.30$, $P < 0.0001$). For females a decline of DBMD (%) was not statistically considerable.
Conclusions
The study age-dependent and gender characteristics of changes in bone mineral density of rats 30 days after hyperthyroidism abolition the exposed, that BMD is restored only at reproductive females of rats and a partial (70%) at reproductive males. The decline of BMD is exposed in other investigational groups.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P187

**Features of bone mineral density losing in ukranian woman of different ages with thyrotoxicosis**

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Disorders of bone tissue as a complication of hyperthyroidism now become accepted fact. On other side decreasing of serum levels of estrogens in postmenopausal women potentially may increase effects of thyroid hormones on bone tissue.
The aim of our study was investigate with DXA the features of loss of bone mass in women suffering from thyrotoxicosis depending of age and thyrotoxicosis duration.
Material and methods
There were examined 192 Ukrainian women with age from 25 up to 72 years: 95 with thyrotoxicosis, which have a history of disease from 3 month to 20 years and 97 healthy women. Subjects were divided to five age groups (20–29, 30–39, 40–49, 50–59 and 60–72 years). BMD measurements of the lumbar spine (L1–L4), proximal femur, radial shaft (utradistal and 33% site) and total skeleton were determined by DXA using a densitometer Prodigy (GE Medical systems, Lunar). Data were analyzed using ANOVA and Student's $t$-test to examine differences among the groups.
Results
In womens with thyrotoxicosis BMD was significantly decreased in all age groups in whole skeleton and ultradistal radius level, except group 20–29 years at lumbar spine and radius 33% levels and 40–49 years at proximal femur level. Rate of osteoporosis in different age groups was 25, 28.6, 36.8, 62.5 and 89.5% in females with thyrotoxicosis against 15, 0, 0, 10 and 52.9% in control group. Deficiency of BMD rises with age since 50 years old up to 17.9% at lumbar spine, 12.2% at proximal femur and 23.4% at radial shaft levels. Most losing of bone tissue was observed in radial shaft in all age groups. Moreover patients with thyrotoxicosis duration over 5 years compare to rest patients has significant decreased BMD at site 33% radius level ($0.772 \pm 0.016$ g/m$^2$ against $0.681 \pm 0.035$ g/sm$^2$, $t = 2.69$, $F = 1.806$, $P = 0.009$).
Conclusion
Most deficiency of BMD in womens with thyrotoxicosis is observed in radial shaft especially. It increases with age and duration of thyrotoxicosis. Thus it is necessary to perform a measurement of BMD in radial shaft during DXA examination for thyrotoxic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P188

### Comparison of the effects of hormone replacement therapy on bone mineral density, lipid profiles, and biochemical markers of bone metabolism

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Objective
To assess the effects of hormone replacement therapy on bone mineral density (BMD), biochemical markers of bone turnover, and lipid profiles in postmenopausal women.
Methods
We retrospectively reviewed the medical records of 199 postmenopausal women who had received care at the Department of Obstetrics and Gynecology of Catholic University Seoul St. Mary's Hospital between January 1994 and December 2008. The patients were divided into the following three groups: group 1 received combined estrogen and progesterone therapy ($n=91$); group 2 received estrogen only ($n=65$); and group 3 received tibolone ($n=43$). We compared the changes in biochemical markers of bone turnover, lipid profiles, and BMD during therapy.
Results
The BMD of the lumbar spine increased in groups 1 and 3 by 2.0 and 1.2%, respectively, and the BMD of the total femur increased in groups 1 and 2 by 2.3 and 0.5% from the initial values after 3 years, respectively. However, the BMD of the femoral neck and total femur decreased significantly in group 3 by 4.8 and 1.9%, respectively, 3 years after treatment initiation ($P<0.05$). Serum osteocalcin and urinary deoxypyridinoline decreased in all groups 1 year after treatment. In groups 1 and 3, the total cholesterol level decreased and the triglycerides level increased. However, there were no definite changes in the total cholesterol and triglycerides levels in group 2. The HDL-cholesterol level increased in groups 1 and 2, but decreased in group 3. As a result, the BMD of the lumbar spine increased and the total cholesterol level decreased in the combined therapy and tibolone groups. Tibolone had no beneficial effect on the BMD of the femoral neck.
Conclusions
Our results suggest that each therapy has different effects on BMD, biochemical markers of bone metabolism, and lipid profiles. A prospective study involving a larger group, and considering multiple factors, will be required to obtain more clinically meaningful conclusions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P189

### Bone mass in diabetic patients measured by lumbar spine and hip densitometry

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Introduction
It is known that diabetes mellitus and its complex yet insufficiently known mechanisms affect bone strength and increases the fracture risk.
Objective
The aim of this study was to assess bone mass measured by lumbar spine and hip DXA, in patients suffering from diabetes mellitus according to sex and type of diabetes.
Material and methods
It was a prospective study. Included 150 diabetic patients of both sexes.
Results
In type 1 diabetes mellitus were 11 men, age $40.82\pm8.99$ years, BMI $23.24\pm3.45$ kg/m$^2$ and duration of diabetes mellitus $12.67\pm9.30$ years. Also in type 1 diabetes mellitus were 20 women of age $42.81\pm11.77$ years, BMI $24.33\pm2.99$ kg/m$^2$ and duration of diabetes mellitus $23.65\pm11.57$ years. In type 2 diabetes mellitus were 21 male age $58.57\pm11.12$ years, BMI $29.51\pm7.67$ kg/m$^2$ and duration of diabetes $9.57\pm7.21$ years. Also in type 2 diabetes mellitus were 98 women, age $64.15\pm9.28$ years, BMI $29.99\pm5.57$ kg/m$^2$ and duration of diabetes mellitus $11.99\pm8.34$ years. In diabetes mellitus type 1 in four men has osteopenia (57%) and in seven (43%) normal results. The value of L1-L4 BMD was $1.233\pm0.147$ g/cm$^2$, total femur $0.998\pm0.149$ g/cm$^2$ and femoral neck $0.982\pm0.165$ g/cm$^2$. In women with type 1 diabetes mellitus was 6 (30%) with normal findings, 10 (50%) with osteopenia and 4 (20%) with osteoporosis. The value of L1–L4 BMD was $1.088\pm0.151$ g/cm$^2$, total femur $0.878\pm0.132$ g/cm$^2$ and femoral neck $0.864\pm0.138$ g/cm$^2$. In men with type 2 diabetes mellitus normal finding was identified in 12 (57.1%), osteopenia in 8 (38.1%) and in 1 (4.8%) osteoporosis. The value of L1–L4 BMD was $1.191\pm0.163$ g/cm$^2$, total hip $1.064\pm0.179$ g/cm$^2$ and femoral neck $0.895\pm0.457$ g/cm$^2$. In women with type 2 diabetes mellitus had normal findings 27 (27.6%), osteopenia 41 (41.8%) and osteoporosis 30 (30.6%). The value of L1–L4 BMD was $1.071\pm0.201$ g/cm$^2$, total femur $0.914\pm0.162$ g/cm$^2$ and femoral neck $0.858\pm0.153$ g/cm$^2$.
Conclusion
Osteopenia predominates in all patients with type 1 diabetes and in women with type 2 diabetes. It was determined a lower BMD at the femur, primarily in the neck of the femur, in all patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P190

### Efficacy, safety and adherence to treatment of teriparatide: an observational study

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Teriparatide is a skeletal anabolic drug that represents the first and new class of bone anabolic agents able to increase bone formation, bone mineral density and strength. Several studies demonstrated the efficacy of this drug in post-menopausal and glucocorticoid-induced osteoporosis, but few data are available on the effects of the drug in long-term therapy in humans, so far.
We analyzed a population of 135 women in post-menopausal age treated with teriparatide for 18 months between 2005 and 2011, with the aim to evaluate efficacy, safety and adherence to treatment. The efficacy of teriparatide was evaluated as the incidence of new fractures, which was low (0.74%). The overall tolerability of teriparatide was good. Treatment discontinuation because of adverse effects was 2.22% (1.48% for dispnoea, 0.74% for nausea and myalgia). Adherence and compliance to treatment were high (94.07%). Only eight women (5.93%) stopped treatment and only three of these (37.3%) due to an adverse reaction. In view of the most frequently occurring adverse effects, the levels of calcium, phosphorus, calciuria, uric acid, alkaline phosphatise and PTH were assessed at the beginning and at the end of treatment. The reduction of back pain was evaluated by using the visual analogic scale (VAS). We observed only a significant increase of phosphorus (10.9% $P<0.04$) and uric acid (57% $P<0.05$) levels; a significant reduction of back pain (VAS at the begin of treatment $9.0\pm2$; VAS at the end of treatment $4.2\pm1.8$; $P<0.0001$) was observed. None of the patients who completed 18 months therapy had significant side effects. These data show that teriparatide is a safe, well tolerated and effective therapy in post-menopausal osteoporosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P191

### Thyroxin treatment of multinodular and normofunctional goiter and bone mass.

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Objective
To research changes in bone mass during treatment on MNG with thyroxin.
Material and methods
For two years, patients and their clinical histories were examined when each ambulatory patient was personally visited by the author. MNG by Hashimoto's disease and classic MNG were included. The patients with MNG in treatment were placed in the problem group, while the patients who were visited for the first time, and the patients with other endocrine diseases, were placed in the control

group. The patients who presented high blood levels of parathyroid hormone (PTH) (above 64 pg/ml) and patients with less than one year evolution were excluded; also excluded were those with pathological causes or suspected of having lost bone mass for other causes, even local. 172 patients were in the treated group (9 males and 163 females) and 105 (8 males and 97 females) in the control group.
Thyroxin doses were adjusted in this way: TSH blood levels were around 1 μU/ml, and always below 3 μU/ml; the same dose was given each day to each patient, only excepting the adjustments.
Bone masses were compared by densitometry.
Results
There was no correlation between time of treatment and bone mass, neither for *Z*-score nor *T*-score ($P<0.01$). Bone mass was better in the treated group than in the control group ($P<0.01$), by both the mean and the frequency comparisons ($P<0.01$). This may be explained by the bone protection caused by calcium and cholecalciferol prescription or by the increase in bone matrix thyroxin mediate through protein synthesis.
Conclusion
Treatment of MNG by thyroid hormone in this dosage procedure does not cause osteoporosis, in accordance with the results above expounded. The prescription of calcium and cholecalciferol may be additional bone protection against matrix reabsorption.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P192

**Vitamin D status in patients treated with intravenous ibandronate: a cohort study**

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In 100 consecutive osteoporotic women referred to our clinic we assessed 25OHD3 and PTH levels. Patients suffered from postmenopausal osteoporosis and intolerance of oral bisphosphonates, were treated at least for 6 months with calcium and vitamin D. The reasons of intolerance of oral medication were gastritis (53), esophagitis (18) or peptic ulcer (29 cases). Patients were therefore chronically treated with proton pump inhibitors (PPI). They were referred to our Clinic in order to take intravenous ibandronate and were all assessed by referring physician as 'ready to medication' with it. We found, however, that only in eight of them the levels of 25OH D3 were proper (i.e. $>30$ ng/ml). Generally 25OH D3 levels were very low – on average 17.91 ng/ml ($\pm 4.04$), and in 69 patients were lower than 20 ng/ml. Ten patients had 25OHD concentrations even lower than 10 ng/ml with evident symptoms of muscle weakness. In all of them the levels of PTH were elevated (78.05 pg/ml $\pm 4.31$), however in all group we haven't found an evident correlation between PTH and 25OH D3 ($r=-0.48$; $P=0.11$).
The reason of these low vitamin D levels was using too low doses during supplementation. All, except 10 women were treated only with 400 – 800 j D3 daily. According to the data presented above, these doses are evidently too low to achieve recommended concentrations (i.e. $>30$ ng/ml), especially in subjects with gastritis or peptic ulcer and prolonged therapy with PPI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P193

**Correlations between the bone matrix markers and bone mineral density in postmenopausal osteoporosis**

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Objectives
We assessed the implications of PINP (procollagen type I N-terminal propeptide), NTX (cross-linked N-telopeptides of type I collagen), $E_2$ (estradiol), calcium ($Ca^{2+}$), phosphorus (PHOS(2−)), and their correlation with bone mineral density (BMD) in the process of bone remodeling of postmenopausal osteoporosis.
Methods
The study was performed on two groups of women with postmenopausal osteoporosis (with different degrees of estrogenic deprivation): group I ($n=48$, with $<15$ years of estrogenic deprivation) and group II ($n=26$, over 15 years of estrogenic deprivation), compared with a control group ($n=20$, postmenopausal women without osteoporosis). Serum levels of the ions were measured by VitrosSlides quantitative technique. Bone levels of these ions were assessed by bone flame atomic absorption spectrometry (FAAS). BMD was measured by dual-energy X-ray absorptiometry (DXA) technique. Serum levels of the enunciated markers were measured by ELISA technique.
Results
Serum PINP levels were increased in group I ($+17.2\%$, $P<0.001$), and decreased in group II ($-66.2\%$, $P<0.001$), as compared to the controls. Serum levels of NTX were significantly higher (group I: $+33.5\%$, $P<0.001$ and group II: $+58.3\%$, $P<0.002$) vs control. Serum $Ca^{2+}$ and PHOS(2−) levels were increased in group I ($+12.06\%$, $P<0.005$ - calcium and $+36.78\%$, $P<0.004$ - phosphorus), and decreased in group II ($-85.65\%$, $P<0.006$-calcium and $-57.22\%$, $P<0.003$ - phosphorus). The bone levels of these ions were lower in both groups ($P<0.001$, respectively $P<0.002$, vs controls). Estradiol levels were significantly lower in both groups ($P<0.01$, respectively $P<0.02$), associated with low BMD.
Conclusions
The levels of these ions increase transitory in serum, as a result of bone demineralization through hidroxiapatite microcrystal solubilization and mobilization of these ions in the circulation. This imbalance produces a decrease in bone formation and an increase in bone resorption, leading to bone demineralization and increased risk for osteoporotic microfractures.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P194

**Zoledronic acid treatment improve bone strenght parameters assessed by BMD**

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Introduction
Bisphosphonates therapy is the standard of care in postmenopausal osteoporosis. In the HORIZON-RFT, annual i.v. Zoledronic acid (ZOL) 5 mg infusion significantly reduced the rate of new fractures by increased bone mineral density (BMD). We proposed to evaluate the effect of ZOL treatment in BMD change in a group of postmenopausal osteoporotic women assessed by DXA–BMD. We have proposed also to assess the adverse events of this treatment.
Methods
Between 2008 and 2009 we have registered 520 postmenopausal osteoporotic women (PMOW). Among them, 22 were treated with ZOL i.v. 5 mg/yearly and 1000 mg calcium /day plus Alfacalcidol 1 μg/day for 24 months or more. DXA–BMD measurement for BMD change was used at baseline and after 12 and 24 months.
We analyzed also: changes in serum calcium, 25OHD, PTH and bone alkaline phosphatase (BAP) at baseline and one and two-years post ZOL. The mean age of the patients was $64.5\pm3.7$ years.
Results
No significant change in serum calcium, serum 25OHD, BAP. PTH level rested also normal. Clinical significantly reduced the hyperalgesia after a mean of 6.7 weeks (6–12 weeks). Adverse events after infusion were flu-like syndrome transient 18%, arthromyalgias 9%, arterial hypertension 4.5%. No cardiac

**Table 1**

| Time/ BMD ($g/cm^2$) | Baseline | 12 months | 24 months |
|---|---|---|---|
| Lumbar spine | 0.673 | 0.726 | 0.734 |
| Gain % | | +7.8 | +9.0 |
| Total hip | 0.773 | 0.809 | 0.818 |
| Gain % | | +4.6 | +5.8 |
| Femoral neck | 0.606 | 0.645 | 0.648 |
| Gain % | | +6.4 | +6.9 |

arrhytmias, no fractures. BMD changes at lombar, total hip and neck are presented in the Table 1.
Conclusions
In our experience, iv infusions of ZOL (5 mg) have shown to be effectively in increasing BMD. The other biologic parameters remained in normal level. The adverse effects only in the first administration without consequences.
Keywords: osteoporosis, Zoledronic acid, BMD.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding

## P195

**Therapeutical management in hypogonadal osteoporosis**
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Hypogonadal osteoporosis may occur early, asymptomatic and diagnosis is long etiology is often laborious. Early diagnosis of gonadal failure prevention measures require changes already in the prepubertal bone, puberty or postpuberal to ensure adequate peak bone mass sex and age.
Casuistry is represented by 57 patients, of which the: late puberty (26 cases) and premature ovarian failure (31 cases). To elucidate the etiologic diagnosis was clinical and laboratory criteria used.
The paper suggests two major objectives in the strategic management of hypogonadal osteoporosis:
a) early diagnosis of gonadal failure in order to adopt preventive measures bone changes;
b) the estrogen-progestive substitution associated the antiresorbtion or proformation medication to prevent fragility fractures.
Declaration of interest
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# Calcium & Vitamin D metabolism

## P196

**PTH levels are associated with insulin resistance in PCOS women**
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Introduction
Accumulating evidence suggests an association of vitamin D with insulin resistance (IR) in women with polycystic ovary syndrome (PCOS). The role of PTH in insulin metabolism is less clear. We aim to study the association of PTH and 25-hydroxyvitamin D (25(OH)D) levels with metabolic and endocrine parameters in a cohort of PCOS women.
Methods
We measured PTH and 25(OH)D levels in 544 PCOS and 139 healthy control women within the same age range. Metabolic, endocrine, and anthropometric measurements and oral glucose tolerance tests were performed.
Results
PCOS women had higher PTH levels than controls in age-adjusted analysis ($P=0.018$), but results were attenuated after adjustment for BMI ($P=0.073$). PCOS women with higher PTH levels were significantly older, had higher BMI, waist-to-hip ratio (WHR), systolic and diastolic blood pressure, fasting and stimulated insulin levels, homeostatic model assessment-IR (HOMA-IR), CRP levels, and Ferriman–Gallwey-scores and significantly lower 25(OH)D, SHBG, quantitative insulin sensitivity check index (QUICKI), serum calcium, and phosphorus ($P<0.05$ for all). In linear regression analysis, BMI ($P<0.001$), age ($P<0.001$), and PTH ($P=0.016$) were independent predictors of AUCinsulin, whereas 25(OH)D was not. In linear regression analysis using the same covariates, BMI ($P<0.001$) and PTH ($P=0.006$) independently predicted HOMA-IR, whereas age and 25(OH)D did not. Likewise, in linear regression analysis, BMI ($P<0.001$) and PTH ($P=0.037$) independently predicted insulin sensitivity assessed by QUICKI, whereas age and 25(OH)D were not related to insulin sensitivity.
Discussion
We present evidence that PTH levels are independently associated with IR and insulin sensitivity in a large cohort of PCOS women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P197

**Dietary effect of calcium and vitamin D on the gene expressions of tight junction in the duodenum and kidney of calbindin-D9k, -D28k and -D9k/D28k knockout mice**
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Introduction
Calcium transport is regulated by active transcellular and passive paracellular pathways which are responsible for calcium (re)absorption in the intestine and kidney. The activity of passive transport is determined by the permeability of tight junction. In general, the permeability of tight junction is inversely proportional with the expression of tight junction genes. Claudin and occludin among the tight junction genes are concerned directly with permeability of the tight junction. In this study, we examined the effect of reduced dietary supply of calcium and vitamin D on paracellular gene expression in the calbindin-D9k (CaBP-D9k), -D28k (CaBP-D28k), and -D9k/D28k knockout (KO) mice.
Methods
The tissue-specific mRNA and protein expressions of tight junction genes in the duodenum and kidney, the main organs for calcium (re)absorbtion, were examined using real-time PCR and western blot analysis. The localization of tight junction genes was also investigated by immunohistochemistry.
Results
The expression levels of tight junction genes showed similar patterns in CaBP-D9k and -D28k single KO mice. The most of tight junction genes in the intestine were less expressed in CaBP-D9k KO mice than wild-type (WT), while they were over expressed in the kidney. When the calcium or vitamin D deficient diet was given, this difference was accelerated. In addition, both calcium and vitamin D deficient diet in the KO mice showed additive effects on the tissue-specific regulation of tight junction gene expression. Immunohistochmical staining results indicated that the tight junction genes were mainly localized in apical site of paracellular region in both intestine and kidney.
Conclusions
The ablation of CaBPs and/or reduced dietary calcium resulted in the decreased expression of paracellular barrier genes, therefore, this may lead to acceleration of the paracellular transport of calcium.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P198

**Serum IGF1 concentration is positively associated with 25-hydroxy-vitamin D level and increases upon treatment with vitamin D**
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IGF1 concentrations have been reported to increase with 25-hydroxyvitamin D (25(OH)D) values in population studies, but it is unclear whether this association expresses a cause-effect relationship. Mice genetically deficient of steroid receptor coactivator 3, a transcriptional coactivator for the nuclear vitamin D receptor, have significantly lower circulating IGF1 levels than wild-types.
We measured serum fasting 25(OH)D and IGF1 by enzyme- and radioimmuno-assay, respectively, in 42 internal medicine outpatients, aged $61.7\pm8.9$ years, at follow-up visits for stable or recovering medical conditions. The study was performed in Genova, Italy, at latitude 44° North, over Spring and Summer.
Mean ($\pm$S.D.) 25(OH)D and IGF1 concentrations were 15.7 ($\pm$9.1) ng/ml and

182.6 (±77.8) ng/ml respectively. There was a positive correlation between 25(OH)D and IGF1 values ($r$ 0.33, $P<0.05$).
To understand in principle whether vitamin D status does affect GH/IGF1 axis, in 20 patients we measured 25(OH)D, IGF1, and GH (the latter by immunoradiometric assay) before and after 12 weeks of 5000 IU cholecalciferol/week (six subjects), 7000 IU cholecalciferol/week (seven subjects), or no treatment (controls, seven subjects). A significant increase in serum 25(OH)D was observed after supplementation with both cholecalciferol dosages (+16.2–17.7 ng/ml vs baseline, $P<0.01$). Nonsignificantly higher GH levels were also found after either cholecalciferol dose (+1.17–1.19 mIU/l). Seven thousands, but not 5000 IU/week significantly raised IGF1 concentration of 41.1 ng/ml ($P<0.05$ vs baseline).
A significant increase in 25(OH)D values of 7.8 ng/ml vs baseline ($P<0.05$) was also observed in controls after 12 weeks, likely because of sun exposure during normal outdoor life. GH and IGF1 levels did not change significantly.
These preliminary results suggest that treatment with at least 1000 IU/day vitamin D results in increased circulating IGF1, and that the relationship between 25(OH)D and IGF1 concentrations described in cross-sectional analyses may be causal.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P199

### Incidence of diabetes and vitamin D deficiency: a prospective cohort study

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Background
Vitamin D deficiency is an important public health problem because of its great impact on bone metabolism and the possible implication in cardiovascular outcomes, diabetes, cancer and mortality. Cross-sectional studies have related vitamin D deficiency with the prevalence of diabetes but there are only a few prospective cohort studies on the incidence of type 2 diabetes.
Objective
To investigate the relationship between levels of 25-hydroxyvitamin D and the incidence of type 2 diabetes in a Spanish population.
Methods
We undertook a population-based prospective study in a population from southern Spain. The first phase of the study (1996–1998) included 1226 individuals. Of this original cohort, 988 persons were reassessed in 2002–2004 and 961 in 2005–2007. At the second evaluation, we measured 25-hydroxyvitamin D and intact parathyroid hormone (iPTH), glycosylated hemoglobin and did an oral glucose tolerance test (OGTT) to 855 subjects. For the incidence study we excluded 172 subjects with diagnosed diabetes. After 4 years of follow up, 412 subjects were re-evaluated in 2005–2007 with an OGTT and glycosylated hemoglobin. All the participants completed a clinical survey, underwent an anthropometric study and provided a venous blood.
Results
The incidence of diabetes in subjects with 25-hydroxyvitamin D levels $\leq$18.5 ng/ml (percentile 25) was 12.4 vs 4.7% in subjects with levels $>$18.5 ng/ml. The likelihood of having diabetes during the four years of follow-up was significantly lower in the subjects with higher levels of 25-hydroxyvitamin D (OR=0.17 (0.05–0.61)). None of the subjects with levels higher than 30 ng/ml developed diabetes.
Conclusion
In this prospective study, we found a significant inverse association between serum 25-hydroxyvitamin D levels and the risk for type 2 diabetes in a population from the south of Spain.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P200

### Tissue-specific expression of transcellular calcium transport and paracellular tight junction genes in the placentas of calbindin-D9k, -D28k, and -D9k/D28k knockout mice

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Introduction
Placenta has many essential roles to maintain pregnancy and homeostasis. Calcium transport and its regulation are also important for vital roles of placenta. In general, calcium transport is regulated by active transcellular and passive paracellular pathways. Transient receptor potential cation channel subfamily V member 5/6 (TRPV5/6), calbindin-D9k (CaBP-9k), calbindin-D28k (CaBP-28k), and Na+/Ca2+ exchanger (NCX1) are involved in the transcellular pathway. Paracellular pathway is determined by expression of tight junction genes such as occludin, claudin, and ZO-1.
Methods
We examined whether the transcellular and paracellular calcium transport genes are differentially modulated in the placenta of CaBP-9k and/or CaBP-28k knockout (KO) mice on gestational day 19 (GD 19). The expressions of transcellular calcium transport and paracellular tight junction genes in the placenta were examined using real-time PCR and western blot analysis.
Results
The mRNA and protein expressions of calcium transport genes appeared to be increased in a compensatory manner. The expression levels of NCX1 and TPRV6 were increased at both mRNA and protein in KO compared to wild-type (WT) mice. CaBP-9k mRNA and protein were significantly induced in CaBP-28k KO mice, whereas these levels of CaBP-28k were reduced in the placenta of CaBP-9k mice compared to WT mice. Each tight junction gene was shown to be similarly expressed after ablation of CaBP-9k or CaBP-28k. Occludin and claudin levels were increased in single, CaBP-9k and CaBP-28k, KO mice, but not in double KO mice.
Conclusions
The disability of calcium buffering due to CaBP-9k and/or CaBP-28k KO, may lead to accelerate transcellular pathway of calcium, and also to decrease paracellular permeability through the tight junction to maintain calcium homeostasis in placenta of mice.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P201

### Gitelman's syndrome presenting with hypercalcaemia due to severe primary hyperparathyroidism

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Introduction
The combination of hypokalemia and hypercalcaemia is uncommon but potentially lethal. In primary hyperparthyroidism hypokalaemia most commonly occurs due to incomplete distal renal tubular acidosis. We present the first case of simultaneous primary hyperparathyroidism with Gitelman's syndrome resulting in profound electrolyte imbalance.
Case
A 48-year old man, referred with hypokalaemia (2.5 mmol/l) presented with a short history of extreme fatigue, myalgia, nausea and constipation. His adjusted serum calcium was raised (2.84 mmol/l). Patient was otherwise fit and well. He denied any regular medications or liquorice intake. He had no contributory medical family history and never smoked. There was nothing to suggest underlying tuberculosis, sarcoidosis or malignancy. He had normal stature and was normotensive. There was no stigmata of hypercortisolism or lymphadenopathy. Cardiovascular, respiratory and abdominal examinations were all normal. Subsequent bone profile check noted adjusted serum calcium rise to 4.01 mmol/l and prompted urgent administration of bisphosphonates.
Further investigations confirmed diagnosis of primary hyperparathyroidism secondary to large single parathyroid adenoma. Following parathyroidectomy patient's bone profile normalised, hypokalaemia however, persisted. It was accompanied by hypomagnesaemia and metabolic alkalosis. Battery of further endocrine investigations including random glucose (5.1 mmol/l), HbA1c (5.4%), thyroid, anterior pituitary, overnight dexamethasone suppression test, calcium/creatinine urine excretion ratio, fasting gut polypeptides and MEN genotyping all

proved normal. Chest and abdominal radiology were also normal. Notably, significant hyper-reninaemic hyperaldosteronism was discovered and confirmed twice on both supine and ambulatory sampling. Diagnosis of Gitelman's syndrome was made and patient was commenced on Amiloride with a desirable effect.

Conclusion

It is known that primary hyperparathyroidism can cause hypokalaemia, but we report this rare combination of Gitelman's syndrome in an otherwise healthy patient.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P202

### Vitamin D pathway in non-functioning pituitary adenomas

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Introduction

In addition to its well known effects on calcium homeostasis and bone metabolism, growing evidences shows that vitamin D plays an important role in regulation of cancer angiogenesis, cell apoptosis, differentiation, and proliferation. Vitamin D acts through vitamin D receptor (VDR), an intracellular nuclear receptor. It was found an association between different cancer histotypes and single nucleotide polymorphisms (SNPs) of VDR. Despite the close relationship between VDR and PIT-1 (pituitary trascription factor) suggests a potential role of vitamin D pathway in the onset of pituitary adenomas, however, to date no data are available on this issue.

Aim

This study evaluated 25OHvitD serum levels and VDR-SNPs expression in patients with non-functioning pituitary adenomas.

Patients and methods

We evaluated 14 patients with non-functioning pituitary adenomas and 14 healthy controls age and sex matched. Serum 25OHvitD levels were measured by solid-phase chemiluminescent enzyme immunoassays. The SNPs-VDR evaluated included Fok -1 and Taq-1, whose assessment was performed by PCR-restriction fragment length polymorphism (PCR-RFLP) analysis.

Results

Serum 25OHvitD levels were significantly lower in patients ($17.4 \pm 2.4$ ng/ml) than in controls ($31.3 \pm 1.7$ ng/ml) ($P < 0.01$). Allelic variants of Taq-1 VDR polymorphism had a different distribution in patients than in controls, with the variant TT and Tt present in 66 and 30% of patients and in 50 and 47% of controls ($P =$ NS), respectively. Allelic variants of Fok-1 VDR polymorphism were distributed similarly in patients and in controls.

Conclusions

Serum 25OHvitD levels are significantly lower in patients with non-functioning pituitary adenomas than in controls, suggesting a potential etiopathogenetic and therapeutic role of vitamin D pathway in these patients. The significance of different distribution of allelic variants of Taq-1 VDR polymorphism between patients and controls requires further investigation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P203

### A new cause of malignancy associated hypercalcemia (MAH): PTH releasing breast cancer

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Introduction

Here, we present a case of epithelial breast cancer releasing iPTH thereby causing MAH.

Case report

A 48-year-old woman with metastatic breast cancer was consulted for hypercalcemia. Previous medical record was insignificant except for nephrolitiasis. She had a left mastectomy + left axillary lymph node dissection in another hospital. Prior to surgery no calcium levels were obtained. Pathology revealed infiltrative ductal carcinoma. Postoperatively total calcium (tCa) was 12.04 mg/dl (8.4–10.2), albumin: 4.43 g/dl, inorganic phosphate (iP): 3.21 mg/dl. Simultaneous iPTH level was 94.3 pg/ml (0.5–75) and vitamin D was 20.8 μg/l (low). After forced diuresis, tCa was 11.01, albumin: 4.4. Bone scintigraphy was negative. Primary hyperparathyroidism was suspected. Parathyroidal pathology was not detected with MIBI scintigraphy and USG. Bone mineral dansity showed osteoporosis (lomber $T$ score: $-4.2$); pamidronate was begun. Prior to chemotherapy for remaining lymph nodes and liver metastasis her tCa: 9.87, albumin: 3.7. As to verify the source of iPTH, specimens were stained with PTH-antibodies. Focal positivity was observed (Figure 1). Plasma PTH-rp levels were non-detectable. Thus the diagnosis was iPTH releasing breast cancer. iPTH level was 254 on the tenth day of chemotherapy then declined to a level of 103 after 4th week.

Conclusion

In our knowledge this is the first case of iPTH releasing breast cancer. In patients without bone metastasis, release of iPTH as a reason for hypercalcemia should be kept in mind.

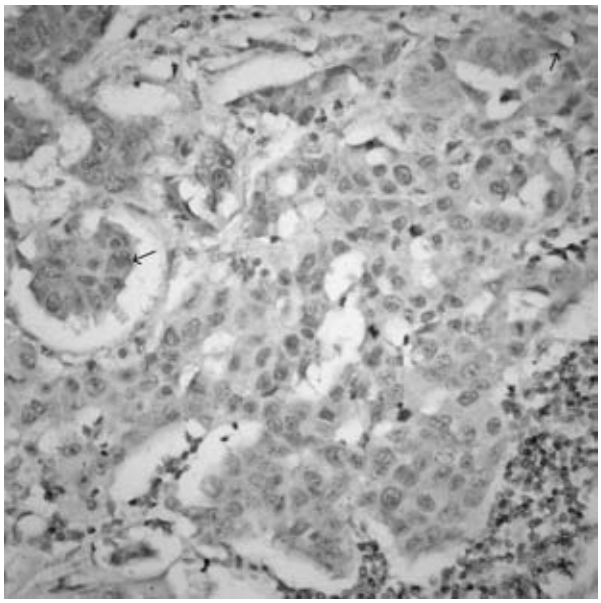

**Figure 1** Parathyroid hormone stain focal cytoplasmic positivity was observed in the neoplastic cells (arrows) (×40) (immunohistochemistry, Human Ab-1, Clone PTH01, Labvision).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P204

### Two cases of alendronate induced atypical fracture of femur shaft

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Studies show conflicting results regarding possible excess risk of atypical fractures associated with bisphosphonate (BSP) use. Since BSP reduces bone remodeling, they freeze the skeleton allowing accumulation of microcracks over time leading to atypical fractures. We present two cases of atypical fracture and discuss on monitoring therapy and drug holiday in chronic BSP users.

Methods

Over the period of last 8 months we diagnosed two cases of alendronate induced atypical fracture of femur shaft the details of which are given below. Case 1 A 48-year-old postmenopausal female presented with limp and right anterior thigh pain. X-ray revealed a focal beak of cortical thickening on lateral side of cortex which after 3 months resulted in to bicortical femoral shaft fracture. She was on BSP since last 10 years. Bone turnover markers were done. Stabilization of the fragile bone was done with intramedullary nailing. However, the femur cracked during surgery.

Case 2 A 52-year-old female patient presented with bilateral thigh pain and on evaluation showed cortical thickening and hairline fracture on both sides. She had a history of longterm use of alendronate since last 5 years. The fracture was stabilized on both the sides with intramedullary nailing. The right sided bone cracked during nailing. Urinary N telopeptide levels were done. And intraoperative bone biopsy was also taken.

Results

Both the patients had very low bone turnover markers and showed bone necrosis in bone biopsy. Tetracycline labeling was not done as was not available in our institute. Both the patients on follow up showed poor healing of fractures and were started on teripartide once daily.

Conclusion

Optimal protocols for dosage, duration of BSP therapy deserve consideration. Benefit of BSP use should be balanced against potential risk of atypical fractures. Painful pyramidal projection of cortical bone in subtrochanteric femoral shaft should be recognized as an impending fracture and treated expediously. Additional therapy after BSP discontinuation is needed to promote healing.

Declaration of interest

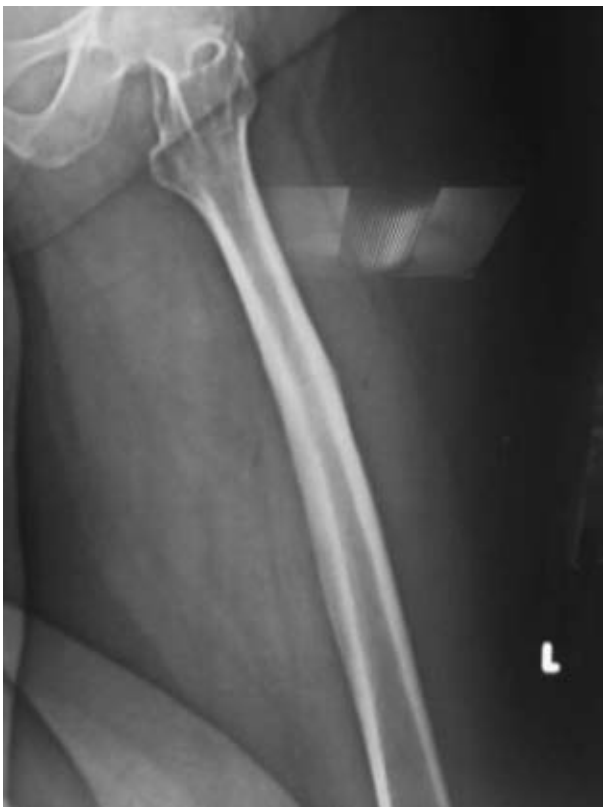


**Figure 1** Showing transverse fracture on left femur diaphysis with lateral cortical thickening.

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P205

### Vitamin D levels and carbohydrate metabolism

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Introduction and objective

The aim of this study has been to evaluate the relationship between vitamin D and iPTH levels and the status of carbohydrate metabolism in a representative sample of the Spanish population.

Material and methods

The study was conducted in two population of northern and southern Spain, evaluated in the same period of time and with a similar methodology: Pizarra Study (Málaga) and Asturias Study. Subjects in both groups underwent clinical and anthropometric assessment survey, a blood test (fasting glucose and insulin, creatinine, calcium, phosphorus, 25OH vitamin D, iPTH) homeostatic model assessment-insulin resistance (HOMA-IR) and an oral glucose tolerance test (OGTT). The number of subjects included was 1182. To calculate the statistical difference between the means of continuous variables we used the ANOVA test for quantitative and the $\chi^2$ test for qualitative variables. Strength of association between two variables was measured using odds ratio (OR).

Results

The mean age of participants was $50.3 \pm 14.4$ years (range: 20–83 years), with 57% female and 43% male. The mean of 25-hydroxyvitamin D and iPTH were: 22.46 ng/ml and 42.29 pg/ml, respectively. 65.6% had normal fasting glucose and OGTT, 6.45% impaired fasting glucose, 8.15% carbohydrate intolerance, 9.85% new diagnosed diabetes and 9.93% known diabetes.25-hydroxyvitaminD levels in these groups were, respectively: 23.43, 21.45, 22.97, 21.82 and 23.22 (*P* 0.034) and iPTH: 45.1, 48.26, 45.99, 49.31 and 41.08 (*P* 0.001) both adjusted for age, sex, BMI and season. The percentage of patients with 25-hydroxyvitaminD levels below 20 ng/ml was different in the five groups: 30.9, 35.5, 37.5, 49.1 and 36.8% (*P* 0.014).Vitamin D levels were significantly different for different values of HOMA-IR: quartile 1 (HOMA $<$ 1.07): 25.85; quartile 2 (HOMA 1.07–1.73): 22.94; quartile 3 (1.74–2.95): 22.54 and quartile 4 ($>$ 2.95): 22.05 (*P*0.041, all adjusted for age, sex, BMI and season).

Conclusions

1-One-third of the subjects studied have 25hydroxyvitaminD levels below 20 ng/ml,

2-The higher the insulin resistance (HOMA-IR) the lower the levels of vitamin D,

3-Patients with undiagnosed diabetes have 25hydroxyvitaminD levels significantly lower and iPTH significantly higher than normal population.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P206

### A single metastatic lesion in the leg of a parathyroid carcinoma

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Introduction

We report a case of a parathyroid carcinoma with very unusual and revealing features.

Case report

DAB, male aged 66 was subjected to subtotal gastrectomy because of a localized gastric adenocarcinoma (intestinal type). A mild primary hyperparathyroidism (PTH $<$ 200 pg/ml) was simultaneously found. Calcium oxalate nephrolythiasis at 50 years was noted. Sonography revealed a nodular lesion in the right lower thyroid pole, while NMR showed a nodular lesion at the left lower thyroid pole and in the sestamibi scan late hyperfixation at the right lower pole. Bilateral inferior parathyroidectomy was performed and revealed on both sides adenoma with moderate nuclear polymorphism and no mitotic figures; on the left side a central cystic area was found. Clinical and biochemical cure apparently followed. Six months later a presumed unrelated nodular lesion in the right thigh muscular mass was removed and found to be a metastatic lesion of a carcinoma, with numerous mitotic figures and immunoreactivity for PTH. A sestamibi scan showed marked hyperfixation in the right leg and PET- FDG-F18 hyperfixation at the right quadricipital area.

Discussion

Some unusual features may shed light on the pathologic process. There is evidence for a long standing disease since nephrolytiasis was found 14 years before. Long standing hyperparathyroidism and increased gastric acid secretion can lead to chronic gastritis, intestinal metaplasia and gastric adenocarcinoma. There is evidence for evolution along bilateral hyperplasia, adenoma and carcinoma since bilateral disease was present and a left cystic nonfunctioning

lesion was found. Neither clinical nor pathological a priori suspicion of a carcinoma was present. Most surprisingly a single metastatic lesion was later found in a very unusual location and this lesion was highly undifferentiated in sharp contract with the primitive lesion.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P207

### Pseudohypoparathyroidism type Ia, hypothyroidism and insulin resistance

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Introduction

Pseudohypoparathyroidism type la (PHP-1a) is an uncommon disorder that results from an inactivating mutation in the GNAS gene. It can present with resistance to several hormones, in addition to parathyroid hormone (PTH). A characteristic skeletal phenotype also results, referred to as Albright's hereditary osteodystrophy, that occurs because of the crucial role of Gs-α signaling in the growth, differentiation, and structure of these tissues.

Objective

To report a rare case of pseudohypoparathyroidism type Ia, presenting with hypothyroidism and insulin resistance, and review the related literature.

Case report

A male patient with PHP type 1a caused by a heterozygous mutation of the GNAS gene (c.568_571del) is presented.

In addition to PHP, the patient presents hypothyroidism and insulin resistance. GH levels are within normal range. The phenotype includes obesity, mild mental retardation, psychosis, calcifications of basal ganglia and Albright's hereditary osteodystrophy.

The genetic study of the mather is still pending.

Discussion/conclusion

Patients diagnosed with PHP1a should be further evaluated for associated endocrinopathies. Hypothyroidism is the most common endocrinophathy in this context, that is frequently subclinical in infancy.

Severe obesity is characteristic of PHP1a. It has been postulated that paternal imprinting of Gsα occurs in the hypothalamus such that maternal Gsα mutations lead to loss of the melanocortin signaling cascade, which is important for energy balance.

Insulin resistance has not commonly been described. It most probably results from the combined effects of obesity, family history, and abnormal melanocortin signaling.

Soft-tissue calcification has been reported in various body parts, especially in the subcutaneous tissues, and rarely in the brain and cardiac septum.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P208

### A diagnostic conundrum associated with hypercalcaemia

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Northwick Park Hospital, NWLH NHS Trust, London, UK.

A 72-year-old woman was initially referred by her GP to the colorectal service of our Hospital. She had a 4-month history of weight loss, fatigue and appetite loss. The initial blood tests at the GP surgery revealed a normochromic normocytic anaemia.

She had a diagnosis of SVT and she was on sotalol. She had never smoked nor she had a history of excess alcohol.

In her initial blood tests: Hgb 9 g/dl, albumin 27 g/l, ALP: 187 IU/l (40–150), adjusted serum calcium 2.61 mmol/l (2.15–1.58), creatininine 51 μmol/l, normal serum electrophoresis.

She first saw a colorectal surgeon.

A gastroscopy showed gastritis and the colonoscopy was difficult therefore she had a CT virtual colonoscopy which did not show any tumour in the bowel. However it revealed a 15 mm lesion in the left kidney. She was then referred to the Urologists. A CT chest was organised and this was clear. She also had an isotope whole body scan and this did not show any evidence of bone matastases. Her case was discussed at the Urology MDT and it was deemed that the lesion is unlikely to be the cause of her systemic symptoms.

She was seen in the Endocrinology clinic for an opinion about her high calcium. Her repeat cCalcium was 2.59 mmol/l, PTH 3.7 pmol/l (1.2–6.2), 25(OH) Vitamin D low at 33 nmol/l, ACE 350 U/l (13–55), ESR: 115 mm in the first hour and a CRP 105 mg/l. Her autoantibody screen was negative.

Based on the results above we proceeded with a PET scan which identified a large vessel vasculitis.

The patient was started on high dose steroids at the Rheumatology clinic. She reported a great improvement in her symptoms and all the repeat laboratory values including cCalcium had normalised.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P209

### Expression of 25-hydroxyvitamin D3-1α-hydroxylase and 24-hydroxylase in human vascular smooth muscle cells

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Medial calcification is a major cause of premature cardiovascular mortality in chronic kidney disease. Vascular smooth muscle cells (VSMC) play a key role in this process and studies have suggested a protective role for vitamin D. The exact mechanism for this is unclear but local synthesis of 1,25-dihydroxyvitamin D (1,25D) may be important. Production of 1,25D from 25-hydroxvitamin D (25D) is catalyzed by 25-hydroxyvitamin D-1α-hydroxylase (1α-OHase). 1,25D is metabolized by 24-hydroxylase (24-OHase). This study used normal arterial tissue (kidney donors; ethical approval obtained) and primary cultures of human aortic smooth muscle cells (HAoSMC) to examine the expression and regulation of 1α-OHase, 24-OHase and vitamin D receptor (VDR) mRNA (real-time PCR), protein (western blotting/immunohistochemistry) and synthesis of 1,25D. HAoSMC expressed 1α-OHase and 24-OHase mRNA. The PCR transcripts were similar to those in HKC-8 cells (a kidney cell line expressing both hydroxylases). Western blot analyses of 1α-OHase and 24-OHase identified single protein bands ($\sim$56 kDa) in HAoSMC and arteries, which were identical to those seen in HKC-8 cells and human kidney tissue. VDR mRNA and protein was detected in all samples. Immunohistochemistry of arteries indicated expression of 1α-OHase, 24-OHase and VDR in the medial layer. Incubation of HAoSMC with 1,25D (6 h) resulted in a dose dependent increase in VDR protein (1–100 nM). 1α-OHase mRNA and protein were significantly decreased (1 nM; $P<0.05$). Conversely, 24-OHase mRNA was significantly increased (10 nM; *P*lt;0.05). Importantly 24-OHase mRNA was also increased and 1α-OHase protein decreased by 25D3 (100 and 10 nM respectively; 6 h; *P*lt;0.05) indicating 1α-OHase activity in HAoSMC. These data demonstrate that arteries and VSMC cells express functional vitamin D signaling. We believe this is the first demonstration of 24-OHase in human arteries and VSMC. We have also shown that VSMC express 1α-OHase and are able to synthesize sufficient 1,25D to stimulate a local response.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P210

**Longitudinal changes in serum 25-hydroxyvitamin D levels of older persons during 6 and 13 years of follow-up**

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Background
Vitamin D deficiency is very common in older persons. It is not clear how serum 25-hydroxyvitamin D (25(OH)D) levels change during aging.
Objective
To examine longitudinal changes in serum 25(OH)D levels in two representative cohorts of Dutch older persons during 6 and 13 years of follow-up, respectively.
Methods
Data of the Longitudinal Aging Study Amsterdam (LASA) were used, an ongoing cohort study in older persons. Two different cohorts were included: i) younger cohort: aged 55–65 years at baseline, $n=738$, follow-up of 6 years; ii) older cohort: aged 65–88 years at baseline, $n=1320$, follow-up of 13 years. Linear mixed models were used to analyze the longitudinal change in serum 25(OH)D in both cohorts. Age and body mass index were added to the model. Seasonal variation was modeled by adding a cosine and sine function with a period of 1 year to the model.
Results
At baseline, average levels were 56.5 nmol/l in the younger cohort and 51.1 nmol/l in the older cohort. The seasonal variation was $\pm 11$ nmol/l in the younger cohort, and $\pm 7$ nmol/l in the older cohort. In the younger cohort, a longitudinal increase in mean serum 25(OH)D levels of 5 nmol/l in 6 years was observed after adjustment for age, sex, season and body mass index (BMI). In the older cohort, a longitudinal decrease in mean serum 25(OH)D levels of 5 nmol/l in 13 years was observed.
Conclusions
Serum 25-hydroxyvitamin D levels changed during follow-up with increasing levels in persons aged 55–65 years, and decreasing levels in persons aged 65–88 years. This implicates that vitamin D supplementation becomes more important in older age groups.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
This work was supported, however funding details unavailable.

## P211

**Primary hyperparathyroidism, vitamin D deficiency and quality of life**

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Background
The study aimed to evaluate quality-of-life (QoL) aspects and impact of vitamin D deficiency in primary hyperparathyroidism (pHPT).
Method
One hundred and fifty consecutive patients (119 women) with pHPT (mean age $60\pm11$ years; BMI $27\pm5$ kg/m$^2$; ionized calcium $1.44\pm0.08$ mmol/l; PTH $130\pm69$ ng/l) were included. Six weeks postoperatively, the pHPT patients were reexamined and randomized (double-blind) to daily substitution with calcium carbonate (1 g), with or without cholecalciferol (1600 IE). Self-estimation questionnaires (SF-36) were completed at baseline and repeatedly during follow-up. A gender- and age-matched reference group ($n=459$) was randomly selected from the Swedish SF-36 norm database.
Results
Vitamin D deficiency, defined as 25-hydroxyvitamin D $<50$ nmol/l, was present preoperatively in 59% of the patients. The 25-hydroxyvitamin D level increased postoperatively (mean $49\pm18$ vs $52\pm17$ nmol/l, $P=0.019$). The calcium level normalized after parathyroidectomy in all patients, while the PTH level decreased but was not normalized in 45% of the cases. Compared to the reference population baseline, the patients scored significantly lower on all eight domains of the SF-36. Postoperatively, patients improved significantly but reached the level of the reference population in only two domains: GH (general health perceptions) and BP (bodily pain). No correlation was found between the SF-36 scores and the calcium, PTH or vitamin D levels at baseline. The baseline scores were inversely correlated to body mass index (BMI; $r=-0.226$–$r=-0.386$; $P<0.01$). The study will be deblinded on January 15 2012 and further follow-up data will be presented.
Conclusions
Patients with pHPT had worse QoL scores than the reference population on all eight domains of the SF-36. The outcome after parathyroid surgery was not predictable from preoperative calcium, PTH- or vitamin D status. The effects of vitamin D supplementation are yet to be evaluated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P212

**Cinacalcet treatment in MEN1-associated hyperparathyroidism: a clinical trial**

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Introduction
In type 1 multiple endocrine neoplasia (MEN1), primary hyperparathyroidism (PHPT) is a challenging problem due to the high post-surgery recurrence rate. Cinacalcet is a calcimimetic agent which showed to be effective in patients in whom surgery is contraindicated or refused. In this study we assessed the efficacy and the safeness of cinacalcet in MEN1 patients, in comparison with patients with sporadic PHPT (sPHPT). Moreover, the influence of Arg990Gly CASR polymorphism was also evaluated.
Design and methods
A randomised, cross-over, double-blind study was set up in University Hospitals context. Fifteen patients with PHPT MEN1-associated were randomised in two groups, one treated with 30 mg daily cinacalcet, titrated until calcium normalization, and one with placebo. Patients were reassessed after 3 months and switched to the other treatment once washed out. Twenty patients with cinacalcet treated sPHPT, having similar calcium levels, were considered as comparison group. Calcium/phosphate metabolism, bone turnover markers, liver and kidney functions, and perceived quality of life were evaluated at each assessment step. Arg990Gly CASR polymorphism was genotyped on leukocyte DNA by direct sequencing.
Results
Cinacalcet was able to normalize calcium levels and to reduced PTH in all patients, without significant effects on bone metabolism markers and quality of life. Cinacalcet dosage in MEN1 was not significantly different to sPHPT patients ($45\pm21$ vs $54\pm25$ mg/day). Few mild adverse events were observed in both groups, not requiring drug withdrawal. No association between Arg990Gly CASR polymorphism and response to cinalcalcet was found.
Conclusions
Although this is a short-term prospective study, it demonstrated that efficacy and safety profile of cinacalcet was similar both in patients with MEN1-associated PHPT and in those with sporadic PHPT, with no 990Gly CASR variant influence. Thus, in MEN1 patients, cinacalcet treatment might be considered an effective and safe option.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P213

**Serum levels of soluble secreted α-Klotho are decreased in the early stages of chronic kidney disease, making it a probable novel biomarker for early diagnosis**

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Background
α-Klotho was first identified as an aging gene and was later shown to be a regulator of phosphate metabolism. Fibroblast growth factor 23 (FGF23) is the key regulator of phosphate metabolism. Serum levels of soluble α-Klotho in

chronic kidney disease (CKD) patients have not previously been determined, especially in relation with FGF23 and creatinine levels. This study was designed to investigate whether serum soluble α-Klotho levels are modulated by renal function, age, and FGF23 level in CKD patients. This study is the first report on the utility of measuring soluble α-Klotho levels in human CKD.
Methods
A total of 292 CKD patients were enrolled. Serum samples were collected, and FGF23 and soluble α-Klotho levels were measured using enzyme-linked immunosorbent assay kits. In addition, serum creatinine, hemoglobin, albumin, calcium, and phosphate levels were measured.
Results
Serum soluble α-Klotho levels were associated positively with estimated glomerular filtration rate (eGFR) ($P<0.0001$) and inversely with serum creatinine level ($P<0.01$). Interestingly, α-Klotho levels were significantly decreased in stage 2 CKD compared with stage 1 ($P=0.0001$). Serum FGF23 levels were associated positively with serum creatinine and negatively with eGFR. FGF23 levels were significantly increased in stage 5 compared with stage 1 CKD. Soluble α-Klotho was associated inversely with log-transformed FGF23 level ($P<0.01$).
Conclusion
Our data indicate that soluble α-Klotho levels are significantly decreased in stage 2 CKD compared to stage 1, and not only in the advanced stages of the disease. Soluble α-Klotho may thus represent a new biomarker for the diagnosis of CKD, especially in the early stage.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P214

**Lack of effects of high dose colecalciferol (D3) on insulin sensitivity and metabolic markers in type 2 diabetic patients: a double-blind, randomised, placebo-controlled trial**

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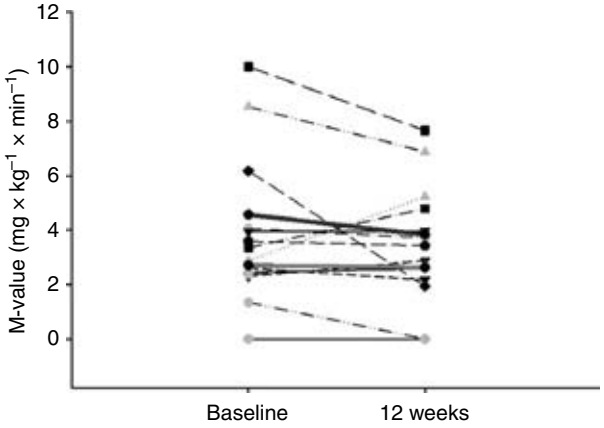
Introduction
Vitamin D insufficiency is common in subjects with type 2 diabetes. Observational studies suggest that vitamin D plays a role in the pathogenesis of type 2 diabetes. However, results of intervention studies have been inconsistent. We investigated the effects of improving vitamin D status on insulin sensitivity and inflammatory markers in type 2 diabetic patients with vitamin D insufficiency.
Methods
A double blind, randomized, placebo controlled, intervention study was conducted. Sixteen patients with type 2 diabetes and vitamin D insufficiency ($\leq$50 nmol/l) were recruited. Eight patients were randomized to supplementation with cholecalciferol for 12 weeks (11 200 IU (280 μg) daily for 2 weeks followed by 5600 IU (140 μg) daily for 10 weeks), and eight patients received identical placebo tablets for 12 weeks. To assess insulin sensitivity the hyperinsulinemic euglycemic clamp method was used. DEXA scan, 24-h-ambulatory blood pressure monitoring (ABPM) and fasting blood samples were performed at baseline and at the end of the 12 weeks intervention.
Results
Serum levels of 25(OH)vitamin D and 1,25$(OH)_2$vitamin D after 12 weeks increased significantly from baseline values in the intervention group ($P=0.01$ and $P=0.004$). Serum 25OHD concentrations increased from 31.0$\pm$4.9 nmol/l at baseline to 104.9$\pm$19.0 nmol/l in the intervention group, whereas a decrease from 34.8$\pm$3.8 to 32.1$\pm$3.4 nmol/l was observed in the placebo group. The change in serum 25OHD concentrations between the two groups differed significantly ($P=0.02$). However no significant changes in insulin sensitivity (*M*-value), inflammation, blood pressure, lipid profile, or HbA1c were found.
Conclusions
Significant improvement in vitamin D status after 12 weeks treatment with colecalciferol does not reduce insulin resistance, blood pressure, inflammation or glycosylated haemoglobin in patients with established type 2 diabetes.




Grey circles = Placebo, black = Vitamin D. Δ Vitamin D vs. Δ placebo: $P=0.431$ Blue line = Mean value Vitamin D, Red line = Mean value Placebo.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P215

**Blood pressure after parathyroidectomy in patients with primary hyperparathyroidism and hypertension**

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Introduction
Primary hyperparathyroidism (PHPT) is emerging as a cause of secondary hypertension. Between 20 and 50% of patients with PHPT have hypertension, this being directly proportional to the calcemia. The elevation of blood pressure in PHPT is mediated by hypercalcemia that acts directly stimulating the contraction of muscle fibers of the arterial vessel wall, and increases plasma renin levels and the release of catecholamines.
Objectives
(1) Determine the rate of normalization of blood pressure (BP) in hypertensive patients with PHPT after parathyroidectomy.
(2) Showed a reduction in the number of drugs if hypertension persists after surgery.
Materials and methods
A retrospective study of hypertensive patients with PHPT operated between 2005 and 2010. We use the Excalibur database, and look for the following key words: PHPT, hypertension, and parathyroidectomy. The following data were collected: age, sex, BMI, time with hypertension, pre-surgical PTH, calcium, phosphorus, 25OHVitamin D, creatinine, GFR, mean pre-surgery BP, pre-surgery anti-hypertensive drugs, mean post-surgery BP and post-surgical anti-hypertensive drugs. All patients included had to have biochemical criteria of cure PHPT.
Results
There were 88 patients with the search criteria: 18 (20.4%) were male and 70 (79.6%) were women. There was no statistically significant gender differences when comparing the other variables. The average values of the sample were: BMI: 28.2 kg/m$^2$, duration of hypertension: 8.8 years, pre-operative PTH: 124.6 pg/ml, serum calcium: 11.1 mg/dl, phosphoremia: 2.2 mg/dl, 25OH Vitamin D: 18 ng/dl. Creatinine 1.2 mg/dl, GFR 58 ml/min, pre-surgical BP 136.2/88.6 mmHg vs postoperative BP 130.3/82.2 mmHg ($P<0.005$), pre-surgical anti-hypertensive drugs 2.7 vs 2.2 in the post-surgery ($P<0.005$). 26.4% normalized their BP after surgery and did not require antihypertensive therapy. 54.4% of patients reduced the number and doses of drugs.
Conclusions
Although most patients probably had essential hypertension, PHPT appears to increase BP probably by serum calcium-dependent mechanisms. This could explain normalization tension and decrease in the number and doses of antihypertensive drugs presented by patients after parathyroidectomy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P216

**Serum cystatin C is a reliable predictor of renal function and cardiometabolic risk in primary hyperparathyroidism**

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Primary hyperparathyroidism (PHPT) negatively affects renal function. It is known that chronic kidney disease is a predictor of cardiometabolic diseases. Cystatin C (cystC), an alternative measure of renal function, has been associated with adverse cardiovascular events and cardiometabolic risk factors. This study was aimed to evaluate serum cystC, its relationships with PTH and cardiometabolic risk in patients with PHPT.
The following parameters were measured in 193 consecutive patients (46 males, 147 females, age 59.4±15 years, mean±S.D.) affected with PHPT: weight, height, arterial blood pressure, serum calcium, phosphate, PTH, 25-hydroxyvitamin $D_3$, glucose, insulin, triglycerides, HDL cholesterol, creatinine and cystC; BMI and the insulin-resistance index HOMA were calculated and the glomerular filtration rate was estimated with the Modification of Diet in Renal Disease Study formula (eGFR).
Mean eGFR was 88.7±26.6 ml/min×1.73 $m^2$ in PHPT patients, where only two patients showed an eGFR <30 ml/min×1.73 $m^2$. Median cystC value was 0.87 mg/l (range 0.45–3.13, 25th–75th percentile 0.73–1.04 mg/l) and it negatively correlated with eGFR ($r=-0.69$, $P<0.001$). Moreover, cystC showed a significant positive correlation with total and ionized calcium as well as PTH levels ($P<0.05$). In multiple regression analysis, cystC was a stronger determinant of PTH ($P<0.001$) than eGFR ($P=0.06$). Patients with cystC levels >75th percentile were older, they had higher BMI, insulin, HOMA, triglycerides, lower HDL and a greater prevalence of arterial blood hypertension, diabetes mellitus and dyslipidemia ($P<0.05$). After adjusting for age, cystC levels correlated with BMI, insulin, HOMA, triglycerides and HDL cholesterol levels ($P<0.05$).
In conclusion, in PHPT patients serum cystC demonstrated a good correlation with eGFR. Moreover, CystC seems a better predictor of PTH elevation than eGFR in PHPT patients. Elevated cystC levels identify a subgroup of PHPT patients with a cluster of cardiometabolic risk factors. CystC might be a reliable marker to evaluate renal function and cardiometabolic risk in PHPT patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P217

**Usefulness of MS-MLPA for detection of genetic and epigenetic states of GNAS complex in Pseudohypoparathyroidism type1b**

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Objective
Pseudohypoparathyroidism type 1b (PHP-1b) is rare disorders resulting from genetic and epigenetic aberrations in the GNAS locus. PHP-1b usually defined by isolated renal resistance to PTH, is due to a maternal loss of GNAS exonA/B methylation, leading to decreased Gs'$\alpha$ expression in specific tissues. To clarify the usefulness of methylation specific multiplex ligation-dependent probe amplification (MS-MLPA), we evaluate the genetic and epigenetic changes of GNAS comlex in PHP1b patients.
Patients and measurements
We studied a total of 13 subjects with PHP-1b (three families with nine affected members and four sporadic cases). The methylation status of GNAS DMRs was then evaluated using MS-MLPA. The presence of deletion mutation in GNAS complex and STX16 were assessed by using MLPA.
Results
In all the familial PHP1b cases, 3 kb deletion of STX16 and de-methylation of A/B domain were identified. In contrast, no deletion had been detected thoughout GNAS regions, however, methylation abnormalities were widely observed in the GNAS DMRs.
Conclusions
MS-MLPA facilitates precise and rapid analysis of methylation status in GNAS DMRs as well as detection of micro deletion mutation in PHP-Ib. MS-MLPA assays is useful molecular tools for understanding the molecular bases and confirming the diagnosis of PHP-1b.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P218

**Pathophysiological changes in extracellular pH regulate calcium-sensing receptor responsiveness in HEK-293 and isolated parathyroid cells but not as a result of altered histidine residue ionisation**

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Parathyroid hormone (PTH) secretion maintains extracellular calcium ($Ca^{2+}_o$) homeostasis under the control of the calcium-sensing receptor (CaR). Supra-physiological $pH_o$ changes (±1 pH unit) significantly alter CaR activity in a heterologous expression system. Interestingly, metabolic acidosis and hyperparathyroidism are concomitant in both ageing and chronic kidney disease. Therefore, here we examined whether smaller, pathophysiological $pH_o$ changes modulate CaR activity and in isolated (bovine) parathyroid cells as well as in CaR stably-transfected HEK-293 (CaR-HEK) cells.
Fura2-loaded CaR-HEK cells were stimulated with 2.5 mM $Ca^{2+}_o$ (pH 7.4) to elicit CaR-induced $Ca^{2+}_i$ mobilisation and then switched to the same buffer at either pH 7.2 (acidosis) or 7.6 (alkalosis) resulting in rapidly attenuated (−59±7%; $P=0.012$) or elevated (+31±9%; $P=0.011$) responses respectively. Furthermore, in isolated parathyroid cells, acidosis (pH 7.2) elicited a similar inhibitory effect on CaR-induced $Ca^{2+}_i$ mobilisation (−42±9%; $P=0.017$) while, again, alkalosis (pH 7.6) increased it (+35±10%; $P=0.039$). All responses were rapidly reversible upon return to pH 7.4 and are unlikely to result from altered intracellular pH as 0.4 unit $pH_o$ changes (5 min) failed to alter $pH_i$ in BCECF-loaded cells. The abundance of serum albumin *in vivo* might be expected to attenuate this effect, however, the $pH_o$ effects on CaR activity were preserved in the presence of 5% albumin (equivalent to serum concentration). Furthermore, acidosis also attenuated the CaR-induced activation of extracellular signal-regulated kinase (3.5 mM $Ca^{2+}_o$) by 56±11% ($P<0.05$) while alkalosis potentiated the response (+125±52; $P<0.01$). Finally, the most likely explanation for CaR's $pH_o$-sensitivity is extracellular histidine residue ionisation. However, serial mutation of all 15 histidine residues (to valine) failed to significantly attenuate CaR $pH_o$ sensitivity.
Therefore, pathophysiological changes in $pH_o$ significantly modulate CaR sensitivity with extracellular alkalinisation potentiating and extracellular acidification inhibiting CaR activity. This could indicate a mechanistic link between metabolic acidosis and hyperparathyroidism in ageing and renal disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P219

**Normocalcemic hyperparathyroidism: a personal experience**

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There are limited data on the natural history of normocalcemic PHPT and it is unclear how the patients should be regarded vis a vis parathyroid surgery. Among the 1500 admissions/year for 10 years referred to our outpatient endocrine clinics for a variety of diseases, we collected 481 subjects with high serum levels of PTH.

In the presence of hypercalcemia, a diagnosis of primary hyperparathyroidism was performed in 127 (26%). Other 354 (74%) subjects were normocalcemic: 186 (51%) were vitamin D deficient, 94 (27%) had renal failure, 39 (11%) suffered from secondary hyperparathyroidism due to different causes (mainly use of lithium and thiazides); the remaining 37 subjects (11%) (33 females, 4 males, age range 71 ± 11 years) had serum calcium and creatinine in the normal range and had a high PTH level. Among them 10 dropped out and the other 27 were followed up for 1–10 years. Over time 10 out of the 25 hyperparathyroid subjects became hypercalcemic (group A, followed up for 4–10 years, mean 7 years) while the others(17 pt) continue to be normocalcemic (group B, followed up for 1–10 years). The two groups were not different at baseline for calcium, creatinine, BMD expressed as *T*-score at lumbar spine (LS) and femoral neck (FN) while subjects in group A were significantly older than in group B. In group A (normocalcemic hyperparathyroid group) followed over time, nine patients had a significant reduction of BMD, three had also a fracture (two vertebral and one Colles) and one a kidney stone, none deterioration of renal function. Eight patients were operated on and a parathyroid adenoma was removed. In group B, followed over time, five subjects had a significant reduction of BMD. In conclusion our retrospective longitudinal study suggest that patients with normocalcemic hyperparathyroidism have a more important involvement of bone than not in mild primary hyperparathyroid patients. More studies are needed to identify patients who may develop features of PHPT over time.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P220

### Cardiac structure and functions in patients with asymptomatic primary hyperparathyroidism

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Introduction

Primary hyperparathyroidism is associated with cardiovascular disease. In addition, data about cardiovascular abnormalities in patients with asymptomatic primary hyperparathyroidsm (APHPT) is'nt clear. In this study, we aimed to examine the cardiac structure–functions and hs-CRP levels in patients with APHPT.

Materials and method

Thirty-one patients with APHPT and 32 sex- and age-matched control cases with similar cardiac risk factors were included.

Cardiac structure and functions were evaluated by two-dimensional Doppler echocardiography (PWD) and tissue Doppler echocardiography (TDI).

Results

Serum parathormone (PTH) and calcium (Ca) levels were higher in the APHPT group compared to the controls (181.38 (79–736) vs 58.36 (26.20–60.00) pg/ml $P<0.001$; 10.95 ± 0.62 vs 9.46 ± 0.30 mg/dl $P<0.001$). Left ventricular mass index (LVMI) was found to be significantly higher in APHPT group compared to the controls (112.09 ± 25.74 vs 93.42 ± 26.52 g/m$^2$, $P=0.006$). This statistical significance remained unchanged after the adjustment has been made according to the cardiovascular risk factors. LVMI was found to be correlated with the serum Ca level ($r=0.260$, $P=0.038$). Myocardial performance index (MPI) was significantly higher in the patient group compared to the controls (0.49 ± 0.64 vs 0.39 ± 0.48, $P<0.001$). MPI was found to be correlated to serum Ca ($r=0.493$, $P<0.001$), PTH ($r=0.494$, $P<0.001$), and LVMI ($r=0.334$, $P=0.007$). No difference was determined between two groups in terms of serum hs-CRP levels (2.38 (0.01–9.41) vs 3.07 (0.05–9.41) mg/l $P=0.063$). Also, no correlation was found between hs-CRP levels and LVMI and MPI ($P>0.005$).

Conclusion

Cardiac structure and functions are affected in patients with APHPT. These changes seem to occur independent of traditional cardiac risk factors, and correlated with serum PTH and Ca levels. Prognostic and clinical significance of these results should be investigated in larger and prospective studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P221

### Perioperative management difficulties in parathyroidectomy for primary vs secondary and tertiary hyperparathyroidism

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Background

In patients with hyperparathyroidism, parathyroidectomy is the only curative therapy. Anesthetic management differs function of etiology (primary vs secondary or tertiary hyperparathyroidism) and surgical technique (minimally invasive or classic parathyroidectomy).

Aim

To evaluate peri-operative management in parathyroidectomy for hyperparathyroidism of various etiologies, in a tertiary center.

Patients and methods

Two hundred and ninety-two patients who underwent surgery for hyperparathyroidism between 2000 and 2010 were retrospectively reviewed; 96 patients (77F/19M) presented with primary hyperparathyroidism (group A) and 196 (116F/80M) with secondary and tertiary hyperparathyroidism due to renal failure (group B). Biochemical parameters (serum calcium, phosphate, creatinine) were determined by automated standard laboratory methods. Serum intact PTH was measured by ELISA (IPTH – normal range: 10–71 pg/ml).

Results

Median surgery duration was 30 minutes in group A (minimally invasive or classic parathyroidectomy) and 75 min in group B (total parathyroidectomy and re implantation of a small parathyroid fragment into the sternocleidomastoid muscle).

During anesthesia induction, arterial hypotension developed significantly more frequent in group B (57 out of 196 pts, 29.1%) than in group A (8 out of 96 pts, 8.34%), $P<0.0001$, especially in patients receiving Fentanyl -Propofol.

During surgery and anesthesia maintenance, bradycardia was significantly more frequent in group A (67 out of 96 pts, 69.8%) than in group B (26 out of 196 pts, 13.3%), $P<0.0001$, especially during searching of parathyroid glands. By contrary, ventricular premature beats were less frequent in group A (25 out of 96 pts, 25.25%) than in group B (84 out of 196 pts, 42.85%), $P=0.003$. There were no statistically significant differences between the studied group regarding frequency of arterial hypertension and hypotension, paroxysmal atrial fibrillation.

Conclusions

Anaesthetic management in parathyroid surgery may be difficult because of cardiac arrhythmias (bradycardia in primary hyperparathyroidism and ventricular premature beats in secondary and tertiary hyperparathyroidism, respectively) and arterial hypotension during anesthesia induction in patients with secondary and tertiary hyperparathyroidism.

Declaration of interest

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## P222

### Evaluation of glucose metabolism in primary hyperparathyroid patients

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Aim

In this study, our aim was to investigate frequency of impaired glucose tolerance (IGT), undiagnosed diabetes mellitus (DM) and presence of insulin resistance by HOMA.

Material and method

Fifty-five primary hyperparathyroid patients without known glucose tolerance were included in this study. The control group was with similar BMI, normocalcemic, without known glucose tolerance. Seventy-five gram oral glucose tolerans test was performed to PHP patients and control group. Insulin resistance was calculated by HOMA-IR.

Results

Mean body mass index (BMI) was 29.16 ± 4.92 kg/m$^2$ in PHP group, 29.54 ± 4.38 kg/m$^2$ in control group ($P=0.676$). Serum Ca level was 10.46 ± 0.81 mg/dl

in patient group, 9.26±0.39 mg/dl in control group ($P=0.000$). While IGT was detected in 6 PHP patients (%11.3), there is no IGT in control group ($P=0.013$). Diabetes mellitus (DM) didn't detect in OGTT in PHP patients. HOMA-IR was 1.51±0.89 in PHP group, 1.36±0.92 in control group ($P=0.458$). Second hour glucose OGTT value was significantly higher than control group (respectively, 99.28±30.86 mg/dl, 81.05±24.89 mg/dl, $P=0.001$). Second hour insulin OGTT value was significantly higher than control group (respectively, 22.84 ±17.92 μIU/ml, 14.94±14.10 μIU/ml, $P=0.02$). In both groups there were positive correlation between Ca levels and fasting plasma glucose, OGTT second hour glucose, OGTT second hour insulin (respectively, $P=0.011$, $r=0.241$; $P=0.000$, $r=0.400$; $P=0.004$, $r=0.296$).

Conclusions

Frequency of IGT is increased in PHP patients. Increased levels of serum Ca affects the glucose metabolism and leads to glucose intolerance.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P223

### The prevalence of hypocalcaemia with low parathyroid hormone level in Polish elderly population

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Introduction

The postoperative and idiopathic (autoimmune) hypoparathyroidism are the most frequent causes of hypocalcaemia with low parathyroid hormone level. Moderate chronic hypocalcaemia frequently is asymptomatic, thus the prevalence of hypocalcaemia with low parathyroid hormone level in the general population remains unknown. Therefore, the aim of this study is to assess the prevalence of hypocalcaemia with low parathyroid hormone level in a representative sample of Polish elderly population.

Description of methods/design

The study was carried out as a part of the nationwide PolSenior project (Medical, psychological and socioeconomic aspects of aging in Poland) in the population of randomly selected 5695 participants (2899 males and 2796 females) using the national PESEL database (the National Electronic System of Population Records). Serum calcium, phosphates, albumin, creatinine, 25-OH-D3 and serum intact PTH concentrations were assessed in 4270 participants accounting for 75.0% of the study group. Serum calcium corrected for albumins lower than 8.4 mg/dl was scored as hypocalcaemia. Hypoparathyroidism was diagnosed when hypocalcaemia was accompanied by low serum intact PTH (<15 pg/ml) concentration.

Results

Only in 10 participants hypocalcaemia with low PTH were identified including 6 of 2213 (0.27%) males and 4 of 2057 (0.19%) females. The prevalence of primary hypoparathyroidism in the Polish elderly population was estimated at 0.25%. The median levels of corrected calcium and phosphates in participants with hypocalcaemia and low parathyroid hormone level were 7.99 (7.02–8.25) mg/dl and 3.44 (2.98–5.68) mg/dl, respectively. Only in two subjects serum calcium level was below 7.0 mg/dl. Two out of 10 participants with hypoparathyroidism had serum vitamin D level lower than recommended (<30 ng/ml). None of the participants with hypoparathyroidism were receiving calcium or vitamin D supplementation.

Conclusions

The prevalence of such a rare disease as primary hypoparathyroidism is quite high in the polish elderly population. In most subjects hypocalcaemia was mild, asymptomatic and remained undiagnosed.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P224

### Obesity, carbohydrate, lipid metabolism and polymorphisms (Fok, Bsm) of vitamin D receptor gene in aging population: POLSENIOR study

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Pleiotropic effects of vit. D in the etiology of obesity, metabolic syndrome and diabetes are still debated. So far published reports considered only changes in vit D plasma level in individuals with obesity and diabetes, and did not take into account the role of polymorphisms in the vit. D receptor gene.

The aim of the study was to evaluate obesity, carbohydrate and lipid metabolism and vitamin D level in two VDR polymorphisms (Fok and Bsm) in Polish aging population – POLSENIOR Study.

The study involved randomly selected out of 4737 individuals 982 subjects (456 women and 526 men) aged above 65, in whom the following measurements were performed: BMI, waist circumference, and blood serum HDL cholesterol, triglycerides, vitamin D, glucose and insulin (HOMA index) levels. In order to apply the genetic material, PCR was used. To identify polymorphisms, RFLP technique was used. The reaction products were analyzed by electrophoresis on 2% agarose gel. Statistical significance of the relationships between investigated metabolic parameters in relation to investigated polymorphisms was evaluated by means of the ANOVA test.

Results

Our findings revealed only statistically significant elevated levels of glucose for the polymorphic form digested with the Bsm enzyme of the VDR gene, both in homozygotes as well as heterozygotes, however when gender was taken into account, the statistical significance disappeared. The remaining metabolic parameters did not reveal and relationship to the investigated polymorphisms.

Conclusion

The polymorphisms VDR (Fok, Bsm) don't plays key role in metabolic disorders in aging polish population implemented under publicly-funded project no. PBZ-MEIN-9/2/2006.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P225

### Quality of life in premenopausal women with vitamin D deficiency and vitamin D insufficiency

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Introduction

The aim of this study was to compare quality of life among premenopausal women with vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency.

Methods

Premenopausal women with chronic fatigue, myalgia and nonspecific body pain were divided according to their vitamin D levels: ≤20 ng/ml (vitamin D deficient, $n=30$), 21–29 ng/ml (vitamin D insufficient, $n=30$) and ≥30 ng/ml (vitamin D sufficient, $n=20$). The groups were compared regarding the scales of short form-36 (SF-36). Higher scores in SF-36 were associated with better quality of life. Women with depression, thyroid disorders, diabetes mellitus, obesity, renal and hepatic disorders and other chronic diseases were exluded.

Results

Women with vitamin D deficiency had lower physical function scores than women with vitamin D insufficiency ($P=0.001$). Women with vitamin D insufficiency had lower physical component ($P=0.025$), mental component ($P=0.025$) and vitality ($P=0.02$) scores than women with vitamin D sufficiency. When women with vitamin D deficiency and insufficiency were combined ($n=60$) and compared with vitamin D sufficiency group, they had lower physical component ($P=0.002$), mental component ($P=0.01$), physical function ($P=0.0002$), social function ($P=0.04$) and vitality ($P=0.01$) scores. Vitamin D levels were positively correlated with physical component ($r=0.3$), physical function ($r=0.4$) and role physical ($r=0.3$) scores.

Conclusion
Vitamin D status affects quality of life in premenopausal women without other chronic disorders even in the insufficiency level. Impairment in quality of life is seen in both physical and mental components, but physical component seems to be more affected. This study is the first study evaluating quality of life in premenopausal women according to vitamin D status and the results may have an impact on daily clinical practice, espacially when evaluating women with nonspecific symptoms.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P226

**Intraoperative parathormone monitoring allows successful minimally invasive parathyroidectomy in patients with non concordant preoperative imaging**

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Introduction
Sestamibi (SM) and ultrasound (US) are routinely used for preoperative localisation of abnormal parathyroids in patients with hyperparathyroidism. Minimally invasive parathyroidectomy (MIP) is recommended if localisation is concordant, otherwise neck exploration is the method of choice.
Aim
To assess whether introduction of intraoperative parathormone (IOPTH) monitoring allows us to perform MIP successfully in patients with non concordant findings.
Method
Retrospective review of patients operated between 2006–2011.
Results
158 patients with hyperparathyroidism (120 female, 38 male) with median age 60 (13–85) had parathyroidectomies with IOPTH monitoring.
152 patients had US of which 118 identified an abnormal parathyroid and 153 patients had SM of which 106 identified an abnormal parathyroid. 73 (46.2%) patients had concordant imaging qualifying them for MIP but 85 patients (53.8%) with non-concordant findings were also scheduled for MIP.
114 patients (72.1%) had MIP of which 6 were converted to neck explorations (5%). 44 patients (27.8%) who had simultaneous thyroidectomies, sternotomies or mediastinoscopies for ectopic glands or all 4 glands removed had neck exploration.
Overall, 154 patients (97.4%) were cured. 107 (99%) of patients who underwent MIP and 41 (93%) patients who had neck explorations were cured.
Conclusions
Introduction of IOPTH monitoring allowed successful MIP not only in patients with concordant findings but also in patients with non concordant preoperative imaging. Use of IOPTH monitoring doubled the number of Minimally Invasive Parathyroidectomies in our series.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P227

**Vitamin D status, chronic kidney disease and secondary hyperparathyroidism in Polish elderly population**

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Introduction
Secondary hyperparathyroidism (SHPT) develops more frequently in the elderly. The high prevalence of chronic kidney disease and inappropriate vitamin D status are among the predisposing factors. The aim of this study is to assess the prevalence of SHPT, chronic kidney disease (CKD) and vitamin D status in a representative group of Polish elderly population.
Objective
The study was carried out as a part of the nationwide PolSenior project (medical, psychological and socioeconomic aspects of aging in Poland) in the population of randomly selected 5695 participants (2899 males and 2796 females) using the national PESEL database (the National Electronic System of Population Records). Serum calcium, phosphates, creatinine, 25-OH-D3 and PTH concentrations, as well as albumin to creatinine ratio in urine were assessed in 4270 participants accounting for 75.0% of the study group. SHPT was diagnosed in participants with normal (8.4–10.0 mg/dl) or decreased ($<$8.4 mg/dl) corrected serum calcium concentration and increased ($>$65 pg/ml) intact PTH level. Estimated glomerular filtration rate (eGFR–CKD–EPI) below 60 ml/min per 1.73 $m^2$ or albuminuria $\geq$ 30 mg/g creatinine was interpreted as CKD. Low vitamin D status was ranked as hypovitaminosis (20–29.9 ng/dl), insufficiency (10–19.9 ng/dl) or deficiency ($<$10 ng/dl).
Results
Among the studied participants, 540 (12.6%) subjects were identified as having SHPT. The prevalence of SHPT in the Polish elderly population was estimated at 10.43%. It was increasing with age from 4.39% in the subjects aged 55–59, through 6.07% in those aged 65–69, 8.61% in those aged 70–74, 13.49% in those aged 75–79, 15.48% in those aged 80–84, 17.786% in those aged 85–89, to 25.97% in the group aged 90 years or more. Impaired eGFR was found in 267 (49.4%), albuminuria in 127 (23.5%) and CKD in 310 (57.4%) of participants with SHPT, while hypovitaminosis D was demonstrated in 150 (27.8%), vitamin D insufficiency in 147 (27.2%), and deficiency in 32 (5.9%). CKD or low vitamin D status was found in 82.6% of SHPT causes. Improper serum vitamin D concentration was found in 62,3% of participants with CKD.
Conclusions
Low serum vitamin D concentration is frequently observed in elderly patients with chronic kidney disease and secondary hyperparathyroidism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P228

**Vitamin D status in primary hyperparathyroidism: a 'Southern European' observational study**

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Introduction
It has been reported that vitamin D deficiency is common in patients with primary hyperparathyroidism (pHPT), and this could affect the clinical expression of the disease. However only few studies addressed this issue and refer to American or North European patients. In these studies, only about one third of the patients showed a vitamin D replete state.
Subjects and methods
In 207 consecutive pHPT patients (M/F=45/162, age (mean $\pm$S.D.): 60.1$\pm$13.6 years, PTH=216.1$\pm$202.8 pg/ml, calcium=11.1$\pm$1.2 mg/dl, asymptomatic/symptomatic=89/118)) we have assessed vitamin D status by measuring plasma 25-OH vitamin D (25-OHD) levels. Vitamin D deficiency was defined as 25-OHD $<$20 ng/ml, whereas vitamin D insufficiency was defined as 25-OHD $>$20 to $<$30 ng/ml.
Results
In our series 36.2% of the patients showed 25-OHD deficiency and 21.3% showed 25-OHD insufficiency. 37% of female patients resulted 25-OHD deficient and 19.8% insufficient. 33.3% of male patients resulted 25-OHD deficient and 26.7% insufficient. 38.1% of symptomatic patients resulted 25-OHD deficient and 22.1% insufficient. 33.7% of asymptomatic patients resulted 25-OHD deficient and 20.2% insufficient. 25-OHD levels were significantly related with BMD at forearm, at femur and at spine ($R=0.27$, $P<0.0003$ at forearm; $R=0.16$, $P<0.04$ at femur, $R=0.20$, $P<0.009$ at lumbar spine). Moreover 25OHD-levels were negatively related with PTH ($R=-0.38$, $P<0.000001$), calcium ($R=-0.27$, $P<0.00009$) and bone turnover markers levels ($R=-0.21$, $P<0.003$ for osteocalcin, $R=-0.38$, $P<0.0004$ for cross-links). No seasonal variability was observed in 25-OHD levels.

Conclusions
In this 'Southern European' pHPT case series the proportion of 25OHD deficiency is lower than that reported for North American and North European studies. These differences could be due to the different latitude/skin UV exposure. 25-OHD status is related to biochemical indexes of the disease but it does not seem to affect classical pHPT manifestations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P229

**Vitamin D and rheumatoid arthritis**

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Vitamin D deficiency has been implicated in the pathogenesis of autoimmune diseases, such as diabetes mellitus type 1 and multiple sclerosis. Reduced vitamin D intake has been linked to increased susceptibility to the development of rheumatoid arthritis (RA) and vitamin D deficiency has been found to be associated with disease activity in RA patients. The aim was to evaluate vitamin D status in RA patients and to assess the relationship between vitamin D levels and disease activity.
In a cohort of 44 patients suffering from RA 25(OH)D3 levels, parathyroid hormone levels, C-reactive protein and ESR were measured. Disease activity was evaluated by calculating the DAS28 score. A control group ($n=44$), matched for age and sex, was evaluated as well. All patients fulfilled the American College of Rheumatology criteria for the classification of RA.
In the cohort of 44 RA patients 25(OH)D3 levels were found to be low as compared to the control group, 25(OH)D3 being $15.36\pm1.09$ and $24.9\pm1.2$ ng/ml, in the patient and control group respectively (Student's $t$-test, $P<0.05$). Parathyroid hormone levels were $70.82\pm7.22$ pg/ml (normal values 10.0–65.0 pg/ml), CRP $7.59\pm1.64$ mg/l (normal values $<3$ mg/l) and ESR was $36.7\pm4.5$ mm/h in the group of RA patients. Levels of 25(OH)D3 were found to be negatively correlated to the DAS28 score, correlation coefficient being $-0.065$. Levels of 25(OH)D3 were also found to be negatively correlated to CRP and ESR, correlation coefficient being $-0.11$ and $-0.16$ respectively.
It appears that vitamin D deficiency is highly prevalent in RA patients, and that vitamin D deficiency may be linked to disease severity in RA. As vitamin D deficiency has been linked to diffuse musculoskeletal pain, these results have therapeutic implications. Vitamin D supplementation may be needed both for the prevention of osteoporosis as well as for pain relief in rheumatoid arthritis patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P230

**The prevalence of primary hyperparathyroidism in Polish elderly population**

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Introduction
Primary hyperparathyroidism (PHPT) develops more frequently in the elderly with marked predisposition for female gender. The prevalence of PHPT in Poland is unknown. Therefore, the aim of this study is to assess the prevalence of PHPT in a representative sample of Polish elderly population.
Description of methods/design
The study was carried out as a part of the nationwide PolSenior project (medical, psychological and socioeconomic aspects of aging in Poland) in the population of randomly selected 5695 participants (2899 males and 2796 females) using the national PESEL database (the National Electronic System of Population Records). Serum calcium, phosphates, 25-OH-vitamin D and PTH concentrations were assessed in 4588 out of 4737 obtained blood samples, constituting 80.6% of the study group. PHPT was diagnosed in participants with increased corrected serum calcium concentration ($>10.6$ mg/dl) and increased ($>65$ pg/ml) or normal (15–65 pg/ml) intact PTH level or corrected serum calcium $>10.0$ mg/dl and increased intact PTH concentration.
Results
Fifty-six participants including 18 out of 2358 (0.76%) males and 38 out of 2230 (1.70%) females with PHPT were identified. The prevalence of PHPT in the Polish elderly population was estimated at 0.99%. Female gender significantly increased the risk for PHPT (OR = 2.25(1.28–3.96); $P=0.005$). The higher prevalence of PTHP was found in the oldest subjects: aged 85–89 (1.31% of males and 3.01% of females) and 90 years. or above (1.83% of males and 3.93% of females). Thirty-six out of 56 participants with PHPT had vitamin D level lower than recommended ($<30$ ng/ml). None but one of the participants with PHPT had prior episode of stone expulsion or lithotripsy.
Conclusions
The prevalence of PHPT is increased in the very old. PHPT in the elderly does not seem to increase the risk of kidney stones.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P231

**Markers of bone turnover and polymorphism of vitamin D receptor gene in aging population: POLSENIOR study**

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The levels of bone turnover markers are elevated e.g. in osteoporosis, neoplastic diseases with bone involvement (e.g. myeloma). Recently bone markers have been found to play an important role in other diseases, like cardiovascular diseases (CVD). Vitamin D, which plays a role in body metabolism, is also an important hormone maintaining bone homeostasis.
The aim of the study was to evaluate the levels of bone turnover markers (osteoprotegerin and ICTP) in vitamin D receptor gene polymorphisms in the population of responders of the POLSENIOR project.
Out of a group of 5695 randomly selected responders of the POLSENIOR study, blood was collected from 4737 individuals. Out of this group, 982 individuals (including 456 females and 526 males) aged above 65 had the levels of 25-OH-D3, ICTP and OPG (RIA method) measured. In order to amplify the genetic material, PCR was used. To identify polymorphisms of the VDR (Fok and Bsm) RFLP technique was used. The reaction products were analyzed by electrophoresis on 2% agarose gel.
No differences in the vitamin D level were found among the investigated polymorphisms in the whole investigated group. Among bone turnover markers, only in case of ICTP subjects with the bm polymorphic form (digested by BSM 1 enzyme) were characterized by lower ICTP level (on the borderline of significance = 0.08). When the group was divided into genders, it appeared that in males the bm polymorphic form revealed also lower level of OPG, which reached the level of statistical significance ($P=0.027$).
Conclusions
Our studies demonstrated that the bm polymorphic form of vitamin D receptor may play a protective role against bone resorption.
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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P232

**Endothelial functions of the patients with hyperparathyroidism**

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Introduction
Primary hyperparathyroidism (PHPT) is associated with increased risk of mortality from cardiovascular disease and this appears to decrease with time after parathyroidectomy. However, data on hyperparathyroid patients are conflicting and there is no data regarding secondary hyperparathyroid patients. Our aim in this study was to determine the association between flow-mediated dilatation (endothelium dependent, FMD), nitroglycerine-induced dilatation (endothelium independent, NID) and carotid artery intima media thickness (IMT) in primary hyperparathyroidism (PHPT), secondary hyperparathyroidism (SHPT) and the control group.
Material and methods
Twenty patients with primary hyperparathyroidism, twenty with secondary hyperparathyroidism and 12 healthy subjects were included in the study. Both groups were matched with respect to age. Serum calcium level, parathormone(PTH) and daily urinary calcium excretion (UCE) were calculated and FMD, NID and IMT were also evaluated for all subjects.
Results
Serum Ca levels were significantly higher in patients with primary hyperparathyroid patients compared to both patients with secondary hyperparathyroidism and the control group ($P<0.001$ and $P<0.001$ respectively). As expected, urinary calcium excretion levels of the patients with primary hyperparathyroidism were higher than both the control group and the patients with secondary hyperparathyroidism ($P<0.001$, $P<0.001$ respectively). Serum PTH levels were also significantly higher in patients with primary and secondary hyperparathyroidism than the control group ($P<0.05$ and $P<0.05$ respectively). FMD, NID and IMT levels were not significantly different between the groups. But there was a negative correlation between serum calcium levels and FMD ($r:-0.41$ $P<0.01$).
Conclusion
This finding suggests that the cause of the cardiovascular diseases seen in hyperparathyroid patients is the increased calcium levels rather than the hyperparathyroidism itself.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P233

**The importance of citrate and the calcium/citrate ratio in patients with calcium renal lithiasis and severe lithogenesis**

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Introduction
One of the factors influencing a predisposition to calcium renal lithiasis is a lack of crystallization inhibitors, the most important of which is citrate. The aim of our study was to analyze the importance of urinary citrate and the urinary calcium/citrate ratio in patients with calcium renal lithiasis and severe lithogenesis in comparison with control group patients without lithiasis.
Methods/Design
We conducted a cross-sectional study of 115 patients in eastern Andalusia (Spain). The patients were divided into two groups: groups A and B. Group A: 56 patients aged 25 to 60 years without calcium renal lithiasis. Group B: 59 patients aged 25 to 60 years presenting with calcium renal lithiasis and severe lithogenesis. We analyzed and compared the citrate levels and the calcium/citrate ratio in the patients' urine and the relationship of these two factors to lithiasic activity. In addition, we examined the bone mineral density across groups.
Results
In group B, 32.2% of the patients presented with hypocitraturia, compared with 14.3% of the patients in group A ($P=0.02$). The urinary citrate levels were lower in group B compared to group A ($P=0.001$), and the calcium/citrate ratio was higher in group B compared to group A ($P=0.005$). Our results suggest that a patient urine calcium/citrate ratio $\geq 0.25$ indicates serious lithogenesis (sensitivity of 89%, specificity of 57%). After a linear regression analysis, we found that the urinary citrate level is an independent factor associated with changes in bone densitometry *T*-score values of patients.
Conclusion
Compared to the control group, the patients with severe lithogenesis presented with hypocitraturia, which was associated with lower bone mineral density. The calcium/citrate ratio, which is linearly related to the bone resorption marker β-crossLaps, could be useful in evaluating the risk of serious lithogenesis when its value is exceeds 0.25.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P234

**The diagnostic values of serum intact parathyroid hormone, calcium and phosphorus for the prediction of vitamin D deficiency**

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Background
The results of studies evaluating the relationship between serum 25-hydroxyvitamin D (25OHD) with intact PTH (iPTH), calcium (Ca) and phosphorus (P) levels are controversial.
Objective
To determine the diagnostic values of iPTH, Ca and P for the prediction of vitamin D deficiency.
Methods
We analysed iPTH, Ca and P levels in otherwise healthy 700 subjects (619 (88.4%) female, 81 (11.6%) male, mean age $47.44\pm16.58$ years). To define deficiency status, 25OHD were further classified into four groups, ($<25$ nmol/l as severely deficient, 25–49.9 nmol/l as deficient, 50–74.9 nmol/l as insufficient and $\geq 75$ nmol/l as sufficient, respectively).
Results
The mean 25OHD, iPTH, Ca and P levels were $39.42\pm29.43$ nmol/l, $66.16\pm34.57$ pg/ml, $9.63\pm0.46$ and $3.35\pm0.52$ mg/dl respectively. 271 (38.7%) of the subjects were classified as severely 25OHD deficient, 237 (33.9%) as deficient, 91 (13.0%) as insufficient and 101 (14.4%) as sufficient respectively. iPTH were inversely correlated with 25OHD ($r=-0.264$), while no correlation was found between Ca, P, age and gender with 25OHD ($r=0.099$; $r=0.065$; $r=0.003$ and $r=0.012$ respectively). Secondary hyperparathyroidism (sPTH) were present in 277 (46.24%) of the subjects with low 25OHD levels (in 167 (61.6%), 81 (34.2%) and 29 (31.9%) of the subjects with severely deficient, deficient and insufficient 25OHD levels respectively). In post-hoc analysis iPTH were significantly higher in subjects with severe 25OHD deficiency compared to those with deficient, insufficient and sufficient 25OHD levels ($P<0.0001$ for all). Nevertheless, iPTH were not significantly different when subjects with deficient 25OHD were compared with those who had insufficient and sufficient 25OHD levels ($P>0.05$ for all). In ROC analysis the overall sensitivity and specificity for the best iPTH cut-off value (62.5 pg/ml) in subjects with low 25OHD were 65.3 and 64.8% respectively.
Conclusions
Despite of a significantly higher iPTH levels in subjects with severe 25OHD deficiency, neither iPTH nor Ca and *P* have sufficient sensitivity and specificity to precisely predict vitamin D deficiency.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P235

**Vitamin D and gestational diabetes**

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Background and aims
We aimed to establish the frequency of vitamin D deficiency and analyse its association with gestational diabetes (GDM).

Material and methods
In this descriptive, case control and cross sectional study, pregnant women were screened at 24–28 weeks of pregnancy using a 75 g, 2 h oral glucose tolerance test. Fourthy-six GDM diagnosed (case, group 1) and 43 healthy pregnants (control, group 2) were recruited. Plasma 25-hydroxyvitamin D levels of less than (20 ng/ml) were defined as deficiency. Groups were matched according to their serum magnesium levels, multivitamin use and hypertensive status. Mann–Whitney *U* test and Pearson correlation test were used for statistical comparisons.
Results
The mean ages were 33.3 ± 4.9 years (18–44 years) in group 1, and 29.9 ± 4.4 years (21–39 years) in group 2. Mean vitamin D levels were 20.5 ± 10.1 ng/ml (min. 4–max. 39.3) in group 1 and 23.1 ± 12.6 ng/ml (min. 6.6–max. 73) in group 2 ($P = 0.3$). Frequency of vitamin D deficiency status was not significantly difference between groups (51.4% ($n = 18$) in group 1 and 44.7% ($n = 17$) in group 2).
Plasma vitamin D levels were negatively correlated with OGTT (50 gr) and PTH levels (respectively $r = -0.7$, $P = 0.006$, $r = -0.8$, $P = 0.002$). There was no association between vitamin D levels and serum insulin, fasting glucose, HbA1c levels and BMI. Eleven of vitamin D-deficient pregnants (30.6%) were veiled and only seven pregnants (19.4%) were engaged in physical activity of at least 30 min/day duration.
Conclusion
Vitamin D deficiency is prevalant in our GDM patients as in our general population. This may be mostly attributable to lifestyle and genetic factors. As GDM and hypovitaminosis D continue to increase all in the world, we suggest that establishing potential factors that affect GDM and improving vitamin D status in pregnancy may prevent their possible adverse health outcomes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P236

**Bone biomarkers, body composition and metabolic profile in patients with primary hyperparathyroidism**

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Objective
Primary hyperparathyroidism (PHPT) is systemic disease affecting bone metabolism and glucose homeostasis. Patients with PHPT are insulin resistant and have atherogenic dyslipidaemia. Osteocalcin (OC) is bone biomarker associated with increased insulin secretion and insulin sensitivity and decreased visceral fat The aim of our study was to evaluate the body composition and levels of bone biomarkers (osteocalcin and betaCTx), insulin sensitivity and lipids in patients with PHPT.
Material and methods
In 25 patients with PHPT (Group 1 – age: 55.08 ± 11.62 years, BMI 26.75 ± 4.44 kg/m$^2$, PTH 144.27 ± 138.17 ng/l, Calcium 2.94 ± 0.26 mmol/l) and 8 healthy controls (Group 2 – age: 55.00 ± 6.90 years, BMI 224.70 ± 3.94 kg/m$^2$, PTH 35.46 ± 10.82 ng/l, Calcium 2.52 ± 0.15 mmol/l) OC(ng/ml), betaCTx(ng/ml), glucose(mmol/l), insulin(IU/ml), phosphate (mmol/l), total cholesterol (TC, mmol/l), HDL-C, LDL-C, triglyceride (TG, mmol/l), ApoA1, ApoA2, ApoB, ApoE, Lp(a) and vitamin D levels were determinated. HOMA IR was calculated as a marker of insulin resistance, body composition was evaluated using DEXA.
Results
There was significant difference between Group 1 and Group 2 in total (35.00 ± 4.84 vs 29.90 ± 4.77%, $P < 0.05$) and truncal fat percent (33.59 ± 6.72 vs 24.60 ± 6.38%, $P < 0.05$), TC (6.47 ± 1.34 vs 5.32 ± 0.88, $P < 0.05$), LDL (4.27 ± 1.14 vs 3.26 ± 0.93, $P < 0.05$), TG (1.83 ± 0.82 vs 1.14 ± 0.61, $P < 0.05$) and Apo B (1.20 ± 0.0.29 vs 0.85 ± 0.23, $P < 0.05$). In Group 1 OC (67.93 ± 14.39 vs 30.08 ± 15.54, $P = 0.05$), betaCTx (1.11 ± 1.00 vs 0.61 ± 0.39, $P = 0.05$) and Apo E (50.17 ± 14.62 vs 40.37 ± 10.79, $P = 0.05$) were also higher than in Group 2. There was no difference in HOMA IR between groups (3.29 ± 1.24 vs 3.12 ± 1.38, $P > 0.05$). There was significant correlation between betaCTx and trunkal fat percent ($R = -0.44$, $P < 0.05$), insulin ($r = -0.42$, $P < 0.05$), OC ($r = 0.95$, $P < 0.01$) and PTH levels ($r = 0.62$, $P < 0.01$), as well between OC and phosphate ($r = -0.42$, $P < 0.05$) and PTH levels ($r = 0.68$, $P < 0.01$).
Conclusion
Patients with PHPT have atherogenic lipid profile, elevated bone biomarkers and more visceral fat in comparision to healthy controls. In our group of patients with PHPT betaCTx (but not the OC) levels suggest possible interrelation between bone, glucose and lipid metabolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P237

**The daily calcium intake is associated with sarcopenia in older, non-obese koreans: the fourth korea national health an nutrition examination surveys (KNHANES IV) 2009**

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Backgroud
The association with daily calcium intake and sarcopenia has not been well established.
Objective
The objective was to assess the associate of daily calcium intake and sarcopenia.
Methods
We analysed the fourth Korea National Health and Nutrition Examination Survey (KNHANES) in old adult (over 50 years), conducted in 2009. A total of 1964 non-obese (body mass index (BMI) <25 kg/m$^2$) old adults (871 men and 1093 women) were enrolled. Dietary variables were assessed using a 24 h recall method in the nutrition survey. The intake of daily calcium from the consumption of each food item was calculated. Sarcopenia was defined as an appendicular skeletal muscle mass divided by body weight that was less than two S.D. below the sex-specific mean for young adults. Obesity was defined as a body mass index (BMI) of 25 kg/m$^2$ or higher.
Results
Daily calcium intake correlated negatively with appendicular fat mass, although not significant ($P = 0.201$). However, we found that Daily calcium intake correlated positively with appendicular skeletal mass ($P < 0.001$). Our study showed that participants with sarcopenia have significantly lower daily calcium intake compared to participants without sarcopenia ($P < 0.001$). The unadjusted prevalence of sarcopenia according to tertiles of daily calcium intake were 52.2, 31.9 and 15.9% in tertile1 – tertile 3. After adjustment for age, sex, BMI, total energy intake and lifestyle factors, compared with those in the lowest tertile of daily calcium intake, participants in the highest tertile had an odds ratio for sarcopenia of 0.311 (95% CI, 0.129–0.748).
Conclusion
Our study suggest that there is a strong inverse association between daily calcium intake and sarcopenia in non-obese old adult Korean.
Adjusted Odds ratiom and 95% CI of sarcopenia by tertiles of daily calcium intake.

| Tertile of daily calcium intake (mg/d) | T1(<274.8) *n*=654 | T2(274.6–488.7) *n*=656 | T3(>488.8) *n*=654 | P for trend |
|---|---|---|---|---|
| Unadjusted | 1(ref) | 0.596 (0.346–1.024) | 0.294 (0.148–0.582) | <0.001 |
| Model 1 (age and sex) | 1(ref) | 0.677 (0.387–1.185) | 0.352 (0.173–0.719) | 0.003 |
| Model 2 (age, sex, BMI and total energy intake) | 1(ref) | 0.712 (0.392–1.293) | 0.421 (0.192–0.921) | 0.029 |
| Model 3(age, sex, BMI, total energy intake and serum vitamine D) | 1(ref) | 0.549 (0.291–1.033) | 0.345 (0.152–0.783) | 0.008 |
| Model 4 (age, sex, BMI, total energy intake, HOMA-IR, regular exercise, occupation, region, smoking, alcohol drinking, and vitamin, mineral supplement use) | 1(ref) | 0.596 (0.309–1.149) | 0.317 (0.133–0.756) | 0.008 |
| Model 5 (age, sex, BMI, total energy intake, serum vitamine D HOMA-IR, regular exercise,occupation, region, smoking, alcohol drinking, and vitamin, mineral supplement use) | 1(ref) | 0.590 (0.303–1.148) | 0.309 (0.129–0.742) | 0.007 |
| Model 6 (age, sex, BMI, total energy intake, serum vitamine D, PTH HOMA-IR, regular exercise, occupation, region, smoking, alcohol drinking, and vitamin, mineral supplement use) | 1(ref) | 0.589 (0.303–1.147) | 0.311 (0.129–0.748) | 0.007 |

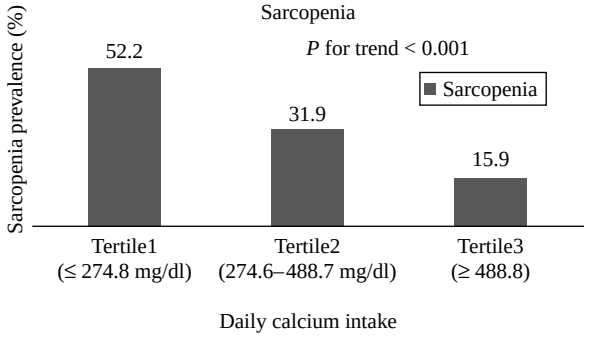


**Figure 1.** Unadjusted prevalence of sarcopenia by tertiles of daily oral calcium intake

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P238

### Role of chromogranin-A in the truncation of parathyroid hormone in cultured parathyroid cells from patients of the secondary hyperparathyroidism

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Introduction
Two major assay systems have been used for evaluation of parathyroid hormone (PTH) secretion. Whole PTH (wPTH) recognizes full-length 84 amino acid residues [PTH(1–84)], and Intact PTH (iPTH) recognizes NH2-truncated forms, such as PTH(7–84), in addition to PTH(1–84). Patients with chronic renal failure have increased amount of PTH(7–84) in circulations, so that the ratio of wPTH/iPTH indicates 0.7 or below usually. On the other hand, chromogranin-A (ChgA) is known to be a major protein costored and cosecreted with PTH in parathyroid glands. So, a role of ChgA in the process of biosynthesis of PTHs in cultured parathyroid cells was studied by using RNA interference (RNAi).
Method and result
RNAi was performed for 24 h to suppress the expression of ChgA in parathyroid cells. We observed that 50 nM of siRNA suppressed both values of wPTH and iPTH moderately, and importantly, they kept being almost equal for two weeks. Representatively, the mean values and S.D. of secreted wPTH and iPTH from cells transfected with siRNA for ChgA were $466.3 \pm 48.0$ ng and $477.0 \pm 18.4$ ng, respectively (wPTH/iPTH = 0.98), whereas those of cells transfected with a negative control siRNA were $602.0 \pm 168.9$ ng and $860.0 \pm 191.8$ ng, respectively (wPTH/iPTH = 0.70). The result indicates that almost no truncated PTH(7–84) was produced in cells of ChgA suppression.
Conclusion
It is suggested that ChgA plays a role in production of truncated forms of PTH. Parathyroid cell culture system with suppression of CgA is useful to examine processes of PTH biosynthesis.
Declaration of interest
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## P239

### Correlations between vitamin D supply and PTH-intact and PTH-bio-intact levels in dialysis patients

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Parathormone (PTH) is the marker for bone remodelling in chronic renal failure. The biologically active PTH 1–84 intact molecule (PTHibio) is supposed to better correlate with the degree of remodelling than PTH-intact (PTHi) which also binds to the 1–34 fragment. Our aim was to examine the PTH levels in dialysed patients with these two methods, considering also the total 25-hydroxi-vitamin-D (t-25OHD) levels.
Methods
patients (age $63 \pm 15$ years) on hemo- (HD) and 37 on peritoneal dialysis (PD, age $62 \pm 20$ years) were enrolled. PTH was measured by an electro-chemiluminescent immuno-metric assay, while t-25OHD by a protein binding assay (Cobas e411, Roche). Total protein (TP), albumin (ALB), protein electrophoresis and ionized calcium were also determined. The time period spent in dialysis was significantly ($P < 0.001$) lower in PD than in HD ($2.1 \pm 1.7$ vs $4.7 \pm 3.8$ years).
Results
The t-25OHD level was significantly ($P < 0.001$) lower, while PTH levels (with either method) were significantly higher in PD (PTHi: $302 \pm 176$ pg/l, PTHibio: $186.3 \pm 82.2$ pg/ml) than in the HD patients (PTHi: $149.8 \pm 89.6$ pg/ml and PTHibio: $88.5 \pm 50.0$ pg/ml). We recorded significantly ($P < 0.001$) lower PTHibio levels compared to PTHi concentrations. Ionized calcium, as well as Alb, TP, and alfa-2-globulin levels were significantly ($P < 0.01$) lower in the PD group. In HD, a significant ($P < 0.01$) negative correlation ($-0.52$) was confirmed between PTHibio and t-25OHD levels, while no such correlation was present between PTHi and t-25OHD. Conclusions: PD patients suffer from severe vitamin D deficiency. PTHi and PTHibio levels in the PD patients are higher, which could be the consequence of lower ionized calcium and more frequent adynamia, common in PD, despite the relatively shorter time period spent in PD treatment. The lower serum protein levels in PD may explain the particularly low t-25OHD levels. In conclusion, PTHibio seems to be more suitable for monitoring bone metabolism, than any other parameter measured.
Declaration of interest
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## P240

### Relationship among time outside, sun exposure, clothing worn, adiposity and serum 25(OH)D in overweight and obese adults

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Introduction
Obese persons may have low vitamin D levels due to behaviors (clothing, time outdoors) that can reduce synthesis of vitamin D. In overweight and obese individuals there are limited quantitative data regarding relationships among serum 25(OH)D, time spent outdoors, sun exposure, and clothing worn.
Methods
We assessed environmental and behavioral factors that contribute to cutaneous synthesis of serum 25(OH)D in 63 overweight and obese individuals enrolled in a 12-week controlled feeding clinical trial. Vitamin D content in the food was known and varied based on treatment group. Sun exposure was recorded bi-weekly from sun exposure logs that included date, time of day, location, activity, minutes in direct sun, clothing worn, and sunscreen use. UV-B radiation measured by UV-B Broadband Pyranometer and converted to J/m$^2$. Total sun exposure (J) was measured as a product of UVB and total body surface area exposed from sun logs. Serum 25(OH)D was measured by RIA. Total body fat (TBF) and % body fat (%BF) was by DEXA. Intra-abdominal adipose tissue (IAAT) was by CT Scan.
Results
Sun exposure and % body surface area exposed (%BSAE) significantly correlated with serum 25(OH)D ($r = 0.2545$, $P = 0.0441$ and $r = 0.4614$, $P = 0.0001$, respectively). Time outside did not correlate with serum 25(OH)D ($r = 0.0387$, $P = 0.763$), but did relate to TBF ($r = -0.3935$, $P = 0.0014$), %BF ($r = -0.4132$, $P = 0.0008$), and BMI ($r = -0.3987$, $P = 0.0012$). Sun exposure significantly correlated with only BMI ($r = -0.2946$, $P = 0.0191$). %BSAE significantly correlated only with IAAT ($r = -0.3526$, $P = 0.0053$).
Conclusion
We found a significant association between serum 25(OH)D and indices of cutaneous sun exposure, indicating that these variables should be considered when addressing the etiology of often-reduce vitamin D status in obese individuals.

Declaration of interest
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## P241

**Prospects of patients with oligosymptomatic primary hyperparathyroidism**

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The more sophisticated laboratory methods are used, the more likely a disease is found at its asymptomatic stage. In particular, diagnosis of primary hyperparathyroidism (PHPT) is currently based not only on the presence of clinical symptoms but on the typical upswing of the serum parathyroid hormone level. Outlooks of patients with PHPT and normal serum calcium level have not been quite clear so far. We tried to contribute to this question in a prospective study that has been in progress in the Czech Republic at a tertiary endocrinology center from January 1st, 2007, till December 31st, 2011. A total of 178 out-patients were diagnosed with PHPT, i.e. parathyroid hormone level was higher than 7.1 ng/l. Initially, the serum calcium level was not higher than 260 mmol/l in 154 cases (normocalcemic patients) and got over this limit in 24 cases (hypercalcemic patients). During a follow-up period, the serum calcium level exceeded 2.60 mmol/l in 22 originally normocalcemic patients. In 19 patients it raised to 2.85 mmol/l or less (moderate increase) and in 3 patients it transcended this value (high increase). In these patients, the increase followed after 1, 2 and 5 years of watching respectively. In a group of 24 patients in whom hypercalcemia was present from the very beginning, parathyroidectomy was performed in 16 cases (66.7%) during the study. In a group of 154 originally normocalcemic patients, parathyroidectomy was performed in 8 patients (5.2%). In normocalcemic patients, parathyreoidectomy was suggested mainly for parathyroid adenoma evidenced by MIBI scintigraphy, exceptionally by a surgeon recommendation finding parathyroid adenoma during thyreoidectomy.
Conclusion
Normocalcemic hyperparathyroidism is a strong reason for a 'watchful waiting' above all. Imaging methods, and rarely careful surgeon performing a thyroidectomy, may help to discover a parathyroid adenoma. One must be aware of risks and benefits of all attitudes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P242

**Cross-reactivity of 25-hydroxyvitamin D2 in ADVIA centaur vitamin D assay**

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Background
Vitamin D3 is the common supplemented form, vitamin D2 is often provided as prescribed supplement in many countries, such as the USA. In these countries, measuring 25(OH)D in patients treated with vitamin D2 using an assay that measures 25(OH)D2 poorly may confound clinical care. Recently, the ADVIA Centaur vitamin D assay has been commercialized for use in the clinical laboratory. We evaluated the assay's ability of measuring both 25(OH)D2 and 25(OH)D3 and compared the results to established automated and a LC-MS/MS method.
Methods
Adult volunteers, who gave informed consent, took orally 3000 IU vitamin D3 [D3], 3000 IU vitamin D2 [D2], or 1500 IU vitamin D2 plus 1500 IU vitamin D3 [D3+D2] daily for 3 months. Blood was obtained, processed, divided into aliquots, and stored at −20 °C until measurement. The Total 25(OH)D levels were measured with LC–MS/MS, DiaSorin Liaison, IDS-iSYS, and the Siemens ADVIA Centaur Total vitamin D immunoassays (Old and New calibration).
Results
In no supplementation subjects ($n=10$), the immunoassay values were similar to the LC/MS–MS. In D3 ($n=9$), the immunoassays were higher than LC/MS–MS. In D2 ($n=8$), Liaison (Mean + SD) (31.3+8.3) and IDS- iSYS (31.6+7.2) were similar to LC-MS/MS (35.3+8.8). The Centaur (Old and New) produced higher values: 52.7+21.1 ng/ml and 51.4+19.9 ng/ml. In D3+D2 group ($n=3$), we obtained 81.1+39.8 ng/ml (Centaur Old), 75.8+32.6 ng/ml (Centaur New). The LC–MS/MS, Liaison, and IDS-iSYS were: 52.1+13.0, 50.7+11.1, and 56.7+16.7, respectively.
Conclusions
The IDS-iSYS and Liaison 25-OH vitamin D results confirmed their D3 and D2 co-specificity. The elevated results in D2 and D3+D2 supplemented subjects suggested the Centaur method might significantly overestimated 25(OH)D level in patients treated with vitamin D2.

**Table 1** Total 25(OH) D Level (ng/ml)

| | Vitamin D supplementation type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vitamin D2 | | | Vitamin D3 | | | Vitamin D2 and Vitamin D3 | | | None | | |
| 25(OH)D Method | N | Mean | S.D | N | Mean | S.D | N | Mean | S.D | N | Mean | S.D |
| LCMS/MS | 8 | 35.3 | 8.8 | 9 | 48.2 | 13.3 | 3 | 52.1 | 13.0 | 10 | 21.0 | 11.1 |
| Liaison | 8 | 31.6 | 7.2 | 9 | 60.8 | 18.8 | 3 | 50.7 | 11.1 | 10 | 22.0 | 10.3 |
| IDS-iSYS | 8 | 31.3 | 8.3 | 9 | 54.8 | 19.2 | 3 | 56.7 | 16.7 | 10 | 20.1 | 10.6 |
| Centaur Old | 8 | 52.7 | 21.1 | 9 | 59.4 | 20.2 | 3 | 81.1 | 39.8 | 10 | 21.2 | 9.1 |
| Centaur New | 8 | 51.4 | 19.9 | 9 | 62.3 | 20.9 | 3 | 75.8 | 32.6 | 10 | 21.2 | 8.8 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P243

**Primary hyperparathyroidism: association of imaging and pathology**

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Background
We studied a cohort of hyperparathyroid patients in order to elucidate their clinical, laboratory, radiological and histological findings; the role of diagnostic imaging and concomitant thyroid pathologies.
Method
48 patients met our inclusion criteria for hypercalcaemic primary hyperparathyroidism (pHPT). We documented patients' demographic data, symptomatology, associated conditions and treatment and analysed the work-up, management and outcomes for each of these patients.
Results
pHPT patients had a median age of 62 years (range 20–79), median PTH of 145.5 pg/ml (range 27–4660) and mean serum calcium of 2.94 mmol/l (S.D. ± 0.33), while those operated (30/48, 62.5%) had a median age of 60 years (range 20–79), mean calcium of 3.02 mmol/l (S.D. ±0.39) and a median PTH of 176 pg/ml (range 37–4660). Histology showed parathyroid adenoma in 16/30 (53.3%), hyperplasia in 8/30 (26.7%), parathyroid carcinoma in 1/30 and normal tissue in 5/30. 19 of the 30 operated patients had a positive sestamibi scan of which 14/19 (73.7%) had an adenoma, 3/19 (15.8%) had hyperplasia, 1/19 had a carcinoma, and 1/19 had normal histology. Out of the 11 patients who had a negative sestamibi scan, 8/10 also had a negative ultrasound (US) and histologically 2/11 (18.2%) had an adenoma, 5/11(45.5%) had hyperplasia and 4/11 (36.4%) had normal histology.
Thyroid US showed a multinodular goitre in 12/41 (29.3%), solitary nodule in 5/41 (12.2%) thryoiditis in 4/41 (9.8%) and normal thyroid morphology in 20/41 (48.8%).
Conclusion
Parathyroid adenoma is the commonest pathology in patients with positive parathyroid imaging while hyperplasia is commoner in scan negative patients. This study highlights the need to proceed with surgery even when imaging is negative if clinically indicated. It is important to investigate associated thyroid

pathology prior to surgery though our data suggests similar incidence of thyroid pathology as documented in the general population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P244

**Vitamin D modulates composition of extracellular matrix in cultured human vascular cells**

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Vitamin D (VitD) was shown to affect biology of vascular wall by regulating cell proliferation. However, its effects on vascular extracellular matrix (ECM) remain largely unaddressed. We investigated effects of VitD (calcitriol) on composition of ECM deposed by cultured endothelial cells (HAEC) and smooth muscle cells (HASMC) isolated from human aorta, and compared them to effects of ascorbate (VitC). Cellular monolayers were treated with or without vitamins for 72 h, then ECM were exposed by differential cell removal and ECM contents for collagen types I (CoI-I) and IV (Col-IV), elastin, heparan sulfate (HS), chondroitin sulfate (ChS) and hyaluronic acid (HA) were assessed immunoenzymatically. HASMC and HAEC demonstrated a significant difference in composition of ECM deposed under standard cultured conditions. HAEC deposed by one order more of Col-IV as compared to HASMC, whereas deposition of Col-I did not differ. HS deposition by HAEC was two-fold higher, whereas deposition of ChS was by two orders lower than by HASMC. Deposition of both elastin and HA were 30% higher in HAEC cultures. Treatment with 100 μM VitC increased Col-I, Col-IV, HS and HA content in HASMC-ECM by 8, 6, 2.3 and 1.5 folds, respectively. VitD had dose-dependent stimulatory effects on Col-I deposition by HASMC (increase by 42.5% at 1 μM), which were additive to the effects of VitC. In contrast, Col-IV and HS contents in HASMC-ECM were decreased by VitD by 25% each, counteracting stimulatory effects of VitC. HA content in HASMC-ECM was not affected by VitD. Neither vitamin had any effects on elastin nor ChS content in HASMC-ECM. Content of all tested compounds in ECM produced by HAEC was independent of either vitamin treatment. In conclusion, VitD was shown capable to affect composition of vascular ECM. Significance of these effects is a subject for discussion.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P245

**Vitamin D level and ALT level in Chinese-American patients with chronic hepaitits B in the new york city downtown area**

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Background
Recent studies have suggested extensive associations between plasma levels of vitamin D and hepatobiliary diseases including viral hepatitis B or C. In hepatitis C, vitamin D deficiency may lead to a poor prognosis and a poor response to antiviral treatment. CHB has different pathophysiology. There has been no study on the association of vitamin D level and the ALT level in CHB patients, and it is not known whether vitamin D levels are associated with host response to hepatitis B infection.
Aims
To determine the association between serum 25-OH vitamin D3 levels and the severity of chronic hepatitis B based on HBV viral load and ALT levels in chronic HBV infected patients.
Method
One hundred seventy patients who visited the clinic at New York Downtown Hospital between November 4, 2010 and May 30, 2011 and due for other laboratory testing were screened for serum 25-OH vitamin D3. Thirty four Chinese-American patients were with non-cirrhotic CHB. Serum calcium, TSH, and phosphorus were also collected. Student t-test was used for the group comparison analysis. Correlation was analyzed using GraphPad Prism software.
Results
See Fig.1. More than 80% of our ethnic Chinese patient population had vitamin D levels lower than normal ($<30$ ng/ml). The mean vitamin D level (ng/ml) was $23.7 \pm 8.69$ in non-CHB patients and $22.9 \pm 8.6$ in CHB patients. The correlation between Vit D levels and ALT levels were, for all 170 patients: Pearson $r = 0.1664$ (95%CI $-0.0015$–0.00), $P > 0.05$ (not significant), and for 34 hepatitis B patients: Pearson $r = -0.027$ (95%CI $-$ 0.3668 – 0.30), $P > 0.05$ (not significant).
Discussion
The mean level of vitamin D in CHB patients was as low in non-CHB patients in this patient population. Apparently there is no correlation between vitamin D levels and CHB severity based on ALT levels in non-cirrhotic patients.

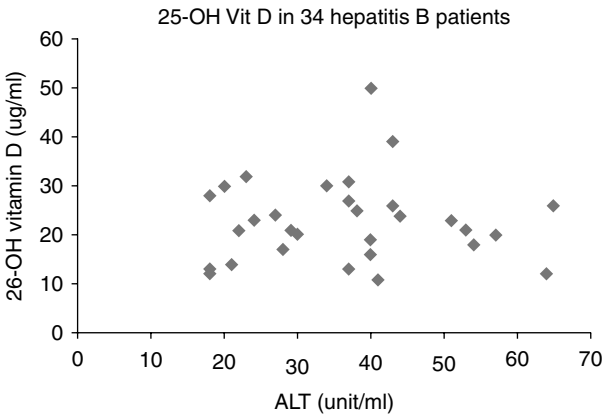


**Figure 1.**

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P246

**High levels of parathormone after parathyroidectomy in primary hyperparathyroidism patients does not always mean disease recurrence**…

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Introduction
Primary hyperparathyroidism (PHPT) patients who undergo parathyroidectomy can develop isolated intact parathormone (iPTH) elevation with normal calcemia. Common causes are: renal insufficiency (RI), vitamin D deficit and hypomagnesemia.
Aim
To identify clinical factors associated with iPTH elevation in patients submitted to surgery treatment.
Methods
The medical records of patients admitted into Santo António's Hospital between January/2000 and July/2011 with ICD-9 diagnosis of 'Hyperparathyroidism', 'Complete Hyperparathyroidectomy' and 'Other Hyperparathyroidectomy' were reviewed. Patients who underwent surgery and had post-operative iPTH elevation and eucalcemia were selected. Demographical and clinical data was collected.
Results
There were 82 patients who underwent surgery treatment, 31.7% ($n = 26$) of them had at least one elevated iPTH level throughout the fallow-up. This group consisted mainly of women (76.9%) with a mean age of $58.3 \pm 14$ years old at surgery. Eleven patients (42.3%) had high iPTH levels since the first measurement after surgery. In 18 patients (69.2%) there was at least one reason for iPTH elevation: 77.8% had low vitamin D levels; 23.5% had hypomagnesemia; 23.1% had RI (creatinine $= 2.5 \pm 1.4$ mg/dl). Three patients had MEN-1 mutation.
At last evaluation (mean follow-up of $4.1 \pm 3.4$ years) 13 patients maintained iPTH levels above normal range ($111.1 \pm 59.1$ pg/ml, 67–260). Four patients maintained RI, 6 low vitamin D levels and 1 hypomagnesemia. There was no analytical,

surgical or pathological differences between these patients and those with iPTH levels normalization.
Discussion and conclusions
Almost 1/3 of the PHPT patients submitted to parathyroidectomy had post-operative iPTH elevation mainly because of vitamin D deficit, hypomagnesemia or RI. The authors emphasize the need to identify those factors that may increase iPTH levels, and to monitor those who, despite supplementation, maintain iPTH elevation because of risk for persistent or recurrent PHPT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P247

**Sporadic pseudohypoparathyroidism with osteitis fibrosa cystica**

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Pseudohypoparathyroidism (PHP) refers to a group of heterogeneous genetic diseases, characterized by resistance to PTH and frequently other hormones activating cAMP-dependent events down-stream of different G protein-coupled receptors. PHP-Ia and Ib, the most frequent forms, are caused by mutations within the GNAS locus that encodes Gαs and several splice variants thereof. We describe a 36-year-old man with sporadic PHP-Ib with renal PTH-resistance, yet severe hyperparathyroid bone disease. The patient presented at age 8 years with limping due to bone deformities, who was treated with multiple osteotomies. Biochemical evaluation showed hypocalcemia (7.5 mg/dl) with high PTH (504 pg/ml) and increased ALP (487 IU/l). After restoring serum calcium to the low-normal range by oral calcium (1 g/day) and calcitriol (0.5–1 μg/day), PTH and ALP progressively increased (2100 pg/ml and 980 IU/l, respectively). Cortical bone mineral density (BMD) was markedly reduced (distal radius T-score: −7.7). Subperiosteal bone resorption with acroosteolysis, salt-and-pepper appearance of the skull, bone cysts and long bone deformities were observed radiographically. Parathyroid ultrasound showed four-gland hyperplasia. Epigenetic analyses of GNAS locus documented loss of methylation at all four DMRs without evidence for patUPD20q; the 3-kb STX16 deletion was excluded. No features of PHP-Ib were present in first-degree relatives. After increasing calcitriol to 4 μg/day, a progressive fall of PTH and ALP occurred over the course of 8 months (130 pg/ml and 97 U/L, respectively), while serum calcium remained within normal limits without evidence for hypercalciuria. Cortical BMD markedly increased (+6.5%) and bone pain vanished. Nine months later hypercalcemia developed, likely because of completion of the remineralization process. Calcitriol was reduced to 1.5 μg/day and normocalcemia was restored. This is an unusual case of sporadic PHP-Ib with marked bone involvement, in whom the standard dose of calcitriol was insufficient. Despite long-standing disease, PTH levels declined dramatically and approached the normal range.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P248

**Vitamin D supply in healthy blood donors: is there relationship with the use of oral contraceptives?**

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Sufficient vitamin D supply is essential for general health. The total 25-hydoxy-vitamin-D (t-25OHD) level refers to the vitamin D supply and can be measured with various methods. Since these methods are not independent of the serum matrix, differences can be obtained among vitamin D levels measured with different methods. Our aim was to study the vitamin D in healthy blood donors, taking also into consideration the use of oral contraceptives (OC).
Methods
t-25OHD and intact parathormon (PTHi) (ECLIA, cobas e411, Roche) were measured in 131 healthy blood donors (mean age: 33.5±10.5 years; 50 men, 81 women: 41 of them taking OC). Calcium and albumin levels were measured with routine methods (Modular, Roche).
Results
The age of the patients correlated negatively with the t-25OHD ($r=-0.29$; $P<0.01$) and albumin ($P=-0.33$; $P<0.001$) levels. There was a negative correlation between t-25OHD and PTHi levels ($r=-0.26$; $P<0.01$), however, the PTHi was below the upper cut off level. Vitamin D supply was significantly more often insufficient in men (60%) than in women (24%). Only 12% of women taking OC had a poor (50–75 nmol/l) vitamin D supply and there was no lower D vitamin level than 50 nmol/l, while in women not taking OC, the suboptimal (<75 nmol/l) t-25OHD occured three times more often (14/40; 35%; $P<0.01$). The level of t-25OHD was significantly ($P<0.001$) higher in women taking OC (101.8±22.9 nmol/l) than in the other group of women (85.6±24.4 nmol/l) or in men (77.9±26.8 nmol/l).
Conclusions
Our results mostly correlate with the literature data; however, the surprisingly good vitamin D supply in women taking OC may suggest the increased production of vitamin D binding globulin in the liver, stimulated by ethynilestradiol.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P249

**Chronic hypercalcemia, an unusual presentation in Q1011E heterozygous CaSR polymorphism**

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Introduction
The extracellular calcium-sensing receptor (CaSR) is a G-protein coupled receptor (GPCR) that is expressed in the parathyroids and kidneys, where it allows regulation of parathyroid hormone (PTH) secretion and extracellular calcium concentrations. Inactivating heterozyogous CaSR mutations result in familial benign hypocalciuric hypercalcaemia. However, the role of CaSR polymorphisms in controlling serum calcium, PTH and bone mineral density (BMD) remains controversial.
Case report
35 years old patient presented with abdominal pain and vomiting. Standard investigations showed a moderate chronic hypercalcemia (2.80 mmol/l). Further analysis showed a mild hypophosphatemia (0.58 mmol/l), mild hypocalciuria (2.4 mmol/24 h), normophosphaturia (15.8 mmol/l) and a normal parathyroid hormone (PTH) level (32 ng/ml). Imaging studies did not show any parathyroid nodule or radio isotope fixation. BMD was normal. Family history showed a sister with persistent hypercalcemia even after parathyroidectomy of 2 parathyroid glands, her grandmother underwent parathyroidectomy of the 4 glands. Genetic screening for CaSR mutations did not show any deleterious mutation, indeed, a heterozygous variation (c3031 C>G P.Gln1011Glu) on the exon 7 of CaSR gene was determined.
Discussion
The CaSR coding region polymorphisms involve evolutionary conserved residues with amino acid substitutions. In our patient, the polymorphysm involves non conservative changes at codon 1011. In the literature, polymorphisms of CaSR are known to be benign without an impact on serum calcium and PTH. We demonstrate an association between a polymorphism in the coding region of CaSR gene and serum calcium, PTH, 25-hydroxyvitamin D, urinary calcium excretion. Indeed, this polymorphism does not affect the BMD. This polymorphism associated with alterations in the dose-response functional modification of the receptor to Ca+2 concentrations explaining this moderate hypercalcemia.
Conclusion
Although studies showed that polymorphisms did not produce significant alterations in calcium homeostasis, it is clear that phenotypes of these polymorphisms could be variable and hypocalciuric hypercalcemia is not unexpected.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P250

**Quantification of serum 25-hydroxyvitamin D: a comparison among immunoassay, HPLC-UV, and HPLC-MS**

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Vitamin D deficiency is widespread among all age and ethnic groups. Serum 25-hydroxyvitamin D [25(OH)D] is the most reliable marker of vitamin D status. Adequate levels of serum 25(OH)D are necessary to sustain the pleiotropic effects of vitamin D, either skeletal (classical) or extra-skeletal (non-classical). Concentration levels $\geqq$50 nmol/L (20 ng/ml) are required for optimal musculoskeletal health. However, levels above 75 nmol/L (30 ng/ml) may be necessary to maximize musculoskeletal benefits and take advantage of the extra-skeletal effects of vitamin D. Traditional assays based on immunoassay display a substantial intra- and inter-assay variability (up to 30%) around the cut off limits, which may cause misclassification of individual vitamin D status. Conversely, Mass Spectrometry coupled to High Performances Liquid Chromatography (HPLC-MS) offers a high quantification accuracy. This is mainly due to its high selectivity, which reduces the contribution of interfering compounds to the final results.

Herein we report the results of measuring serum 25OHD using three different kits from DiaSorin (Liaison), Eureka (HPLC-UV), and PerkinElmer (HPLC-MS). Due to the features of MS, PerkinElmer method/kit was considered as the 'gold standard'. It involves a quick sample preparation, consisting just in the addition of stable-isotope-labeled internal standards (ISs) and protein precipitation. Quantification was performed by a tandem quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source, in positive ion mode and multiple reaction monitoring (MRM). Specific ISs allowed to quantify separately 25(OH)D2 and 25(OH)D3.

The analysis of about 200 blood samples, either from healthy volunteers (controls) or patients with endocrine pathologies, showed the real advantages of LC-MS/MS with respect to the other methods, consisting of a better accuracy due to the absence of cross-reactivity and the possibility of separately detect 25(OH)D3 and 25(OH)D2. The comparison results on real sample will be reported.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P251

**Vitamin D deficiency and insufficiency and secondary hyperparathyroidism in Ukrainian population**

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Introduction
Vitamin D is important for bone and muscle development, function, and preservation. The serum 25OHD concentration is the best available clinical indicator of vitamin D status. The optimal serum 25OHD concentration is considered to be that level associated with maximal parathyroid hormone (PTH) suppression.

The aim
To determine the frequency of vitamin D deficiency and insufficiency in patients of different region of Ukraine and to evaluate the influence of 25OHDto bone mineral density (BMD).

Methods
It was examined 1575 people aged 20–80 yrs. old who lived in different regions of Ukraine. 25OHD and PTH level were evaluated by electrochemiluminescence method (Elecsys 2010, Roche). Vitamin D deficiency was diagnosed in level of 25-OH vitamin D below 49.5 nmol/l, vitamin D insufficiency – between 74.5 and 50.0 nmol/l. BMD was determined by ultrasound densitometry Sahara (Hologic) and DXA (Lunar).

Results
Vitamin D deficiency was registered in 81.8% persons, 13.6% examined had vitamin D insufficiency. It was determined negative significant correlation between PTH and 25OHD ($r=-0.16$, $P<0.0000001$). Secondary hyperparathyroidism was diagnosed in 11.9% patients. The mean level of 25OHD was significantly higher in southern resident of the country ($P<0.001$) and during summer ($P<0.05$). No significant correlation between 25OHD and BMD was found. But, only patients with vitamin D deficiency had significant negative correlations between PTH level and BMD at the level of femur neck ($r=-0.12$, $P<0.004$), dual femur ($r=-0.09$, $P<0.004$), upper and lower extremities ($r=-0.11$, $P<0.01$ and $r=-0.10$, $P<0.01$ accordingly), forearm 33% ($r=-0.20$, $P<0.001$).

Summary
In Ukrainian population the frequency of vitamin D deficiency is 81.8%. Only patients with vitamin D deficiency have significant negative correlations between PTH level and BMD at the level of femur neck, dual femur, forearm 33%, upper and lower extremities.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P252

**Prevalence of hypovitaminosis D in adult patients with hypopituitarism**

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Introduction
Italy is considered a country with high prevalence of 25 OH vitamin D deficiency. Changes in vitamin D serum levels were linked with the onset and progression of several diseases, including osteoporosis and cardiovascular diseases. The hypopituitary patients show several comorbidities like cardiovascular diseases and osteoporosis.

Purpose
To evaluate the prevalence of vitamin D deficiency in hypopituitary patients.

Patients and methods
57 patients were enrolled (27 M and 30 F), 24 with hypopituitarism and GH deficiency (GHD) and 33 non–GHD patients with hypopituitarism, aged between 30 and 80 years and 90 controls age, sex and BMI matched. In all subjects were evaluated serum levels of vitamin D, PTH, serum and urinary Ca and P. The 25 OH vitamin D levels were considered deficient when $<$20 ng/ml, insufficient when between 20–30 ng/ml and normal when $>$30 ng/ml.

Results
25 OH Vitamin D levels were lower in patients than in controls ($21.7\pm10$ ng/ml vs $31.4\pm12$, $P<0.01$). The levels of PTH, Ca, P did not differ between patients and controls. The 50% of patients vs 12% of controls had vitamin D deficiency ($P<0.01$); 28% vs 32% had vitamin D insufficiency ($P=$ns) and 21% vs 56% had normal levels of vitamin D ($P<0.01$), respectively in patiets and controls. There were no differences in both levels of vitamin D and in the prevalence of hypovitaminosis D between the 2 groups of patients ($P=$ns). In addition,Vitamin D levels were inversely related to age ($P<0.05$).

Conclusions
Hypopituitary patients show vitamin D levels lower than controls, so in these patients, vitamin D deficiency represents an additional risk factor for the hypopituitaric comorbidities such as cardiovascular disease and osteoporosis. It would therefore be advisable to ensure a vitamin D supplementation in all patients with inadequate levels.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P253

**Treatment of vitamin D insufficiency with oral loading doses of cholecalciferol**

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Aim
To minimize the duration of vitamin-D insufficiency, current treatment regimes are initiated with an oral bolus dose of cholecalciferol. We have previously developed an algorithm (# Dekristol caps/Vigantol ml =(80 nmol/l – initial [s-25-OH-d-vit])/7 nmol/l) for determining the dosage of this bolus, aimed at reaching a serum 25-OH vitamin-D of 80 nmol/l. Our main aim was to investigate the accuracy of this algorithm.
Materials
A retrospective study of 88 patients attending the out-patient clinic with low vitamin-D status. 60 patients had been treated with a bolus dose of cholecalciferol, estimated by the algorithm, as either capsules (Dekristol, 20.000 IU/capsule) or drops (Vigantol, 20.000 IU/ml), along with supplementation treatment consisting of Unikalk Forte 2 to 4 tbl./day (1520–3040 IU). 28 patients received supplementation treatment alone (no-bolus group).
Results
The average baselines were <25–nmol/l (below the assay's detectable range) in the bolus group and 32,0 nmol/l in the no-bolus group. Follow-up samples were taken after 116 (±70) days. Both groups had received daily supplementation of cholecalciferol, averaging at 1787 IU and 1924 IU respectively. At the follow-up, the bolus group had an increase in s-25-OH-vitamin-D of 34.9 nmol/l – significantly higher ($P=0{,}005$) than in the other group (19.0 nmol/l). The bolus treated patients had a s-25-OH-vitamin-D of 55.9 nmol/l, 95% CI 46.3–58.9 – significantly lower than the desired value of 80 nmol/l ($P<0.001$).
Conclusion
Our findings suggest that our treatment regime wasn't sufficient to increase the serum 25-OH vitamin-D to 80 nmol/l. Based on present data we have developed a new algorithm incorporating patient BMI, which we plan to validate in a prospective study.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P254

**A 10-year study of vitamin D in Moldova**

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For decades vitamin D by its complex action in the human organism is increasingly considered to be a hormone. Therefore, the author presents here the first complex study performed in Moldova for over 10 years examining the correlation between the vitamin D status in pregnant women, newborns, their physical development after birth, the incidence of hypocalcemic states postpartum, and the prevention of rickets within the first year of life by comparing the strategy of specific rickets prevention used in Moldova and the one recommended by the international group researching the phospho-calcic metabolism from Paris. Data on Moldovan neonates confirm the association between low 25(OH)D levels and low serum calcium and show a high frequency of vitamin D deficiency (27% with 25(OH)D levels below 25 nmol/l) in neonates born at the end of winter to mothers not supplemented with vitamin D. Our interventional study of maternal vitamin D prophylaxis shows beneficial impact on fetal growth, neonate vitamin D status and serum calcium, and on the risk to develop rickets, anemia or respiratory diseases during the first year of life of a 2.5 mg dose of vitamin D given orally to mothers on the 6th month of pregnancy. The study shows that the vitamin D status in Moldovan children is not different from that of other European children and that vitamin D prophylaxis of 'at-risk' children and adolescents should be discussed as in other European countries.
Genotypes of Moldovan children regarding variants of VDR gene's regulatory part and of the lactase gene were studied and compared with children of other European origin. Our study of VDR gene variants is the first to analyze a possible interrelation between genetic and environmental factors to achieve an optimal growth and calcium metabolism in children and adolescents.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P255

**25-Hydroxi-vitamin-D levels in clinical conditions with low plasma albumin**

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The majority of circulating total 25-hydroxi-D-vitamin (t-25OHD) is bound to proteins – 90% to vitamin D binding protein (DBP) belonging to the alfa-2-globulin (a-2gl) fraction and 10% to albumin (ALB). Nowadays the t-25OHD level is the most accepted marker of vitamin D supply in physiological states. Our aim was to investigate the t-25OHD concentration in clinical conditions with low albumin levels.
Methods
Seventy-three patients (39 men, 34 women; mean age: 69.3±13.4 years) with low ALB or/and total protein (TP) were studied. 35 patients had chronic renal failure, 8 nephrosis, 19 cirrhosis and 11 malnutrition. The t-25OHD and intact parathormon (PTHi) were determined by ECLIA (Cobas e411, Roche), as well as calcium, TP, ALB were measured, proteinelectrophoresis was estimated (Gelelfo, INTERLAB).
Results
86% of the cases had vitamin D deficiency (<50 nmol/l), while an optimal vitamin D supply was measured in two cases only. In cases where low ALB (<34 g/l) and low TP (<64 g/l) were present simultaneously ($n=52$), significantly lower (20.8±17.3 nmol/l) t-25OHD levels were measured than in the patients with low ALB and normal TP levels ($n=21$; 36.0±26.7 nmol/l). There was a borderline positive correlation ($r=0.33$) between t-25OHD and TP and a significant ($P<0.05$) negative correlation between t-25OHD and a-2gl fraction% ($r=-0.35$). In the group of vitamin D deficient patients ($n=45$) a pathologically high a-2gl fraction was more frequently observed (71%) than in the group of patients with optimal vitamin D supply (30%).
Conclusions
Our results suggest that in clinical conditions with low ALB and TP levels – especially if hypoproteinemia is associated with an a-2gl excess – t-25OHD levels may not only depend on the vitamin D supply, but also on the presence of its binding proteins and binding capacity besides on increased t-25OHD uptake of cells.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P256

**The impact of different vitamin D preparations for the treatment of vitamin D deficiency in primary hyperparathyroidism (PTHP)**

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We have previously demonstrated that 80% of patients with PTHP will have co-existing vitamin D deficiency suggesting an increased metabolism of vitamin D. There are few data assessing the impact and safety of different vitamin D preparations on calcium, parathyroid hormone (PTH) and vitamin D in this group. Here, we report the details of replacement therapy using different vitamin D preparations.
In a retrospective study of 22 (20 F:2 M and 8 Asian:14 Non-Asian) patients with confirmed PTHP and vitamin D deficiency, we assessed the impact and safety of treatment with osteo-D2 50 000 IU after four-eight weeks ($n=8$), calcium and vitamin D (e.g.Adcal-D3) twice daily for three months ($n=6$) and over the counter (OTC) vitamin D 1000 IU/day ($n=6$) or cod liver oil ($n=2$) for three months, on serum calcium, PTH and vitamin D levels.
Results
Mean (±SD) serum calcium was statistically significantly increased after treatment in the group as a whole (2.7(±0.23) vs 2.75 (±0.28) mmol/l; $P<0.05$)

and also in those treated with osteo-D2 50 000 IU (2.69($\pm$0.22) vs 2.76 ($\pm$0.25) mmol/l; *P*,0.05).
Patients with a pre-treatment calcium of $>3$ mmol/l ($n=3$), did not have a clinically significant increase in their mean serum calcium (3.18 vs 3.22 mmol/l). Eighteen of 22 patients were vitamin D replete in 2–3 months.
Conclusion
These data suggest that vitamin D repletion, regardless of the treatment regimen used, in patients with PTHP and co-existing vitamin D deficiency is safe. Moreover, treatment does not clinically significantly exacerbate hypercalcaemia for the majority of patients. We would still however, advocate close monitoring of calcium levels in these individuals. Finally, despite the increased metabolism of vitamin D in PHPT, most patients will be replete within 2–3 months regardless of vitamin D preparation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P257

**Identification of a novel mutation in the calcium sensing receptor gene in FHH**

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Introduction
Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant trait comprising hypercalcemia, hypophosphatemia and an unusually low renal clearance of calcium. The vast majority of FHH is caused by loss-of-function mutations in the gene CASR, which codes for the calcium-sensing receptor (CASR). CASR is a G-protein coupled membrane receptor expressed in the parathyroid glands and the kidneys, among other tissues. It is generally asymptomatic and does not require treatment. Genetic testing for FHH-associated mutations in CASR can help to prevent unnecessary and inappropriate parathyroidectomy in patients with FHH.
Case
A 41-year-old male presented with an elevated serum calcium on routine testing. A non – tourniqued, corrected serum calcium taken showed a reading of 2.91 mmol/l, which remained elevated on repeated testing. He had no past medical history and did not take any regular medications. No symptoms of hypercalcaemia were present. There was no family history of note. Physical examination was unremarkable.
The remainder of his blood work showed a normal serum creatinine, ACE concentration, TFTs and serum protein electrophoresis. His parathyroid hormone was 58 pg/ml (reference range 10 – 65 pg/ml). A urinary calcium/creatinine ratio was abnormally low at 0.002.
Our patient subsequently underwent CASR gene testing. He was found to have a C to T nucleotide substitution in exon 7 of CASR gene (c.2254C$>$T), p.Arg752Cys (Arginine replaced with cysteine). This mutation has not been described previously.
Discussion
Given the biochemical findings it is likely this is an inactivating mutation causing FHH. The patient's mother and brother had normal serum calcium levels and were negative for CASR mutation. Unfortunately his father had died. We speculate that this is a de novo mutation causing FHH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P258

**Vitamin D status in patients with musculoskeletal symptoms in Haryana, India**

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This cross sectional single centre study was performed at an endocrine center in northern India (latitude 29°42′N and longitude 77°02′E) to assess the prevalence of vitamin D deficiency in 234 symptomatic women presenting with musculoskeletal symptoms. The study was conducted during winter (Nov 2010–Jan 2011).
Subjects were recruited from patients attending the OPD for various musculoskeletal symptoms. Inclusion criteria were symptomatic women, without known renal or hepatic disease or malignancy. Patients with history of surgery, hospitalization, or major medical illness within the past one year were excluded from the study. Patients on hormone replacement therapy, glucocorticoids, biophosphonates, teriparatide and other drugs affecting bone metabolism were excluded.
Intake of conventional calcium/vitamin D supplements was not considered an exclusion criterion. Only patients living at the same location, in Karnal district, for at least one year were included. All subjects enrolled after taking a written informed voluntary consent.
The cohort comprised of 165 urban dwellers (70.51%) and 69 (29.48%) rural dwellers. 171 (73.07%) were of Hindu religion, with the rest 63 being Sikh (26.92%). All women wore traditional Indian dress of sari or salwar-kameez. None of them observed purdah. All but 21 (8.97%) were vegetarian. The average age was 45.43$\pm$11.72 years (range 18.0 to 65.0 years).
Twenty-five (10.68%) had hemoglobin below 10 g%. Serum calcium levels were below 8.5 mg% in 24 subjects (10.25%), and were raised (above 10.5 mg%) in one (0.427%). Serum phosphorus was normal in all. Serum alkaline phosphatase was raised in 32 (13.67%) patients.
25-Hydroxy vitamin D levels measured $<10$ mg/ml in 130 subjects (55.55%) and between 10 and 30 mg/ml in 90 subjects (38.46%). Thus, the prevalence of vitamin D deficiency and insufficiency was 94.01% in this cohort of north Indian patients with musculoskeletal complaints. This was significantly higher than the 66.7% prevalence of Vitamin D deficiency/insufficiency reported in healthy asymptomatic postmenopausal women from the same centre.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P259

**Evaluation of the platelet functions in hyperparathyroid patients**

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Introduction
Coagulation and fibrinolysis defects were reported in primary hyperparathyroid(PHPT) patients. However, there is not enough data regarding platelet functions in this group of patients. Platelet functions of hyperparathyroid patients were measured in very few studies and they have conflicting results. Our aim was to evaluate the platelet functions in primary and secondary hyperparathyroid(SHPT) patients and to compare it with the healthy subjects.
Material and methods
In our study 25 subjects with primary hyperparathyroidism and 25 subjects with secondary hyperparathyroidism and 25 healthy control subjects were included. Platelet functions of the subjects were evaluated by using platelet rich plasma and platelet aggregation tests with epinephrine, ADP, collagen and ristosetin. Also, serum P selectin levels that indicates platelet activation level were measured in all subjects.
Results
There was no significant difference between the groups with PHPT, SHPT and the control group regarding the platelet aggregation tests. Also,there was no significant correlation between parathormone levels and aggregation parameters and between P selectin levels. When we separated the group of subjects with high serum calcium levels, there was also no significant correlation between aggregation parameters and serum calcium levels. We could not find any significant correlation between p selectin levels and serum calcium levels in this group of patients.
Conclusion
There is no significant effect of primary and secondary hyperparathyroidism and serum calcium levels on platelet functions evaluated with aggregation tests.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P260

**The relation between endocrine and radiological changes in adolescents with vitamin D deficiency**

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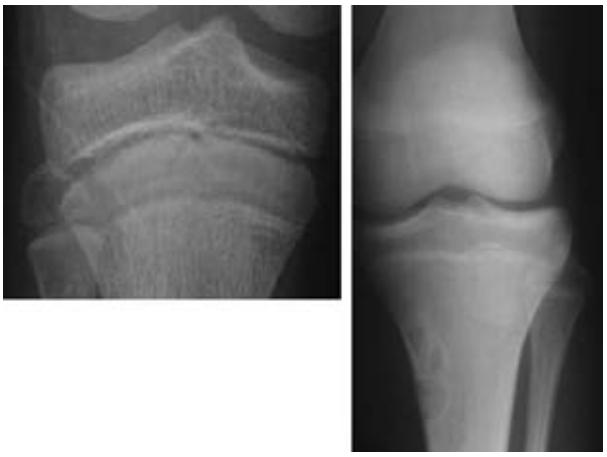
Vitamin D deficiency causes rickets in children and osteomalacia in adolescents. Rickets cases are still being reported in the Arab gulf area and worldwide. The clinical spectrum ranges from subclinical (asymptomatic) form to severe symptoms and signs including progressive bone pains, myopathy, waddling gait and bone deformities.

We recorded the clinical, biochemical and radiological data 50 adolescents with severe vitamin D deficiency. Clinical symptoms included pain in weight bearing joints, back, thighs, knees, and calves (36/50) difficulty walking and/or climbing stairs and/or running (12/50), muscle cramps and/or facial twitches and/or carpopedal spasms (21/50). Significant inverse correlation was detected between serum 25OHD and PTH concentrations ($r=0.447$, $P<0.01$). Two radiological patterns have been identified. In pattern (I) ($n=8$),(Fig -right) the lesions appear as metaphyseal multilocular cystic lesion with sclerotic margins, located subcortically without significant cortical erosions, periosteal reaction, osteoporosis or other metaphyseal manifestations. Whereas pattern (II) ($n=18$)(fig -left) appears as generalized diminished bone density with prominent primary and secondary bone trabeculations, widening of the metaphyseal zone with relatively more lucency (loss of all bone trabeculations). Patients with pattern I had significantly higher body mass index and insulin- like growth factor-I (IGF-I) levels compared to those with pattern II (left). Complete healing of the radiological evidence of both patterns was achieved in all patients after one to 2 years of vitamin D therapy.



Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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Authors have nothing to disclose.

## P261

**Remember calcium! Fahr's syndrome diagnosed after repeated generalized seizures: a case report**

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Introduction
Idiopathic calcification of basal ganglia (Fahr's syndrome) is a rare pathology, mostly incidental finding or familial origin, characterized by cognitive, behavioural and motor impairment, as well as by metabolic changes, such as primary hypoparathyroidism.
Report
We report a case of a 59-years old white lady who was referred to our clinic for an evaluation due to suspected Fahr's syndrome. She had a 20 years long history of moderate radicular low back pain, progressive numbness in hands and feet during last 7 years. Two months before presentation the patient was hospitalized in emergency room (ER) due to a first-time generalized seizure followed by unconsciousness. Biochemical tests had shown normal serum glucose, creatinine, potassium, elevated liver enzymes and creatine kinase. Brain CT scan had demonstrated changes consistent with Fahr's syndrome – diffuse symmetrical parenchymal calcifications, including basal ganglia, subcortical regions of cerebral white matter and cerebellum. After discharge patient was seen by neurologist, but only treatment for vertigo has been prescribed. Multiple blood testing was repeated, but no calcium (Ca) or parathormone (PTH) was checked, fatigue progressed.
Two months after initial episode patient had a sudden fall with unconsciousness and was hospitalized, more detailed investigation was done, showing severe hypocalcaemia, hyperphosphataemia, undetectable PTH level and hypocalciuria. No cognitive or significant motor changes were observed. Chvostek's and Trousseau's signs were negative. In her past history bilateral cataract was diagnosed and operated 7 years ago. Secondary reasons of Fahr's syndrome were excluded. Family history showed no possible hereditary disease.
Based on these findings, diagnosis of Fahr's syndrome and primary hypoparathyroidism was done. Treatment was started with intravenous Ca and followed with oral calcitriol and Ca supplementation, showing gradual improvement in biochemical tests. In spite of a regular treatment, three years later the patient still has low serum Ca and PTH, high phosphates, but urinary electrolytes are normal, hand and feet numbness has disappeared and she has not experienced any seizures.
Conclusion
We would like to stress the importance of mandatory testing of Ca, phosphates and PTH in all patients with seizures or unexplained episodes of unconsciousness.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P262

**Assessment of vitamin D 3 status in general population**

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Objective
The purpose of this study was to investigate the 25-hydroxyvitamin (25(OH)D3) levels in general population.
Introduction
Vitamin D is important for normal bone health. Vitamin D deficiency is a well-known cause of rickets in children and osteomalacia in adults, and it is also of importance for development of osteoporosis. Beyond this, vitamin D deficiency is suggested as a contributing factor in the development of several other diseases and conditions such as diabetes, some cancer types and immunologic diseases.
Materials and methods
We targeted 40 cases who participated in an annual health check-up from September 2011 until December 2011. In our evaluation we defined levels of 25-hydroxyvitamin D3 $<25$ ng/ml as vitamin D deficiency.
Results
The study consisted of 40 adults (35 women (83.4%)) and 5 men (16.6%)) with a mean age $49.66\pm18.22$DS, range from 16–76 years, and body mass index 28.5kg/m$^2$. Mean serum 25(OH)D3 concentration was 17.33 ng/ml. Using a cut-off point of 25 ng/ml, Seventy- two point five (72.5%) of this population had low vitamin D status. The highest 25(OH)D3 concentration was 47.24 ng/ml. We found vitamin (25(OH) D3 $<$ 10 ng/ml) in 40% of our cases. Mean serum level of calcium was normal 9.33 mg/dl (normal range 8.4–10.4). The level of calcium didn't correlated with low level of Vitamin D3.
Conclusion
Vitamin D deficiency was prevalent in our ambulatory patients. Based on our results, we feel that national recommendations for vitamin D intake should be increased in our country. During the study most of the days were sunny.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P263

**Unrecognized severe vitamin D deficiency**

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Introduction
Vitamin D deficiency is a common disorder and may manifest as a musculoskeletal, neurological, mental disorder or neoplastic disease.
Case report
A 47 year old woman of Caucasian origin suffered from back, muscle, bone pain and severe depression for 8 years. She was ineffectively treated at the departments of Neurology, Psychiatry, Pain Clinic several times a year. Brain, bone CT, internal ultrasound, routine biochemistry analyses were normal. X-Ray – lumbar spine osteochondrosis, spondylosis. Finally, she could hardly walk due to muscle weakness and cramps, when ionized calcium (Ca+2) was measured for the first time. With the severe hypocalcemia (Ca+2 0.62 mmol/l (*n* 1.05–1.3)) she was admitted to the Endocrinology Department. Further investigations confirmed secondary hyperparathyroidism with the 'empty bones' syndrome and osteoporosis due to severe longstanding vitamin D deficiency with the plasma alkaline phosphatase (ALP) of 425 U/l (*n* 40–150), phosphorus of 0,99 mmol/l (*n* 0.74–1.52), 24 hour urine Ca of 0.6 mmol/24 hours (*n* 2.5 – 7.5), parathyroid hormone (PTH) of 94.9 pmol/l (*n* 1.2–7.3), serum 25-hydroxyvitamin D of <4 ng/ml (local laboratory reference range 20–32). 99mTC-sestamibi parathyroid and whole body scintigraphy revealed no pathology, spinal Dual-emission X-ray absorptiometry showed T score of −5.3 at L2-L4. The patient was treated with intramuscular cholecalciferol 200 000 U followed by oral alfacalcidol 1 μg/day and oral calcium carbonate 3 g/day for six months. During the follow up period the analyses improved gradually resulting in Ca+2 of 1.09 mmol/l, ALP of 129 U/l, PTH of 10.6 pmol/l. The patient's overall condition improved significantly.
Conclusions
Severe vitamin D deficiency may remain unrecognised for many years, mimicking other disorders and leading to severe disability. Thus it has to be considered in all cases of unexplained, treatment resistant pain and weakness even the subject does not belong to the risk group.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P264

**Evaluation of clinical and laboratory findings of our primary hyperparathyroid patients**

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Aim
In this study our aim was to evaluate clinical and laboratory findings of our primary hyperparathyroid patients and detect complications caused by hyperparathroidism.
Method
Eighty-three patients (73 women, 10 men) were included in this study. Serum calcium (Ca), phosphorus (P), parathormone (PTH), 25(OH) D, 24 hour urine Ca and P levels, bone mineral density (BMD) and renal ultrasonography were evaluated.
Results
The mean age at diagnosis was 52.4±9.6 years. The mean serum Ca level was 10.47±0.67 mg/dl, serum P level was 2.85±0.50 mg/dl, PTH level 254.12 ±140.72 pg/ml, 25-OH vitamin D3 level was 22.97±16.15 μg/l. Serum Ca was <11 mg/dl in 66 patients (%19.3), 11–12 mg/dl in 16 patients, >12 mg/dl in one patient. Mean 24 hour urine Ca was 354.52±177.23 mg/day, P was 868.42 ±385.86 mg/day. Hypercalciuria was detected in 27 patients (%32.5) while nephrolithiasis was determined in 17 patients (%20.5). In 16 patients (%19.3) BMD was normal. In 31 patients (%37.3) osteopenia, in 36 patients (%43.4) osteoporosis was determined. Vit D deficiency was found in 38 patients (%45.8). With these results operation was recommended to 44 patients (%47).
Conclusion
In recent years primary hyperparathyroidism is detected in asymptomatic phase and fewer complications are found. One of the factors that contribute to low bone density in patients with hyperparathyroidism may be lack of Vit D.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P265

**Experience in the treatment of primary hyperparathyroidism with cinacalcet: Preliminary data**

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Introduction
Cinacalcet is an oral calcimimetic indicated in treatment of primary hyperparathyroidism if patients do not accept surgery, do not carry out the surgical criteria, there is failure of previous surgery or serious comorbidity that makes surgery impossible.
Methods
Descriptive study that included 34 patients with primary hyperparathyroidism treated with cinacalcet. We recorded clinical and biochemical data before and after 6 months of treatement, dose and time of treatment, and adverse events.
Results
Median time of treatment was 10 months (2–42 months). 24 patients complete 6 months of treatment. Reasons for treatment with cinacalcet were 8 patients refusal to parathyroidectomy, 4 surgery not possible due to comorbidities and 21 progressive hypercalcemia prior to surgery. Cinacalcet initial dose was 30 mg/12 hours in 94% and 30 mg/24 h in 6%. Serum calcium level decreased significantly from 11.6±0.9 mg/dl at baseline to 10.1±0.9 mg/dl ($P<0.01$) at six months; also did serum phosphorus level (2.4±0.35 mg/dl to 2.75 ±0.35 mg/dl p <0.01) whereas serum PTHi level did not differ significantly before and after treatment (150±101.96 pg/ml vs 162,6±120.79 pg/ml $P=0.583$). Normocalcemia (S-Ca <10.1 mg/dl) was achieved in 44.1% of patients.
Most common adverse events were nausea and vomiting, especially at the beginning of therapy (8.8%). 4 required dose decrease and 3 withdrawal of treatment.
Conclusion
Cinacalcet is an effective alternative in non-surgical treatment of primary hyperparathyroidism in patients with recurrent disease or in case of surgical contraindications. Furthermore, cinacalcet may be useful in the preoperative hypercalcemia management.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P266

**Primary hypoparathyroidism presenting as adult onset seizure: a report of two cases**

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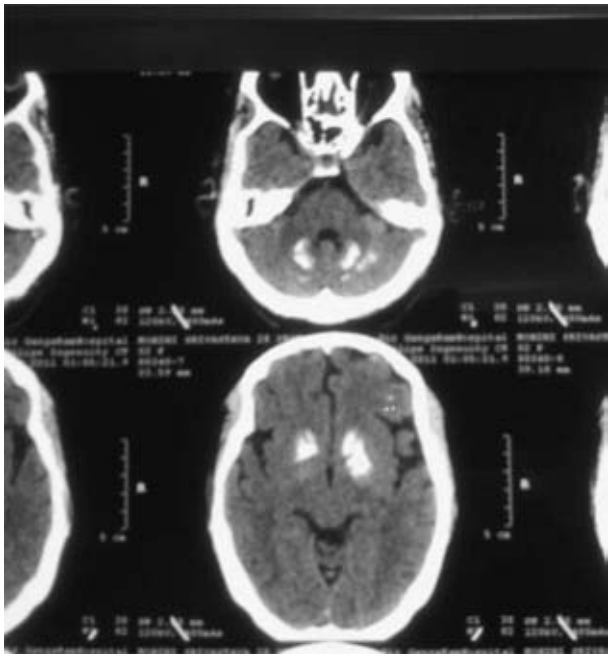
Introduction
Primary Hypoparathyroidism presents most commonly in childhood to early adolescence. Disease may be familial or sporadic.Most commonly hypoparathyroidism is secondary to neck operations especially Thyroid surgeries. Primary can be congenital eg- Digeorge syndrome or inherited by AD, AR or X linked mediated.We report 2 cases of late onset seizure due to Digeorge syndrome and Autosomal Dominant Hypoparathyroidism in a elderly lady.
Case Reports
Case 1
Twenty-seven year old female was brought with statue epilepticus and had hypocalcemia.CT Scan brain showed basal ganglia calcification.She also had mental retardation, cleft palate, posterior tongue tie and recurrent infections. Biochemical profile suggestive of severe hypocalcemia with bicytopenias. FISH for 22qdel11 was positive and diagnosis of Digeorge syndrome was made.

Case 2
Fifty-six year old lady presented with GTCS for first time. She was evaluated and found to have basal ganglia calcification and hypocalcemia.On evaluation her daughter and grand children also had asymptomatic hypocalcemia.
Conclusion
Chronic hypocalcemia may present with seizure, cognitive symptoms or psychiatry symptoms in most of the patients. All these symptoms show marked improvement with treatment. Prevalence of unrecognized Primary hypoparathyroidism in adults is very rare. It is important to evaluate serum calcium in all patients who present with new CNS symptoms.



Declaration of interest
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## P267

**Vitamin D status in primary hyperparathyroidism**

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Vitamin D deficiency seems to be more prevalent in patients with primary hyperparathyroidism (PHPT) than in general population. This association has many clinical and therapeutic implications.
The aim of this study was to assess the vitamin D status of patients with PHPT and to describe the clinical presentation of the disease when vitamin D deficiency is associated.
Methods
We studied in a retrospective manner, the case of seven patients (three men and four women) admitted to our department during the year 2011, for PHPT. During their hospitalization, serum calcium, parathyroid hormone (PTH) and 25-hydroxyvitamin D (25OHD) were measured concomitantly. A bone densitometry was performed for each patient.
Results
The mean age of our patients was $60.8 \pm 7.8$ years. Bone pain revealed the disease in three cases. The mean serum level of calcium was $2.9 \pm 0.4$ mmol/l. Mean PTH concentration was $384.2 \pm 378.7$ pg/ml. The mean serum level of 25OHD was $7.3 \pm 3.3$ µg/l. All the patients had vitamin D deficiency (25OHD $<$ 15µg/l). A single adenoma was localized in four patients with a mean size of $35 \pm 4.5$ mm. Bone densitometry revealed osteoporosis in one patient, osteopenia in 4 patients and was normal in two patients. Renal complications were present in three patients. Other complications (cardiovascular, neuropsychological and digestive complications) were found in three patients.
Conclusion
Our study suggests that vitamin D deficiency is very common in patients with PHPT. Thus, the assessment of vitamin D status should be done in all these patients because the disease seems to be more severe when vitamin D deficiency coexists and the risk of post operative hypocalcemia and hungry bone syndrome is higher.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P268

**Vitamin D deficiency repletion in patients with co-existing vitamin D deficiency and primary hyperparathyreoidism: pro for early postoperative or preoperative treatment: case reports**

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The aim of this work is to present two patients with vitamin D deficiency and primary HPT.
A 51-year old woman (first refer, 2009-jul) was operated for pathohistologically confirmed parathyroid (PT) adenoma seen by ultrasound (US) and scintigraphy (Sc). Patient treated for previous years for hypertension and depression. Calcium (Ca; mmol/l): 3.1; Creatinine (Cre; µmol/l): 67. The results will be shown in following order: pre-/postoperatively, 3rd month, 27th m. (– / –, –, –). Symptoms of depression (yes/yes, yes, without). Ca (ionized; mmol/l): 1.53–1.81/1.0, 1.15, 1.11. Ca (Urine; mmol/d): 7.53/–, 0.81, –. Parathormone (PTH; pg/ml): 488/280, 372, 38.9. VitD3(25OH) (nmol/l): 24.5/–, 10, 51. US of the abdomen: bilateral nefrocalcinosis and nefrolithiasis, BMD (g/cm2) L1-4 T score: $-3.7$ / –, –, $-2.8$. Serum CrossLaps (CL; pg/ml): 2197/–, 521, –. Osteocalcin (OC; ng/ml): 171/–, 45.6, –. Therapy with 1α-OHvitD3 (µg/day): –/0.25, 1.0, 2.0. Second patient a 76-year old man (first presentation, 2011-may) with Sc accumulation in PT, US undetectable PT hipoehogenicity, aortocoronary bypass (2006), Cre 154 µmol/l (Adult Polycystic Kidney Disease), BMD (g/cm2) L1-4 T score: $-0.5$, CL(pg/ml): 243, OC(ng/ml): 22.6. The results: the first visit, after 4 m (–, –). Ca (ionized; mmol/l): 1.33, 0.94. PTH (pg/ml): 244.9, 74.4 (2009-Oct: 169.5). VitD3(25OH) (nmol/l): 13, 10 Therapy with 1α-OHvitD3 g/day: –, 2.0.
Discussion
The chronic vitD deficiency lead to adenoma growth or growth of preexisting adenoma. Adenoma size were not in relationship with 25(OH)D similar as we seen in Indian vs. American patients. Disease stability (Ca, Cre levels) will determine the length and dosage of conservative (nonsurgical) treatment in man with normocalcemic HPT and woman with 'hungry bones'. Losing symptoms of weakness and anxiety only three months after the booster dose of vitD could be the direct effect on the brain tissue.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Cardiovascular Endocrinology and Lipid Metabolism

## P269

**Testosterone replacement therapy inhibits key enzymes of fatty acid synthesis in mouse liver**

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Fatty liver (Hepatic Steatosis) is common in men with type 2 diabetes mellitus (T2DM). Furthermore there is a high prevalence of testosterone deficiency (up to 40%) in this population. Testosterone replacement therapy (TRT) has been shown to reduce elevated serum liver transaminase levels in hypogonadal men. The testicular feminised (Tfm) mouse exhibits a non-functional androgen receptor (AR) and low circulating testosterone levels. We have previously shown that a high-fat diet promotes hepatic steatosis in the Tfm mouse and TRT attenuates

these changes. Our objectives were to determine the effect of testosterone on the key regulatory enzymes of fatty acid synthesis in mouse liver.
Tfm mice were fed a high-fat diet for 28 weeks and received either physiological testosterone replacement (Tfm + t, $n = 12$) or placebo (Tfm + p, $n = 12$) and were compared to wild-type (XY, $n = 12$) littermates. Relative concentrations of mRNA were analysed by qPCR for expression of Fatty Acid Synthase (FAS) and Acetyl-CoA Carboxylase (ACC). Protein expression was analysed by western blotting.
There was an increase in mRNA expression of FAS (Relative fold increase 11.4 $\pm$4.93, $P = 0.06$) and ACC (2.5 $\pm$ 0.64, $P = 0.05$) in the Tfm mice compared to wild-type littermates. In the Tfm + t group, mRNA expression was significantly reduced for FAS, but ACC showed a non-significant decrease relative to the Tfm + p group. Western blotting densitometry confirmed that the Tfm + p group had significantly increased levels of ACC (3.47 $\pm$ 0.27 vs 1.22 $\pm$ 0.22, $P < 0.001$) and FAS (2.43 $\pm$ 0.37 vs 0.88 $\pm$ 0.22, $P = 0.002$) protein expression compared to XY wild-type. In the Tfm + t group ACC protein decreased to a level comparable to the XY wild-type (1.53 $\pm$ 0.21 vs 1.22 $\pm$ 0.22, $P = 0.30$) as did FAS (0.88 $\pm$ 0.22 vs 0.89 $\pm$ 0.17, $P = 0.97$).
This is the first evidence to show that a testosterone deficient state is associated with increased FAS and ACC expression which promotes hepatic steatosis, and TRT suppresses both enzymes. This beneficial action of testosterone is independent of the androgen receptor.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P270

**Effect of testosterone on hepatic liver X receptor and ApoE expression as a potential mechanism of atheroprotection in the testicular feminised mouse**

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Testosterone deficiency is associated with several cardiovascular risk factors. Testosterone replacement therapy (TRT) improves insulin sensitivity, inflammation and cholesterol. Liver X receptor (LXR) is a nuclear receptor which regulates lipid and glucose metabolism, stimulates cholesterol efflux and ApoE, and suppresses inflammation. LXR agonists cause hepatic steatosis but protect against atherosclerosis. TRT attenuates high-cholesterol diet-induced hepatic steatosis in the testicular feminised mouse (Tfm), (which exhibit non-functional androgen receptors and low circulating testosterone) independent of androgen receptor (AR) function. This study investigates the effect of testosterone on LXR and ApoE expression in the Tfm mouse model.
Tfm mice were fed a high-cholesterol diet ad libitum for 28 weeks and received either physiological TRT (intramuscular mixed testosterone esters) or placebo (saline) and were compared to wild-type littermates. Liver tissue was collected and relative concentrations of mRNA and protein were analysed by qPCR and western blotting for expression of Liver X receptor (LXR), as well as Apolipoprotein E (ApoE).
The relative expression of LXR mRNA was significantly decreased in the Tfm mouse compared to wild type littermates (WT) (Relative fold change 0.7 $\pm$ 0.09 SEM, $P = 0.011$) and there was a non-significant decrease in ApoE (0.82 $\pm$ 0.1, $P = 0.07$). Following TRT, mRNA expression increased for LXR (1.3 $\pm$ 0.2 $P = 0.03$) and ApoE (1.2 $\pm$ 0.12, $P = 0.03$) in Tfm mice compared to placebo. Western blot analysis confirmed reduced LXR (0.16 $\pm$ 0.03 vs 0.07 $\pm$ 0.01, $P = 0.05$) and ApoE (3.07 $\pm$ 0.4 vs 2.45 $\pm$ 0.2, $P = 0.029$) protein expression in Tfm mice compared to WT. Following TRT ApoE protein expression increased to wild-type levels (2.92 $\pm$ 0.5 vs 3.07 $\pm$ 0.4, $P = 0.89$), however LXR protein expression was not significantly altered compared to Tfm placebo mice (0.05 $\pm$ 0.0 vs 0.07 $\pm$ 0.01, $P = 0.34$).
This is the first study to demonstrate that testosterone deficiency is associated with decreased expression of LXR and ApoE. TRT increased ApoE and LXR mRNA expression as well as ApoE protein expression, suggesting AR-independent actions. The atheroprotective action of testosterone may in part be mediated through activation of LXR and ApoE.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P271

**The effect of potassium supplementation on the endothelium, the renin-angiotensin-aldosterone system (RAAS) and blood pressure in patients at moderate cardiovascular disease risk**

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There is limited evidence on the effect of potassium (K+) supplementation on endothelial function. Three studies suggest a beneficial effect in healthy volunteers and mild hypertensives. However potassium increases aldosterone due to a direct effect on the adrenal gland and there is evidence that aldosterone excess is detrimental to cardiovascular health. We therefore aimed to determine the effect of potassium supplementation on endothelial function in patients with >10% cardiovascular disease risk. We also aimed to determine the effect of potassium supplementation on brachial and central blood pressure, the RAAS and vascular inflammation.
Forty patients with >10% ten year cardiovascular disease risk were included in a randomised placebo controlled crossover study. Drugs which interfere with the RAAS were stopped and blood pressure controlled with doxazosin. Patients were assigned to either 64 mmol potassium chloride or placebo for 6 weeks with a 6 week washout period. Endothelial function was assessed using global pulse wave analysis (PWA) involving the detection of a change in augmentation index to salbutamol (endothelial dependent) and GTN (endothelial independent) induced vasodilation. Vascular inflammation was assessed using high sensitivity C-reactive protein (hsCRP).
K+ supplementation improved brachial and central systolic blood pressure ($P = 0.013$ and 0.011 respectively) but did not affect endothelial function or hsCRP. Plasma renin activity ($P = 0.048$) and serum aldosterone ($P = 0.001$) both increased significantly with K+ supplementation compared to placebo. Serum K+ increased with supplemental K+ vs placebo (4.1 vs 3.9 mmol/l; $P = 0.012$) but hyperkalaemia did not develop.
These data show that K+ supplementation lowered systolic blood pressure. Interestingly K+ supplementation was associated with an increase in both plasma renin activity and serum aldosterone suggesting that K+ may also stimulate the RAAS via the juxtaglomerular apparatus. Despite this rise in aldosterone K+ supplementation did not affect global PWA or hsCRP.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P272

**(Pro)renin Receptor is essential for filtration barrier in glomerular podocyte**

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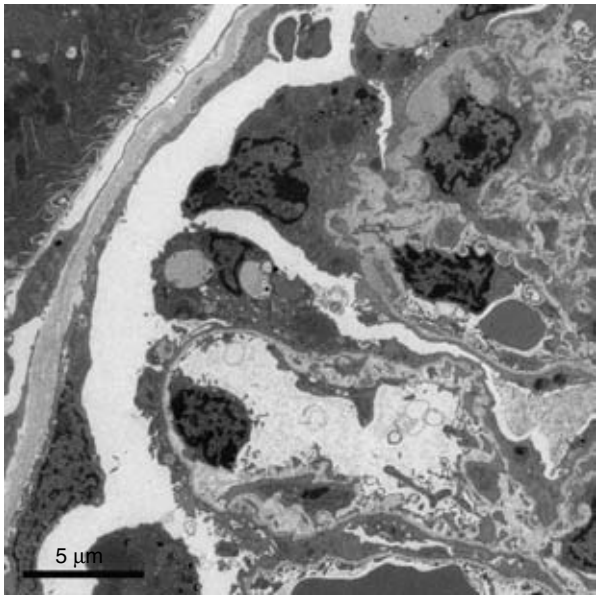
Backgrounds
Podocytes are highly specialized postmitotic kidney cells that contribute to maintaining the filtration barrier and normal structure of glomerular capillaries. The (pro)renin receptor (PRR), as ATP6AP2, is an accessory subunit of the vacuolar H+-ATPase (V-ATPase), implying more fundamental, developmental functions for the PRR in addition to its role in activating the local renin-angiotensin system.
Results
Podocyte-specific conditional (P)RR knockout (CKO) in mice resulted in lethal kidney failure with severe proteinuria, with CKO mice dying within 4 weeks of birth. The histologic examination revealed that as early as on postnatal day 7, the podocytes of CKO mice exhibited foot process effacement and numerous

autophagic vacuoles, along with focal and segmental mesangium cell proliferation. This was confirmed by reduction and altered localization in nephrin and podocin expression, as well as increased expressions of RAB7, lysosomal-associated membrane protein 2 (LAMP2), and microtubule-associated protein 1 light chain 3 (LC3) in CKO podocytes. Notably, over the course of development in CKO mice, nephrin expression became discontinuous and was seen mainly in the cytosol of podocytes. The number of cells positive for WT1, a nuclear marker for podocytes, was significantly fewer in glomeruli from CKO mice on postnatal day 20, implying increased podocyte cell death or detachment from basement membrane. *In vitro* analysis revealed that the treatment with the siRNA knocking down of PRR/ATP6AP2 reduced the protein expression of nephrin in the human cultured podocytes despite the up-regulation of NPHS1 mRNA expression. Also, the inhibition of PRR expression selectively suppressed expression of VO subunit c of the V-ATPase in podocytes, resulting in deacidification of the intracellular vesicles and increased autophagic vacuoles.
Conclusions
A deficiency of the PRR in podocytes resulted in impaired expression of slit diaphragm proteins and increase in the late endosomes, lysosomes, and autophagosomes, due to V-ATPase dysfunction.




Electron microscopic examination of kidneys from conditional (pro)renin receptor-knockout (CKO) mice on postnatal day 14. Kidneys from CKO mice were found to contain prominently enlarged podocytes with extensive foot process effacement and actin filament aggregation. Numerous electron-dense autophagic vacuoles containing partially digested cellular components were observed in the cytoplasm of these podocytes. Mesangial matrix expansion was also noted in CKO glomeruli.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P273

### Testosterone replacement therapy: a safety audit of clinical practice including men with Type 2 diabetes and cardiovascular disease

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Hypogonadism is highly prevalent in men with type 2 diabetes (T2D) and/or cardiovascular disease (CVD). Low testosterone levels are associated with CVD risk factors including obesity, insulin resistance, dyslipidaemia and hypertension. Testosterone replacement therapy (TRT) has been shown to have beneficial effects on these parameters; however, safety needs to be verified. An audit of 308 (50% with T2D, 32.1% CVD, 82.8% ED) hypogonadal men (age 59.02±13.23 years; mean baseline testosterone 7.33±2.85 nmol/l) receiving physiological TRT (86.3% testosterone gels) for up to 27 (mean 4.6±0.10) years in normal clinical practice. BMI, waist circumference, BP, Hb, haematocrit (Hct), lipid profile, liver function, testosterone, estradiol and PSA levels were monitored at 3, 6 and 12 months and yearly thereafter. Hospital admissions, major adverse cardiovascular events (MACEs) and mortalities were recorded.
TRT was associated with a reduction in total cholesterol (−0.26±0.11 mmol/l, $P=0.018$), non-fasting triglycerides (−0.30±0.14 mmol/l, $P=0.033$), liver transaminases (ALT and AST), HbA1c (−0.32% in whole cohort, $P=0.05$) at the primary endpoint. In diabetics with HbA1c >7.0% at baseline; HbA1c sharply fell: 8.65% vs 7.65%, $P<0.001$, $n=151$ after 3 months and 8.65% vs 6.74%, $P<0.001$ after five years with few changes in diabetic medications. HDL-cholesterol fell by 0.06 mmol/l ($P=0.04$). Hb increased by 0.63 g/dl ($P<0.001$), hematocrit by 0.02 ($P<0.001$) and PSA by 0.24 g/l ($P=0.038$). No significant changes in BMI, waist circumference or BP were observed. Adverse events: Hct exceeded 0.52 in eight cases, two new prostate carcinomas, two deaths, 13 MACEs (2 MIs, 2 angina, 5 TIAs, 2 CVAs, 1 CABG and 1 CCF) and 34 hospital admissions. This audit demonstrates that in over 1033 patient years, physiological TRT had beneficial effects on cardiovascular risk factors including glycaemic control, lipids and total cholesterol levels. Importantly, long-term TRT was not associated with any increase in prostate carcinoma, MACEs or mortality over that expected in this morbid population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P274

### Thyroid axis hormones and cortisol are associated with subjective fatigue in patients with coronary artery disease

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Introduction
Subjective fatigue is a common symptom in patients with coronary artery disease (CAD) and has a significant negative effect on quality of life of CAD patients. However, currently there are no studies evaluating possible association between endocrine measures and subjective fatigue in CAD patients. Therefore the aim of the current study was to evaluate possible associations of subjective fatigue with function of adrenal axis and thyroid axis in CAD patients.
Methods
Eighty-three CAD patients (65 men, 18 women; aged 55±9 years) attending a rehabilitation program within two weeks after inpatient treatment for acute coronary syndromes were evaluated for demographic and clinical characteristics, for subjective fatigue using the Multidimensional Fatigue Inventory and Dutch Exertion Fatigue Scale, for depressive and anxiety symptoms using the Hospital Anxiety and Depression Scale, and for serum concentrations of free tri-iodothyronine (T3), free thyroxine (T4), morning cortisol, afternoon cortisol and change in cortisol concentrations (delta-Cortisol).
Results
In univariate regression analysis lower free T4 concentrations were associated with greater general fatigue and exertion fatigue; lower free T3 concentrations were associated with greater general fatigue and physical fatigue; and lower delta-Cortisol was associated with greater mental fatigue.
After adjusting for age, gender, body mass index, hypertension, previous myocardial infarction, heart failure, New York Heart Association functional class, depressive and anxiety symptoms, lower free T3 concentrations remained associated with greater physical fatigue ($\beta=-0.224$, $P=0.03$); lower free T4 concentrations - with greater exertion fatigue ($\beta=-0.219$, $P=0.03$); and lower morning cortisol concentrations - with greater mental fatigue ($\beta=-0.193$, $P=0.03$).
Conclusions
In CAD patients function of adrenal axis and thyroid axis are associated with subjective fatigue independently from cardiac functional class or symptoms of depression and anxiety. Specifically, decreased thyroid hormone concentrations are associated with greater physical fatigue and greater exertion fatigue; and decreased cortisol concentrations are associated with greater mental fatigue.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P275

**Familiar hypercholesterolemia: effects of treatment with rosuvastin**

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Introduction and objetives
There are few data in literature about the effects of rosuvastatin in patients with familial hypercholesterolemia in Spain. The objective of this study has been:
1-to evaluate the efficacy and security of rosuvastatin in patients with familiar hypercholesterolemia
2-to test the hypothesis that patients with diabetes have different response to treatment with rosuvastatin.
Material and methods
Descriptive study of patients followed in a Familial Dyslipidemia Unit, treated with rosuvastatin.
Results
Eighty-two patients with familial hipercholesterolemia. Mean age: 56.16±8.84 años.62,5% heterozygous familial hypercholesterolemia and 37.5% familial combined hyperlipidemia.Indication to initiate rosuvastatin: 68.8% non-achievement of objectives; 15.6% hepatic impairment with other statins, 12.5% patients polypharmacy(more risk of interaction) and 3.1% intolerance to other statins. Mean dose: 14.35±8.82 mg. 90.6% had previously statins, 59.4% ezetimibe and 31.3% fenofibrate.Average values of total cholesterol, LDL, HDL and TG before and after treatment were respectively (mg /dl): 245.75±67.44 vs 198.37 ±62.12(*P* 0, 000),decreased 19%, 159.62±62.36 vs 116.31±57.92 (*P* 0.000), decreased 27.5%, 41.8±10.29 vs 43.93±9.34 (*P* 0.36) and 309±475 vs 291 ±616 (*P* 0.76) Side effects were: 1 elevation of liver enzymes (less than twice normal values) and 3 patients had myalgia forcing to withdraw medication. *P*ercentage of patients achieving LDL target were: 9,4% before vs 62,5% after rosuvastatin. Diabetic patients had a minor decrease in CT and LDL levels than non-diabetics: CT decrease 20.78± 22.57 in diabetic vs 66.26±28.55 in non diabetic (*P* 0.000) and decrease of LDL 17.57±25.01 vs 62.2±30.99 (*P* 0.000) respectively.
Conclusions
Rosuvastatin produced significant decrease in CT and LDL cholesterol in patients with familial dyslipidemia, without significant changes in TG and HDL. Patients with familial hypercholesterolemia and type two diabetes, had a significantly lower decrease in CT and LDL than patients without diabetes despite similar doses of rosuvastatin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P276

**Metabolic and glycaemic profile in patients with acute coronary syndrome**

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Introduction
Components of metabolic syndrome, particularly overweight, dyslipidemia and diabetes mellitus (DM), are well known cardiovascular risk factors. The objective of this work was to evaluate the metabolic and glycaemic profile in patients (pts) with acute coronary syndrome (ACS), treated in our institution.
Methods
A total of 126 pts with ACS (unstable angina, acute myocardial infarction with /STEMI/or without ST-segment elevation/NSTEMI/) were admitted to the Coronary unit (CU) of a Department of Internal medicine, from January to December 2010.
We evaluated the following characteristics: sex, age, waist circumference, total cholesterol serum level, triglyceride level, HDL cholesterol level, LDL cholesterol level, plasma glucose concentration, uric acid, systolic (SBP) and diastolic blood pressure (DBP). All data were recorded at admission to CU. In patients with no previously known DM, and fasting plasma glucose concentration (FPG) $\geq$5.6 mmol/l measured the day after admission, the Oral glucose tolerance test (OGTT) was performed 1–3 days before hospital discharge.
Results
Of 126 patients studied, 73 pts (58%) were male. Five pts (4%) were hospitalized due to unstable angina, 56 pts (44.4%) had STEMI, and 65 pts (51.6%) had NSTEMI. Average age of pts was 64.9±13.65 years (min 33, max 89 years). Waist circumference was 101±3.2 cm in males, and 89.2±1.8 cm in females. Total cholesterol serum level was 4.93±1.28 mmol/l, triglyceride 1.95±0.89 mmol/l, HDL cholesterol 1.13±0.25 mmol/l, LDL cholesterol 2.90±1.08 mmol/l, plasma glucose concentration 7.65±3.78 mmol/l (min 4.4 mmol/l, max 26.9 mmol/l) and uric acid 350.9±114.2 mmol/l. Previously known DM was recorded in 40 pts (32%). According to OGTT test, 8 pts had impaired glucose tolerance (IGT), and 14 pts had newly diagnosed DM type 2.
Conclusion
High percentage of pts with occult or presenting DM, along with other metabolic abnormalities in ACS, suggest the need of early diagnosis and appropriate treatment of this conditions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P277

**Impact of 6-month course of 1 mg finasteride tablets on levels of lipid profile in men with androgenic alopecia**

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Background
Atherosclerosis constitutes one of the most frequent diseases and one of the important predisposing factor for atherosclerosis is lipid profile change. Androgen change lipid profile mainly high densitylipoprotein (HDL), and 1 mg finasteride tablets are used for treating androgenic hair loss through inhibition of 5-$\alpha$ reductase, and preventing convertion of testosterone to dihydrotestosterone (DHT) the most active derivative of testosterone in the other hands androgenic hair loss is considered a risk factor for atherosclerosis. this study was conducted in order to determine the impact of DHT suppression by 1mg finasteride tablets on lipid profile of patients with androgenic hair loss.
Method and materials
Twenty-five patients with androgenic hair loss were studied. The patients were prescribed one 1mg finasteride tablet daily. Fasting plasma levels of LDL, HDL, triglyceride, and total cholesterol of patients before therapy and after 3 and 6 months of therapy were measured. Nutritional status was evaluated. The study was conducted in the form of a before-after clinical trial. Data were analyzed using SPSS Software version 16.
Findings
A significant decreases in fasting plasma level of HDL was observed after 6 months of therapy ($P<0.001$). Moreover, a statistically significant rise in fasting plasma level of triglyceride was observed after 3 months of therapy ($P=0.014$). Plasma levels of LDL and total cholesterol were not altered significantly.
Conclusion
Our study demonstrated for the first time that using 1mg finasteride tablets to treat androgenic hair loss may lead to complications of the lipid profile through reducing HDL and increasing total cholesterol. Finasteride may decrease dihydrotestosterone and increased testosterone this change in lipid profile may be due to testosterone elevation by using finasteride.
Declaration of interest
The authors declare that there is a conflict of interest.
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## P278

**Transient glucocorticoid excess does not cause persisting metabolic changes in mice**

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Introduction
In humans, Cushing's syndrome (CS) is associated with an increased incidence of the metabolic syndrome, increased cardiovascular morbidity and mortality, even after long-term correction of glucocorticoid (GC) excess.
Aim
To evaluate the effects of transient overexposure to GC on the metabolic changes in the long-term in mice.
Methods
Single housed male C57Bl/6J mice were given corticosterone (CORT; 50 μg/ml) or vehicle in the drinking water for 4 weeks, followed by a washout period for 4 or 8 weeks thereafter. Plasma circadian corticosterone levels were assessed at baseline, and at weeks 4, 8, and 12 after the start of exposure. Lipids, insulin, and glucose levels were measured after an overnight fast. Insulin sensitivity was assessed by hyperinsulenemic-euglycemic clamp at week 8 and 12, lean and body- and fat mass by DEXA analysis.
Results
CORT-treatment transiently increased plasma corticosterone by 37-, 13-, 3- and 13-fold at 0700, 1200, 1800 and 2200 h respectively. At week 8, evening peak CORT levels (1800 h) were suppressed, which returned to baseline levels at week 12. CORT-treatment increased food intake and plasma levels of insulin, trigycerides, free fatty acids and cholesterol. Abrogation of CORT normalized food intake, whereas body weight remained unchanged. At week 12, insulin was still significantly higher in CORT treated mice. There were no differences in the lean body or fat mass at week 8 and 12 weeks. Hyperinsulinemic–euglycemic clamp indicated no changes in CORT-treated mice at week 8 or 12.
Conclusion
In mice, transient glucocorticoid excess does not cause persisting metabolic changes. Recovery of these metabolic changes coincides with recovery of the HPA-axis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P279

**Gastrectomy for early gastric cancer is associated with decreased cardiovascular mortality in accordance with amelioration of metabolic abnormalities, visceral adiposity and atherosclerosis**

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Objectives
We investigated cardiovascular and all-cause mortality in patients who underwent gastrectomies for early gastric cancer (EGC), and analyzed the changes in metabolic parameters in these patients after surgery.
Background
Bariatric surgery, including gastric banding and gastric bypass, effectively induces weight loss and resolves cardiovascular comorbidities in obese patients.
Methods
2477 patients who underwent gastrectomies for EGC between 1995 and 2004 were enrolled and followed for mortality through 2007. Standardized mortality ratios (SMRs) were calculated using sex- and age-matched mortality in the general Korean population in 2005. Effects of gastrectomy on changes in metabolic profiles including carotid intima-media thickness (CIMT) were investigated.
Results
During the 15 096.4 person-years of follow-up in 2477 patients, 244 deaths were recorded. The all-cause mortality was not significantly different from that of the general population (SMR (95% CI) = 1.01 (0.89–1.14)); however, cardiovascular mortality was significantly lower (SMR = 0.35 (0.22–0.53)). In the 51 patients included in the second part of the study, significant reductions in body weight and visceral fat areas occurred after surgery, regardless of whether the patients were previously obese. Triglycerides, LDL-cholesterol, and plasminogen activator inhibitor-1 levels were significantly decreased, whereas HDL-cholesterol and adiponectin levels were increased. CIMT was also significantly decreased in previously obese and non-obese patients.
Conclusions
Patients with EGC who undergo gastrectomy have a lower cardiovascular mortality but similar all-cause mortality as that of the general population. In these patients, a significant reduction in body weight and visceral fat after surgery may improve impaired fibrinolytic homeostasis and lipid metabolism and prevent atherosclerotic changes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P280

**The role of testosterone activation of liver X receptor in a human macrophage cell line and its implications for the treatment of type 2 diabetes and atherosclerosis**

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Testosterone deficiency is common in men with type 2 diabetes (T2D) and cardiovascular diseases (CVD), such as atherosclerosis. Previous studies have shown that testosterone replacement improves several cardiovascular risk factors including insulin resistance, glycaemic control, cholesterol and inflammation. The mechanisms by which testosterone mediates these effects are unknown. LXR, a nuclear receptor also regulates these cardiovascular risk factors and the atheroprotective lipoprotein APOE. LXR agonists have been shown to reduce atherosclerosis but can cause hepatic stenosis. The objective of this study is to determine whether testosterone mediates its action via LXRα. Using the human monocyte cell line THP-1, differentiated into macrophages, which we have shown express the androgen receptor, the effect of $10^{-8}$ M testosterone on the expression of LXRα and LXR- targets including APOE, IL6 and TNFα, was investigated following RNA extraction and qPCR. Cells were exposed to testosterone for 24, 48 and 72 h. LXRα gene expression was increased in macrophages following exposure to $10^{-8}$ M testosterone for 72 h. Similarly the LXR-target gene APOE, which encodes the protein apolipoprotein E, involved in binding cholesterol following its removal from the cell, was also upregulated in these cells following testosterone exposure. Conversely, the expression of two pro-inflammatory genes IL6 and TNFα, were suppressed in macrophages following exposure to $10^{-8}$ M testosterone. This is the first study to show that testosterone induces expression of LXRα and APOE in human macrophages. The results suggest testosterone activates LXR and acts through this nuclear receptor to control the expression of LXR- target genes such as APOE, IL6 and TNFα to aid cholesterol efflux and suppress inflammation in human macrophages. We therefore hypothesize that testosterone exerts its anti-diabetic and anti-atherogenic effects in part through the activation of LXR and LXR-target genes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P281

**Chemerin/CMKLR1 and adiponectin/T-cadherin expression in human coronary arterial wall and pericoronary adipose tissue in correlation with atherosclerosis**

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Introduction
Perivascular adipose tissue has been implicated in vascular physiology and pathology, including atherosclerosis. Local adipokine production is one of the

paracrine mechanisms involved in this adipose tissue fuction. We investigated the expression of adipokines chemerin and adiponectin and their receptors CMKLR1 and T-cadherin, respectively, in human coronary arterial wall and pericoronary fat as well as their correlation with coronary atherosclerosis.
Methods
Paraffin embedded samples of human left coronary arteries ($n=38$) including periadventitial fat were evaluated for chemerin, adiponectin, CMKLR1 and T-cadherin expression using immunohistochemistry. AHA classification was used for atherosclerosis assessment. PASW Statistics was used for statistical analysis.
Results
Atherosclerosis was detected in 35/38 coronary arteries. Chemerin was expressed in pericoronary fat (38/38 samples), coronary vascular smooth muscle cells (VSMCs; 38/38 samples) and foam cells in coronary atherosclerotic lesions (32/35 samples). Adiponectin was expressed by periadventitial fat in 37/38 samples. CMKLR1 was expressed in coronary VSMCs (15/38 samples) and foam cells (22/35 samples). T-cadherin was detected in VSMCs (38/38 samples) and endothelia (33/38 samples). Foam cell chemerin expression and CMKLR1 expression were positively interrelated in coronary atherosclerotic lesions ($r=0.406$, $P=0.016$). Periadventitial fat adiponectin expression was positively correlated with coronary VSMC ($r=0.427$, $P=0.007$) and endothelial ($r=0.432$, $P=0.007$) T-cadherin expression. Coronary atherosclerosis was positively correlated with coronary foam cell chemerin ($r=0.365$, $P=0.031$) and CMKLR1 expression ($r=0.349$, $P=0.04$), while negatively correlated with pericoronary fat adiponectin ($r=-0.531$, $P=0.001$) and marginally (not statistically significant) with coronary VSMC T-cadherin expression ($r=-0.307$, $P=0.06$).
Conclusions
Our data lend further support to a possible role for chemerin and its receptor in the atherosclerotic process, probably with pro-atherogenic effects. They also suggest that T-cadherin may serve as a receptor that mediates adiponectin's anti-atherogenic effects in human arterial wall.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P282

**Six loci associated with high-density lipoprotein cholesterol or triglycerides in Chinese pregnant women**

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Background
The serum concentrations of lipoproteins and lipids are heritable risk factors for cardiovascular disease. Pregnancy is associated with increases in plasma lipid profiles. Identifying the genetic determinants could provide novel insights into mechanisms of dyslipidemia and reveal avenues for developing new therapies. The objective of this study was to investigate the impact of six single nucleotide polymorphisms (SNPs) on serum lipid levels during pregnancy.
Method
In this study, 2085 unrelated pregnant women were recruited. Fasting serum lipids were measured in the third trimester of pregnancy. Six SNPs (rs1260326 in GCKR, rs1800775 in CETP, rs515135 in APOB, rs1800588 in LIPC, rs964184 in APOA1-C3-A4-A5 and rs4420638 in APOE-C1-C4-C2) were genotyped using TaqMan allelic discrimination assays. The individual and cumulative effects of the six loci on serum lipids were analyzed.
Results
We found that two variations, rs1260326 ($b=0.180$, 95% CI=0.184 to 0.300, $P=3.91\times10^{-16}$) and rs964184 ($b=0.114$, 95% CI 0.117 to 0.260, $P=2.69\times10^{-7}$) were significantly associated with triglycerides (TG) level. Another two variations rs1800775 ($b=-0.069$, 95% CI $-0.071$ to $-0.016$, $P=0.002$) and rs1800588 ($b=-0.129$, 95% CI $-0.112$ to $-0.055$, $P=8.14\times10^{-9}$) showed relation with high-density lipoprotein cholesterol (HDL-C). Besides, these loci exerted the combined effect on lipids. Subjects with increasing number of risk alleles showed higher TG concentration ($b=0.210$ mmol/l per allele, 95% CI 0.170 to 0.258, $P=2.51\times10^{-21}$) and lower value of HDL-C ($b=-0.140$ mmol/l per allele, 95% CI $-0.082$ to $-0.043$, $P=5.36\times10^{-10}$) in a dose-dependent manner. Other loci did not show the association with related serum lipid level in our population.
Conclusion
Previously identified lipid loci are associated with lipid levels in the Chinese pregnant population. An early lifestyle intervention incorporating exercise training and diet should be encouraged in the pregnant women who harboring risk alleles to keep their lipids in the normal range. Nevertheless more comprehensive investigation is still required to verify the significance of these loci in other population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P283

**Development of primary hyperparathyroidism is closely associated with the onset of dyslipidemia and insulin resistance in a large family harboring a MEN1 gene mutation**

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Introduction
Dyslipidemia is a common finding in patients with primary hyperparathyroidism and together with hypertension and glucose intolerance seems to be responsible by the increased risk of cardiovascular disease in hyperparathyroid patients. In the present work we studied members of a family harboring a MEN1 gene mutation (type 1 multiple endocrine neoplasia syndrome). The penetrance of primary hyperparathyroidism in this condition is close to 100%, starting usually during the second decade of life.
Objective
To study the evolution of carbohydrate and lipid metabolism with the onset of primary hyperparathyroidism as well as with parathyroidectomy.
Patients and methods
All patients enrolled in the present study belong to the same family harboring a new MEN1 gene mutation located in the exon 2 (c124G>C), which leads to substitution of glycine by arginine (Gly42Arg). Three patients with symptomatic hyperparathyroidism exhibited severe combined dyslipidemia. Five young members without clinical and laboratorial evidence of the disease were followed at regular time intervals. Results are presented as the mean$\pm$S.D. of three consecutive determinations in each patient.
Results
Parathyroidectomy was followed by an immediate decline in total cholesterol (from 251$\pm$46 to 225$\pm$27 mg/dl) as well as in triglycerides (from 465$\pm$129 to 238$\pm$65 mg/dl). Fasting glycemia returned to values of $<100$ mg/dl. In young asymptomatic family members, the onset of hyperparathyroidism was closely followed by a twofold increase in insulinemia (from 9$\pm$6 to 21$\pm$7 mU/l) and in triglycerides (from 85$\pm$12 to 167$\pm$37 mg/dl), together with a decline of HDL cholesterol (from 67$\pm$9 to 52$\pm$5 mg/dl). None of these alterations was detected in non-affected family members.
Conclusion
In addition to normalization of serum calcium and PTH, parathyroidectomy corrected, at least partially, alterations in serum lipids as well as in the metabolism of carbohydrates. In previously asymptomatic patients, the onset of primary hyperparathyroidism was associated with an increase in insulin resistance and with the development of a more atherogenic lipid profile.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P284

**Level of adiponectin and vaspin, and adiponectin/vaspin ratio is associated with insulin resistance and clinical expression of metabolic syndrome in type 2 diabetic patients**

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Objective
The goal of this study is to evaluate the relationship between levels of adiponectin and vaspin, adiponectin/vaspin (A/V) ratio and metabolic syndrome, degree of adiposity, and insulin resistance (IR), as well as their correlation with metabolic variables.
Methods
Two hundred sixty patients with newly diagnosed type 2 diabetes (126 male, 134 female) were enrolled. Patients were grouped based on degree of adiposity, IR assessment (homeostasis model assessment of insulin resistance (HOMA-IR), and score of the International Diabetes Federation criteria for metabolic syndrome. Spearman's correlation coefficients and multiple regression analysis were used to determine the association between serum vaspin or adiponectin levels and other continuous variables of interest, and logistic regression analysis to ascertain the association between serum levels of vaspin and adiponectin, with metabolic syndrome as dependent variable.
Results
Metabolic syndrome positive patients had more atherogenic lipid profile, were more insulin resistant, and had higher vaspin compared to metabolic syndrome negative patients. Patient displayed stepwise decrease in adiponectin level and A/V ratio showed, with increasing scores of the criteria for diagnosis of the metabolic syndrome, while an opposite trend was observed for vaspin levels. Using multiple logistic regression, the odds ratio of the metabolic syndrome as protect factor by vaspin was calculated to be 0.469 (95% CI, 0.252–0.873; $P=0.017$). Overweight/obese patients had significantly lower adiponectin levels, lower A/V ratio, and higher vaspin level compared to patients with normal body weight. Insulin-sensitive patients had significantly higher adiponectin levels, higher A/V ratio, and lower vaspin levels than those with IR, after BMI, WC and other variable were adjusted. Receiver operating characteristic analysis demonstrated that vaspin and A/V ratio had significantly higher area under the curve (AUC) for the detection of the metabolic syndrome.
Conclusion
For type 2 diabetes patients, serum levels of adiponectin and vaspin, as well as A/V ratio were associated with obesity, IR, and the expression of the metabolic syndrome. Vaspin and A/V ratio may be a more useful and relevant biomarker for molecular diagnosis of metabolic syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P285

**Insulin mediates the glucagon-induced decrease in atrial natriuretic peptide: a randomized controlled trial**

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Objectives
Atrial natriuretic peptide (ANP) is a cardiac hormone that is known to play an essential role in regulation of blood pressure and vascular endothelial function. However, the metabolic regulation of ANP is not fully understood. In patients with the metabolic syndrome, hyperglucagonemia is a common feature, besides hypertension and hyperinsulinemia. Our aim was to evaluate the impact of glucagon on proANP and the possible mechanisms underlying this effect.
Methods
In this prospective, double-blind, placebo-controlled study, we studied hormonal and metabolic responses to intramuscular glucagon or placebo administration in 13 patients with type 1 diabetes mellitus (DM1; 6/7 males/females; BMI 24.8 $\pm$0.95 kg/m$^2$), in 12 obese healthy subjects (OS; 6/6; 33.9 $\pm$ 1.6 kg/m$^2$) and in 13 lean controls (LC; 6/7; 21.6 $\pm$ 0.5 kg/m$^2$). Furthermore, we studied the response of proANP to insulin (0.15 IU/kg BW) in OS and LC as a possible mediator for the glucagon-induced effects using insulin tolerance test. Finally, we further investigated the impact of hyperinsulinemia under euglycemic conditions on proANP in 32 healthy subjects.
Results
Gender, fasting glucose and glucagon levels were comparable between groups. Glucagon significantly decreased proANP in OS (proANP-AUC240: 203.4 $\pm$ 4.8 (glucagon) vs. 226.6 $\pm$ 6.9 (placebo), $P<0.01$) and LC (205.8 $\pm$ 2.6 vs 245.8 $\pm$ 9.2, $P<0.01$) but failed to affect proANP concentrations in DM1 ($P=0.737$) . Glucagon increased insulin in LS and OS ($P<0.01$) but did not affect it in DM1 ($P>0.05$).
Under hypoglycaemic hyperinsulinemic conditions, insulin markedly decreased proANP in OS and LC ($P<0.01$). Similarly, insulin significantly decreased proANP under euglycemic hyperinsulinemic conditions in healthy subjects ($P<0.01$).
Conclusions
We show that insulin mediates the glucagon-induced decrease in proANP, possibly providing a novel link between hyperinsulinemia and cardiovascular risk.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P286

**The role of β-arrestin proteins in CB1 cannabinoid receptor endocytosis**

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Introduction
β-Arrestins are cytosolic proteins, which play key roles in the desensitization, internalization and signaling of G protein-coupled receptors. Currently, little is known about the role of β-arrestins in the internalization of the CB1 cannabinoid receptor (CB1R). Therefore, the interaction between β-arrestins and CB1R, and its role in receptor internalization were studied.
Materials and methods
The β-arrestin binding of CB1R was studied by confocal microscopy and bioluminescence resonance energy transfer (BRET). To follow CB1R internalization, plasma membrane CB1 receptors were selectively stained using Halo-labeling technique. Internalization of the receptors was also monitored by a BRET assay through measuring non-specific BRET between CB1R and a plasma membrane marker protein (ICAM).
Results
We found that upon activation CB1R binds transiently to β-arrestin2 (β-arr2), but not β-arrestin1. Dominant negative β-arr2 (β-arr2-V54D) or knock-down of β-arr2 by siRNA inhibited the agonist-induced internalization of CB1Rs. Similar inhibitory effects on agonist-induced CB1R internalization were also demonstrated using BRET measurements. In contrast, neither β-arr2-V54D nor β-arr2-siRNA had a significant effect on the constitutive internalization of CB1R.
Conclusions
We conclude that upon activation, CB1R binds β-arr2 in a transient manner (class A GPCR), and this binding is required for the agonist-induced internalization of the receptor. In contrast, constitutive CB1R internalization is independent of the β-arr2 binding of the receptor, suggesting that the molecular mechanisms underlying these two processes are different.
Declaration of interest
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## P287

Abstract withdrawn

## P288

**Plasma aldosterone to plasma renin ratio on an automated analyzer using two novel chemiluminescence immunoassays**

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Background
Screening for primary aldosteronism (PA) using the aldosterone to renin ratio (ARR) is recommended by clinical practice guidelines in patient groups with a high prevalence as the disease is reported by several authors in more than 10% of essential hypertensives (EH).
Methods
Plasma aldosterone concentration (PAC) and direct renin (DR) were measured using two novel automated chemiluminescence assays (IDS-iSYS; Boldon, UK) and compared to the Siemens RIA and Diasorin Liaison (LSN) respectively. Plasma was taken from 20 controls, 26 EH and 36 PA patients. PA was defined by lack of aldosterone salt-load suppression to $<50$ pg/ml. Mineralocorticoid receptor antagonists and b-blockers were excluded, other medication was not restricted. Hypokalemia was controlled in all. Using Siemens PAC (pg/ml)/Liaison DR (mU/l), our laboratory cut-off for diagnosis of PA is 12.
Results
PACs in controls, EHs and PAs were (mean; 25–75%ile) RIA: 157 (84.2–196), 123 (73.3–137), and 274 (190–320) pg/ml and iSYS: 115 (50.5–182), 114 (68.7–131), and 279 (172–348) pg/ml. In the same groups DR using the LSN assay was at 33 (21.2–43.1), 62 (8.9–82.2), and 7.9 (2.0–9.5), and with the iSYS 40.1 (27.4–47.8), 77.9 (13.6–110), and 14.1(4.9–13.7). Using linear regression analysis aldosterone-iSYS $=1.073$RIA $\pm 0.082$, $r^2=0.69$ and renin-iSYS $=1.057$LSN $\pm 0.067$, $r^2=0.77$. Using a diagnostic cut-off of 12 with the RIA and LSN, ROC curve analysis of this cohort gives a sensitivity of 96.7% and specificity of 85.1%. As at this cut-off the iSYS assays provide 93.6% sensitivity, a lower cut-off at 6.3 would provide corresponding 96.7% sensitivity with 84.8% specificity.
Conclusions
The data presented here show good correlation between commonly used aldosterone and renin assays and the newly automated IDS-iSYS system. An overall trend for higher renin values using the iSYS, albeit with equivalent discrimination between the three cohort groups, would require a lower ARR cut-off to provide the same diagnostic power.
Declaration of interest
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## P289

**Hypogonadism as a risk factor for cardiovascular mortality in men: a meta-analytic study**

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Objective
To verify whether hypogonadism represents a risk factor for cardiovascular (CV) morbidity and mortality and to verify whether testosterone replacement therapy (TRT) improves CV parameters in subjects with known CV diseases (CVDs).
Design
Meta-analysis.
Methods
An extensive Medline search was performed using the following words 'testosterone, CVD, and males'. The search was restricted to data from January 1, 1969, up to January 1, 2011.
Results
Of the 1178 retrieved articles, 70 were included in the study. Among cross-sectional studies, patients with CVD have significantly lower testosterone and higher 17-β estradiol ($E_2$) levels.
Conversely, no difference was observed for DHEAS. The association between low testosterone and high $E_2$ levels with CVD was confirmed in a logistic regression model, after adjusting for age and body mass index (hazard ratio (HR) $=0.763$ (0.744–0.783) and HR $=1.015$ (1.014–1.017), respectively, for each increment of total testosterone and $E_2$ levels; both $P$ 0.0001). Longitudinal studies showed that baseline testosterone level was significantly lower among patients with incident overall- and CV-related mortality, in comparison with controls. Conversely, we did not observe any difference in the baseline testosterone and $E_2$ levels between case and controls for incident CVD. Finally, TRT was positively associated with a significant increase in treadmill test duration and time to 1 mm ST segment depression.
Conclusions
Lower testosterone and higher $E_2$ levels correlate with increased risk of CVD and CV mortality. TRT in hypogonadism moderates metabolic components associated with CV risk. Whether low testosterone is just an association with CV risk, or an actual cause-effect relationship, awaits further studies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P290

**Testosterone and cardiovascular risk in patients with erectile dysfunction**

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Background
The relationship between cardiovascular (CV) diseases (CVD) and testosterone levels in men has not been completely clarified.
Aim
To evaluate the association between testosterone levels and CV risk in subjects with erectile dysfunction (ED) and to verify whether their body mass index might (BMI) represents a possible confounder in testosterone-related CV stratification.
Material and methods
A consecutive series of 2269 male patients attending the outpatient clinic for ED was studied. The assessment of CV risk was evaluated using the engine derived from the Progetto Cuore study.
Results
After adjustment and for BMI and associated morbidities, sex hormone binding globulin bound (SHBG) and unbound testosterone levels decreased as a function of CV risk assessed thorough Progetto Cuore risk engine. In addition, a higher prevalence of hypogonadism related symptoms and signs was associated with a higher CV risk. Among factors included in the Progetto Cuore risk engine age, total and HDL cholesterol and diabetes were all significantly associated with CV risk-dependent modification of total and calculated free-testosterone levels. When the relationship between SHBG bound and unbound testosterone and CV risk was evaluated as a function of obesity (BMI $>30$ kg/m$^2$), all the aforementioned associations were confirmed only in non obese patients.
Conclusions
Hypogonadism could be associated either with an increased or reduced CV risk, depending on the characteristics of subjects. Low testosterone observed in obese patients might represent the result of higher CV risk rather than a direct pathogenetic mechanism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P291

**Oxytocin stimulates cardiomyogenesis and cardioprotection via stem cells activation**

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The functional oxytocin (OT) system is present in the heart and stimulate cardiomyocyte (CM) differentiation of embryonic stem cells. Presently, we are reporting that the OT stimulates CM differentiation of stem cells isolated from the rat heart and termed as side population cells (SP). Specifically, the phenotype of SP-CD31(−) cells, but not SP-CD31(+) cells, proliferate in the presence of OT; the OT treatment promote cell aggregation, and then stimulate differentiation into beating cell colonies. These colonies of contractile cells express the markers of a CMs phenotype such as troponin, MLC2V and actinin. Moreover, SP cells stimulated by OT produce endothelial phenotype in the Matrigel culture. Among adult stem cells, mesenchymal stem cells (MSCs) possess unique properties that make them eligible for convenient and highly effective cell therapy of the heart. The rat bone marrow MSCs preconditioned with the OT present a rapid increase and nuclear accumulation of phospho-Akt and of phospho-p44/42 (ERK1/2), indicating an activation of the protective way phosphatidylinositol-3-kinase (PI3K)/AKT and mitogen-activated protein kinases (MAPK) respectively. MSCs carrying OT gene stimulate angiogenesis *ex vivo* and *in vivo* in rat model of hind-limb ischemia. The preconditioning to the OT increases the metabolic activity of the MSCs cells in normoxia, stimulation of cellular trafficking of GLUT 4 transporter and seems to protect against the hypoxia and the serum deprivation as evaluated by the MTT test. As demonstrated by flow cytometry for annexin V and Tunel method, the OT preconditioning reduced apoptosis in rat MSCs exposed to hypoxia. This anti-apoptotic protection was transmitted from MSCs to CMs in co-culture. In conclusion, we propose that stem cells optimized by OT treatment may play the supportive role in injured heart via differentiation to cardiac cells and secretion of paracrine factors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P292

**Metabolism of angiotensin II and angiotensin III in HAC15 cells**

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Angiotensin II (A-II) stimulates aldosterone (aldo) secretion through the zona glomerulosa AT1R. A-II can be metabolized to angiotensin III (A-III) which also stimulates aldo synthesis. In the rat A-III stimulates aldo secretion through the AT2R receptor.

The human adrenocortical HAC15 cells were incubated with A-II or A-III (10 nM) for 24 h and the concentration of aldo and cortisol (F) measured in the media. A-II produced a fourfold and A-III a twofold increase in aldo in the media. A-II produced a twofold increase in F concentration in the media, but A-III did not change F production. Losartan 10 μM inhibited the A-II and A-III stimulated production of aldo, but the AT2R antagonist PD123319 had no effect. Both A-II and A-III stimulated the expression of StAR, CYP11B1 and CYP11B2 mRNA. While A-II had no effect on CYP17A1 mRNA expression, A-III inhibited its expression.

HAC15 cells were incubated with 100 nM of A-II and A-III for 0.5, 1, 3 and 6 h, the media was extracted and angiotensin metabolites measured by HPLC–MS. Approximately 30% of A-II remained after 1 h incubation and was not detectable after 6 h. A-II was converted to angiotensin 1–7, angiotensin-IV and angiotensin 3–7, but not to A-III. A-III was decreased to ∼40% after 30 min and was undetectable after 1 h. A small amount of the A-III was converted to angiotensin 3–7 and angiotensin-IV.

Addition of more A-II (10 nM) at 1, 3, 6 or 12 h did not increase aldo production at 24 h further compared to cells stimulated only once with A-II (10 nM) at time 0.

In conclusion, A-II is not converted to A-III in HAC15 cells. A-III stimulates aldo, but not cortisol production: A-II stimulates both. The half-life of A-II and A-III in the media is short, but stimulation of steroidogenesis persists.

Declaration of interest

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## P293

**Chemerin/CMKLR1 and adiponectin/T-cadherin expression in human aortic wall and periaortic adipose tissue in correlation with atherosclerosis**

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Introduction

Periadventitial adipose tissue has been implicated in vascular physiology and pathology, including atherosclerosis, through paracrine and endocrine mechanisms, mainly adipokine production. We investigated the expression of adipokines chemerin and adiponectin and their receptors CMKLR1 and T-cadherin, respectively, in human aortic arterial wall and periaortic fat as well as their correlation with aortic atherosclerosis.

Methods

Paraffin embedded samples of human aortas ($n=37$) including periadventitial fat were evaluated for chemerin, adiponectin, CMKLR1 and T-cadherin expression using immunohistochemistry. AHA classification was used for atherosclerosis assessment. PASW Statistics was used for statistical analysis.

Results

Atherosclerosis was detected in 31/37 aortas. Chemerin was expressed in periaortic fat (34/37 samples), aortic vascular smooth muscle cells (VSMCs; 37/37 samples) and foam cells in aortic atherosclerotic lesions (25/31 samples). Adiponectin was expressed by periadventitial fat in 34/37 samples. CMKLR1 was expressed in aortic VSMCs (9/37 samples) and foam cells (28/31 samples). T-cadherin was detected in VSMCs (37/37 samples) and endothelia (32/37 samples). Periaortic fat chemerin expression was negatively correlated to adiponectin expression ($r=-0.620$, $P<0.001$). Foam cell chemerin expression and CMKLR1 expression were positively interrelated in aortic atherosclerotic lesions ($r=0.456$, $P=0.01$). Periadventitial fat adiponectin expression was positively correlated with endothelial T-cadherin expression ($r=0.326$, $P=0.049$). Aortic atherosclerosis was positively correlated with periaortic fat chemerin ($r=0.813$, $P<0.001$), aortic VSMC chemerin ($r=0.654$, $P<0.001$), aortic foam cell chemerin ($r=0.639$, $P<0.001$) and aortic foam cell CMKLR1 expression ($r=0.399$, $P=0.026$), while negatively correlated with periaortic fat adiponectin ($r=-0.690$, $P<0.001$), VSMC T-cadherin ($r=-0.413$, $P=0.011$) and endothelial T-cadherin ($r=-0.396$, $P=0.015$) expression.

Conclusions

Our data suggest a putative role for chemerin and its receptor in the atherosclerotic process, probably with opposite effects to adiponectin's. T-cadherin may also serve as a receptor that mediates adiponectin's actions regarding atherosclerosis deceleration.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P294

**In primary aldosteronism, plasma aldosterone concentration is an independent risk factor for albuminuria, and the higher baseline urinary albumin excretion predicts the less glomerular filtration rate after treatment**

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Background
The higher prevalence of urinary albumin was reported in patients with primary aldosteronism (PA) than those with essential hypertension (EH). It remains to be unclear, although 'glomerular hyperfiltration (HF)' was hypothesized as one of the mechanisms of urinary albumin.
Objectives
To clarify the risk factor of urinary albumin and the relationship with HF in PA.
Methods
123 patients with PA were treated according to the result of adrenal vein sampling. Adrenalectomy was performed on patients diagnosed with unilateral disease (APA: $n=68$), while those who were diagnosed with bilateral disease were given medical treatment including with mineralcorticoid receptor antagonists (MRA; IHA: $n=55$).
Blood pressure, estimated glomerular filtration rate (eGFR:ml/min per 1.73 $m^2$) and urinary albumin excretion (UAE:mg/g Creatinine) were followed at baseline and during 24 months after treatment of PA in all patients. Multivariate regression analysis with stepwise procedure was performed to identify the potential risk factor of baseline UAE and the relation factor with the decline of GFR (ΔGFR) between at baseline and at 1 month after treatment. eGFR was estimated by the equation for Japanese established by Japan society of nephrology. UAE was adjusted by logarithm.
Results
Blood pressure, UAE, and GFR significantly decreased at 1 month after treatment and they remained a plateau during the follow-up. Positive relationship was observed between UAE and ΔGFR ($P=0.0003$, $R^2=0.165$). Baseline plasma aldosterone concentration and systolic blood pressure were the risk factors of UAE ($P=0.010$ and $P=0.013$, respectively, $R^2=0.164$). Baseline UAE and serum potassium were the predictors of ΔGFR ($P<0.01$, $R^2=0.275$).
Conclusion
In primary aldosteronism, plasma aldosterone concentration is an independent risk factor for albuminuria, and the higher baseline urinary albumin excretion predicts the less glomerular filtration rate after treatment.
Declaration of interest
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## P295

**Relationships between insulin resistance and cardiovascular risk factors in primary hyperparathyroidism**

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Introduction
Primary hyperparathyroidism (pHPT) is characterized by an increased frequency of glucose tolerance abnormalities associated with insulin-resistance. Few studies evaluated the relationship between insulin resistance and others cardiovascular risk factors in pHPT.
Subjects and methods
In 255 consecutive pHPT patients (M/F=66/189, age (mean±S.D.): 59.9±13.7 years, PTH: 215.6±219.4 pg/ml, calcium: 11.2±1.2 mg/dl, asymptomatic/symptomatic: 115/140) we assessed insulin resistance by means of homeostasis model assessment of insulin resistance (HOMA-IR). Then we evaluated biochemical and clinical features of pHPT subdividing our case series in two groups basing on median HOMA-IR (1.68).
Results
pHPT patients more insulin-resistant showed significantly higher systolic blood pressure values (142.5±20.6 vs 134.8±16.7 mmHg, $P<0.002$), BMI (27.3±4.7 vs 23.1±3.8 kg/$m^2$, $P<0.000001$), PTH (238.2±262.9 vs 193.0±162.9 pg/ml, $P<0.04$), calcium levels (11.35±1.27 vs 11.05±1.06 mg/dl, $P<0.04$) and triglycerides (142.8±63.8 vs 105.6±40.8 mg/dl, $P<0.00001$) than patients with lower HOMA-IR. HDL-cholesterol was lower in patients with higher HOMA-IR (51.6±16.2 vs 63.2±17.4, $P<0.00005$).
Conclusions
In PHPT increased insulin resistance is associated with a more severe alteration of biochemical indices of the disease and a worsening of overall cardiovascular risk profile.
Declaration of interest
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## P296

**Allosteric effects between the protomers of AT1 angiotensin receptor homodimer: role of the conserved DRY motif**

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Introduction
G protein coupled receptor dimerization is proposed to have great impact on receptor signaling. Allosteric communication between the protomers of a dimer can alter ligand binding, receptor conformation and the interactions with different effector proteins. Although the concept of intradimeric interactions is widely accepted, the molecular mechanism behind them is not clearly understood. In this study we investigated the allosteric interactions within the type I angiotensin receptor (AT1R) homodimer transiently expressed in CHO cells.
Methods
To detect the intradimeric interactions, one protomer of AT1R homodimer was selectively stimulated, while the activation process of the other protomer was followed by different bioluminescent resonance energy transfer (BRET) based assays. For the selective stimulation we used an antagonist resistant mutant AT1R. We monitored the activation of the non-stimulated protomer through its interaction with β-arrestin2 and followed the conformation alterations directly with an intramolecular receptor biosensor. Also cooperative ligand binding of the homodimer was examined by ligand dissociation experiments. To evaluate the molecular mechanism behind the allosteric interactions, different mutants of AT1R were investigated in our assays.
Results
We observed that agonist binding of one protomer is followed by the increased ligand dissociation, altered conformation and β-arrestin2 binding of the other protomer. The mutation of the conserved DRY sequence in the activated protomer abolished all the observed effects between the receptors.
Conclusion
The detected effects on the non-stimulated protomer are presumable the consequence of direct intradimeric allosteric interactions. Our results with AT1 receptor mutated in the DRY sequence suggest the crucial role of this motif in intradimeric interactions, and also highlight the molecular mechanisms behind them.
Declaration of interest
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## P297

**Effect of triiodothyronine and angiotensin-II on expression of (pro)renin receptor in the human erythroid cell line, YN-1**

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The renin–angiotensin system is known to enhance erythropoiesis. (Pro)renin receptor ((P)RR), a specific receptor for renin and prorenin, has recently been identified. We have recently shown expression of (P)RR in a human erythroid cell line, YN-1. It is known that triiodothyronine ($T_3$) and angiotensin-II (A-II) stimulate proliferation of erythroid cells. The aim of the present study is to clarify effects of $T_3$ and A-II on expression of (P)RR in the human erythroid cell line, YN-1. YN-1 cells were maintained in Iscove's modified Dulbecco's medium containing 10% fetal bovine serum. To study effects of $T_3$ and A-II on (P)RR expression, the cells were cultured in the medium containing $T_3$ (10 and 100 nM) or A-II (10 and 100 nM) for 24 h. Expression of (P)RR mRNA and protein was examined by real time RT-PCR and western blot analysis respectively. The antiserum against (P)RR was raised in a rabbit by injecting the peptide fragment of human (P)RR corresponding to 224–237 a.a. (Hirose *et al*. 2009). Real time RT-PCR showed that $T_3$ increased expression levels of (P)RR mRNA significantly in a concentration dependent manner. $T_3$ caused a 1.6-fold increase at 100 nM compared with control. Western blot analysis showed increased expression of (P)RR protein by $T_3$ although this increase was not statistically significant. By contrast, A-II had no significant effects on (P)RR mRNA and protein levels in YN-1 cells. In this study, we have found that $T_3$ stimulates expression of (P)RR in YN-1 cells, suggesting a possibility that (P)RR is related to erythropoiesis induced by $T_3$.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P298

**The combined use of serum interleukin 6 and C-reactive protein is better than either alone in predicting cardiovascular events in a Chinese population**

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Objectives

Obesity is associated with an increase in cardiovascular risk. We investigated whether the measurement of obesity-related biomarkers could enhance the prediction of cardiovascular (CVD) events when used in conjunction with traditional risk factors.

Methods

We studied subjects in the Hong Kong Cardiovascular Risk Factors Prevalence Study 2 (CRISPS 2) cohort without previous cardiovascular disease. Baseline serum levels of several biomarkers known to be increased in obesity, C-reactive protein (CRP), interleukin 6 (IL6), and soluble tumor necrosis factor-alpha receptor-2 (sTNFR2; a surrogate marker of TNFα), as well as those of adiponectin, an adipokine with reduced expression in obesity, were measured.

Results

Of the 1848 subjects with no known CVD at baseline, 104 (5.6%) developed CVD events during a median follow-up of 6 years. The CVD group had higher baseline levels of CRP, IL6, and sTNFR2 (all $P<0.001$), but similar adiponectin levels ($P=0.435$), compared to the non-CVD group. Likelihood ratio test showed that elevated levels of CRP and IL6 ($P<0.001$, adjusted for traditional CV risk factors) but not sTNFR2 (adjusted $P=0.05$) were independent predictors of incident CVD. IL6 remained a significant predictor after adjustment for CRP levels. These two biomarkers, alone or in combination, significantly improved the prediction by traditional risk factors, as estimated by c-statistics (Delong $P$ value being 0.0077, 0.035 and 0.0006 for CRP, IL6 and CRP + IL6 respectively). IL6 significantly increased the c-statistics when added to the model already including the traditional risk factors and CRP (Delong $P=0.0393$).

Conclusion

In this prospective study, CRP and IL6 were independent predictors of incident CVD in Hong Kong Chinese without prior CVD. The combined use of serum IL6 and CRP was superior to either alone in predicting CVD events in this community-based cohort.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P299

**Short-term detraining cannot reverse insulin sensitivity improvement and serum retinol binding protein 4 in spontaneously hypertensive rats previously submitted to aerobic exercise**

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Background

Elevated serum retinol binding protein 4 (RBP4) levels were previously described in insulin-resistance states. Recently it was shown that RBP4 contributes, at least partly, to the pathogenesis of insulin resistance in spontaneously hypertensive rats (SHR), but the effects of exercise training and time-course of changes after detraining on RBP4 levels have not yet been explored.

Aims

To examine the effects of exercise training and short-term detraining on serum RBP4 levels and insulin resistance in SHR.

Methods

Thirty-two male SHR, 4 months old, were submitted to 10-week treadmill training, 5 times/week (T) or kept sedentary for the same period (S). Two short periods of exercise detraining were also carried out (2 and 4 days: D2 and D4). Body weight, insulin sensitivity (insulin tolerance test, ITT), functional capacity (maximal exercise test) and serum RBP4 (ELISA) were measured. Repeated ANOVA and Pearson's correlation were used ($P<0.05$).

Results

Rats had the same characteristics at baseline. There was a ~43% gain in body weight over time ($P=0.004$) in all groups. However, in exercise-trained rats there was a ~40% ($P<0.001$) reduction of white fat tissue weight (epididymal) as compared to the S group. Exercise training determined an improvement of insulin sensitivity (S: 3.8±1.0, T: 4.4±1.0, D2: 5.9±1.2, D4: 4.1±1.7% per min, $P=0.001$) and increased function capacity (S: 1.4±0.3, T: 2.4±0.3, D2: 2.6±0.1, D4: 2.7±02 km/h, $P<0.001$), which was not lost after detraining. RBP4 levels were reduced in response to exercise training (~45%, $P=0.015$). There was a negative correlation between insulin resistance and serum RBP4 ($r=-0.690$, $P<0.001$). The short periods of detraining were not enough to change any 10-week training-induced benefits.

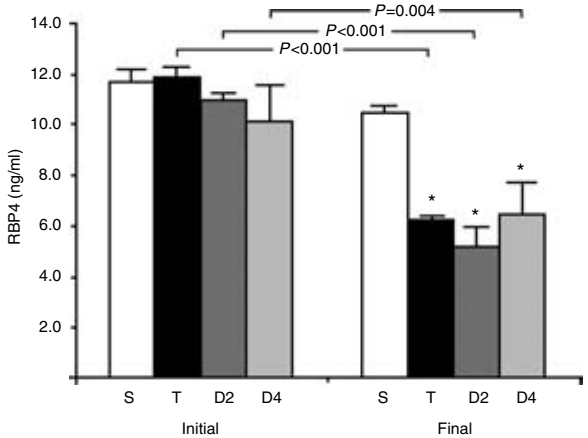


**Figure 1** Serum retinol binding protein 4 (RBP4) in response to exercise training and detraining. S, sedentary; T, 10-week trained; D2, 2 days detrained; D4, 4 days detrained. Initial: pre-training measures. Final: measures after exercise training period or after the respective periods of detraining. Repeated measures ANOVA: $P$ (group) $<0.001$, $P$ (time) $<0.001$ and $P$ (interaction) $=0.015$, followed by post hoc Bonferroni: $^*P<0.05$ vs S in the final period. Significances over time are also presented.

Conclusions
Exercise training determined a decrease in serum RBP4, accompanying the improvement in insulin sensitivity. These benefits were maintained for 4 days of exercise detraining. The inverse association between RBP4 levels and insulin resistance could represent a causal relationship between them.
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Declaration of interest
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## P300

### Multiple cardiovascular risk factors in patients with myotonic dystrophy type 1 and 2

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Introduction
Myotonic dystrophy (DM) type 1 and 2 are multisystemic autosomal dominant disorders with visceral obesity, insulin resistance and hypogonadism. The prevalence of metabolic risk factors for coronary artery disease (CAD) in DM is not well defined, particularly in patients affected by DM2, a milder and clinical challenging condition.
Methods
We assessed the frequency of CAD risk factors in 31 male DM1 ($44 \pm 11$ years) and 13 male DM2 ($54 \pm 9$ years) patients.
Results
DM1 patients showed visceral fat distribution: waist was $>102$ cm in 32% of cases and epicardial fat thickness was increased in 52% of patients ($6.1 \pm 2.8$ mm). Hepatic steatosis with abnormal liver tests occurred in 54% of DM1 patients and dyslipidemia also was frequent (48%). Insulin-resistance was detected in 21% of cases, while diabetes in 3%. Overt and subclinical hypogonadism occurred in 26 and 68% of cases. Arterial blood hypertension was diagnosed in 9% and cardiac dysfunction, including arrhythmias, in 32% of DM1 patients, but CAD was never present. Though visceral body fat and lipid parameters in DM2 patients did not differ from those in DM1 patients, diabetes and insulin-resistance were more frequent in DM2 versus DM1 patients (38 and 61%, respectively, $P=0.006$) and hypogonadism was present in all DM2 (overt in 38%). Hypertension and CAD were also more frequent in DM2 patients (69 and 24%, respectively, $P<0.001$; $P=0.02$). Echocardiography showed higher left ventricular mass index (LVMI) in DM2 vs DM1 patients ($101.1 \pm 18$ vs $86.5 \pm 14$, $P=0.05$), suggesting that ventricular hypertrophy is more common in DM2.
Conclusion
Cardiovascular risk factors, namely diabetes mellitus, hypogonadism, hypertension and ventricular hypertrophy occurred more frequently in DM2 vs DM1 patients, though in DM2 the muscular phenotype is milder. DM2 represents a genetically determined model of metabolic syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P301

### Role of peroxisome proliferator-activated receptor-γ (PPARγ) in regulation of glucocorticoid metabolism in vascular smooth muscle cells

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Glucocorticoids are known to have significant and diverse effects on the cardiovascular system. They have been shown to foster hypertension indirectly due to enhanced vascular tone by potentiation of the vasocontrictor hormones and directly by action on vascular smooth muscle cells (VSMC). The local concentration of glucocorticoids in various tissue including vessels is determined by the enzyme 11β-hydroxysteroid dehydrogenase type two (11HSD2) and 1 (11HSD1) that has been shown to be down-regulated by PPARγ agonists in hepatocytes and adipocytes. Thus it can be hypothesized that PPARγ activation is able to induce a vasodepressor effect. However, PPARγ knock-out mice and animals with VSMC-selective PPARγ deficiency have hypotension. Therefore, in the present study the effect of PPARγ agonist pioglitazone on 11HSDs expression was assessed in primary cultures of VSMC derived form rat aorta. Pioglitazone significantly increased 11HSD1 activity (11-reductase) and mRNA expression in a dose-dependent manner with EC50 243 nM and this effect was not blocked by RU 486, an antagonist of the glucocorticoid receptor. Corticosterone had no effect on 11HSD1 expression. Pioglitazone positively regulated transcription of two CCAAT/enhancer-binding proteins (C/EBPs), specifically C/EBPα a potent activator of 11HSD1 gene transcription in some cells types, and C/EBPζ, whereas C/EBPβ and C/EBPδ were not changed. In contrast, corticosterone stimulated the expression of C/EBPβ and C/EBPδ, but the levels of C/EBPα and C/EBPζ were not changed. 11β-oxidase activity was not detected in VSMC in either control or pioglitazone treated cells even if weak signal for 11HSD2 mRNA expression was found. In conclusion, activation of PPARγ in VSMC up-regulates vascular 11HSD1 and thus reactivates 11-oxo metabolites to biologically active glucocorticoids through a mechanism that seems to involve C/EBPα and C/EBPζ. Our data provide one of the possible explanations for PPARγ agonists' effects on the cardiovascular system.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P302

### Recombinant erythropoietin treatment enhances mitochondrial function in human skeletal muscle

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Erythropoietin (Epo) treatment has been shown to induce mitochondrial biogenesis in cardiac muscle along with enhanced mitochondrial capacity in mice. We hypothesized that recombinant human Epo (rhEpo) treatment enhances skeletal muscle mitochondrial oxidative phosphorylation (OXPHOS) capacity in humans. In six healthy volunteers rhEpo was administered by s.c. injection over eight weeks with oral iron (100 mg) supplementation taken daily. Mitochondrial OXPHOS was quantified by high-resolution respirometry in saponin-permeabilized muscle fibers obtained from biopsies of the vastus lateralis before and after rhEpo treatment. OXPHOS was determined with the mitochondrial complex I substrates malate, glutamate, pyruvate and complex II substrate succinate in the presence of saturating ADP concentrations, while maximal electron transport capacity (ETS) was assessed by addition of an uncoupler. rhEpo treatment increased OXPHOS (from $92 \pm 5$ to $113 \pm 7$ pmol/s per mg) and ETS ($107 \pm 4$ to $143 \pm 14$ pmol/s per mg, $P<0.05$), demonstrating that Epo treatment induces an upregulation of OXPHOS and ETS in human skeletal muscle.
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## P303

### Association of IL4 receptor gene polymorphisms with high density lipoprotein cholesterol

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Abundant evidence demonstrates that long-term cytokine-mediated inflammation is a risk factor for obesity and type 2 diabetes mellitus (T2DM). Our previous study shows a significant association between IL4 genotypes and T2DM, as well as between IL4 genotypes and the lower circulatory levels of high-density lipoprotein-cholesterol (HDL-C). In addition, IL4 has anti-hyperglycemic function by improving insulin sensitivity and glucose tolerance as well as being involved in lipid metabolism by regulating serum levels of adipokines and free fatty acids. Taking the above results together, it strongly suggests that IL4

involves in the regulation of lipid metabolism. Interleukin 4 receptor α chain (IL4Rα) is a crucial component for binding and signal transduction of IL4. Polymorphisms located in IL4Rα which alter the binding affinity to IL4 or downstream signaling pathways and thus contribute to the fine tune of IL4 responsive phenotypes, would also be linked to disease development. The aim of this study is to investigate the correlation between IL4Rα E400A polymorphisms and lipid metabolism. Genomic DNA from 121 type 2 diabetes mellitus patients and 113 non-diabetic non-obese control study subjects were extracted, and their IL4Rα E400A polymorphisms were analyzed by PCR–RFLP. The correlation between IL4Rα E400A genotypes and study subjects' lipid profile was then examined. Significant associations of the IL4Rα E400A genotypes and HDL-C levels among control individuals ($P=0.007$), as well as among the obese T2DM patients ($P=0.029$), were observed. Significant correlations between IL4Rα E400A genotypes with blood pressure, as well as with blood urea nitrogen, were also observed in lean control subjects. Our results reveal that IL4Rα may play certain roles in the lipid metabolism of Taiwanese population and suggest a novel link between lipid metabolism and the cytokine receptor.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P304

### Cardiovascular risk factors in a large cohort of children with GH deficiency

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Background

Adults with GH deficiency (GHD) may have an increased risk of cardiovascular disease. Several studies showed that also GHD children may present cardiovascular risk factors. However results are controversial.

Objective

Aim of this prospective, case-control study on a large cohort of children with GHD was to evaluate the effects of GHD and GH therapy on cardiovascular risk factors, such as lipid profile, clinical measures of visceral adiposity and inflammatory markers in a large cohort of GHD children.

Subjects and methods

Total-, LDL- and HDL-cholesterol, triglycerides, fibrinogen, C reactive protein (CRP), waist circumference, waist-to-height (WHtR) and waist-to-hip ratio (WHR) were evaluated in 60 GHD children (40 males), aged $9.9\pm0.4$ years, before and after 2 years of GH therapy. 60 healthy, age-, sex- and BMI-matched healthy controls were enrolled.

Results

Compared with controls, GHD children at baseline had higher total-cholesterol ($163.8\pm3.0$ vs $150\pm2.8$ mg/dl, $P=0.001$), LDL-cholesterol ($96.7\pm2.7$ vs $83.3\pm3.7$ mg/dl, $P=0.003$), triglycerides ($73.2\pm5.4$ vs $59.5\pm3.6$ mg/dl, $P<0.04$), fibrinogen ($305\pm8.4$ vs $278\pm9$ mg/dl, $P=0.03$) and CRP ($0.4\pm0.03$ vs $0.31\pm0.006$, $P=0.001$).Waist circumference was slightly higher in GHD patients although this difference was not significant. However, GHD children had higher WHtR ($0.54\pm0.01$ vs $0.47\pm0.01$, $P<0.0001$) and WHR ($0.96\pm0.01$ vs $0.92\pm0.01$, $P=0.005$) than controls. Two years of GH therapy were associated with significantly reduced levels of total- ($149.4\pm3.27$, $P=0.001$) and LDL-cholesterol ($79.6\pm3.0$, $P<0.0001$), triglycerides ($60.9\pm3$, $P<0.04$), fibrinogen ($265\pm6.5$, $P=0.0003$), CRP ($0.32\pm0.01$, $P=0.002$), WHtR ($0.49\pm0.01$, $P=0.0006$) and WHR ($0.92\pm0.01$, $P+0.005$).

Conclusions

Our data suggest that in children GHD is associated with cardiovascular risk factors such as adverse lipid and inflammatory profile and increased visceral adiposity. GH replacement therapy improves body composition, lipid profile and it reduces inflammatory risk factors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P305

### Lipid profile in children with persistent idiopathic subclinical hypothyroidism

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Background

There is a great controversy on whether subclinical hypothyroidism (SH) in children should be treated for TSH values between 4.5 and 10 mU/l. In adults SH has been associated with abnormalities in lipid profile and increased risk of atherosclerosis. Data in untreated SH children are scanty.

Objective

The aim of this cross-sectional controlled study was to evaluate in children the effects of long term untreated SH on lipid profile and endothelial function.

Patients and Methods

Twenty children aged $9.7\pm0.6$ years with long-term idiopathic SH (lasting $3.5\pm0.5$ years), and 20 age and sex matched healthy controls underwent clinical examination and lipid profile evaluation. Endothelial function with flow-mediated dilation (FMD), an early marker of atherosclerotic event, was assessed by brachial Doppler ultrasound.

Results

No significant differences were observed between SH children and controls in BMI ($0.2\pm0.2$ vs $-0.3\pm0.3$ SDS), total cholesterol (TC; $149.6\pm7.4$ vs $141.8\pm7.5$ mg/dl), LDL-cholesterol (LDL-C; $85.2\pm5.2$ vs $81.6\pm4.7$ mg/dl), triglycerides (TG; $72.0\pm8.3$ vs $66.0\pm5.1$ mg/dl), atherogenic index ($3.1\pm0.2$ vs $2.8\pm0.1$) and in mean FMD values ($12.3\pm1.2\%$ vs $12.9\pm1.1\%$). A significant difference was only observed in HDL-cholesterol (HDL-C) that was lower in SH children compared to controls ($50.3\pm3.2$ vs $58.0\pm6.8$ mg/dl, $P<0.0001$).

Conclusions

Untreated idiopathic SH in children is not associated with significant abnormalities of lipid profile and endothelial function. However, the mild decrease in HDL-C might represent a first unfavorable change in lipid profile. Further studies on a larger number of patients with long-term follow-up are needed to clarify whether children with SH are at risk of subclinical abnormalities that may require levothyroxine treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P306

### Variants of endothelial nitric oxide synthase gene are associated with components of metabolic syndrome in an Arab population

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[1]King Saud University, Riyadh, Saudi Arabia; [2]University of Milano, Milano, Italy; [3]National Research Centre, Cairo, Egypt.

Genetics plays a crucial role in the development of metabolic syndrome (MetS). Here we examined the association between endothelial nitric oxide synthase (eNOS) gene polymorphisms and MetS in a Saudi Arabian cohort to extend the understanding of the genetic basis of MetS in diverse ethnic populations. Anthropometric, clinical and biochemical parameters as well as genotyping for 894G>T, −786T>C variants of eNOS gene by PCR–RFLP and 4a/b by direct PCR were performed in 886 Saudi Arabians (477 MetS and 409 Non-MetS). The genotype distribution (TT, $P=0.001$; TC, $P=0.001$; TC+CC, $P=0.001$) and allele (T, $P=0.007$; C, $P=0.007$) frequency of the −786T>C SNP were significantly different between Non-MetS and MetS subjects which remained significant after Bonferroni correction. Moreover: i) the GT and GT+TT genotypes of the 894G>T SNP were associated with elevated blood pressure ($P=0.017$, and $P=0.022$ respectively); ii) the ab variant of 4a/b polymorphism was associated with decreased HDL levels ($P=0.044$); and iii) the TC+CC genotype and C allele of the −786T>C SNP were associated with increased fasting glucose levels ($P=0.039$, and $P=0.028$ respectively). Also, G-a-C was identified as the risk haplotype for MetS susceptibility ($P=0.034$). The result suggest a significant association of 894G>T, 4a/b and −786T>C polymorphisms with MetS and its components is present in an Arab population. A genetic predisposition to develop abnormal metabolic phenotypes, consistent with an increased prevalence of metabolic phenotypes can be detected in this ethnic group.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P307

**MRTF-A, a rho-dependent co-activator of SRF, regulates phenotypic modulation of vascular smooth muscle cells and plays an important role in vascular remodeling**

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Myocardin-related transcription factor (MRTF)-A is a Rho signaling-responsive co-activator of serum response factor (SRF). In this study we investigated roles of MRTF-A in the process underlying vascular diseases by using two different mouse models of vascular diseases. MRTF-A expression was significantly higher in the wire-injured femoral arteries of wild-type mice and in the atherosclerotic aortic tissues of ApoE$^{-/-}$ mice than in healthy control tissues, whereas myocardin expression was significantly lower. In MRTF-A$^{-/-}$ mice, neointimal formation induced by wire injury in femoral artery was significantly smaller than that in MRTF-A$^{+/+}$ mice (neointima to media ratio: 2.09$\pm$0.17 in MRTF-A$^{+/+}$ vs 0.96$\pm$0.10 in MRTF-A$^{-/-}$, $P<0.05$). Diet-induced atherosclerotic lesions in MRTF-A$^{-/-}$; ApoE$^{-/-}$ mice was also significantly smaller than those in MRTF-A$^{+/+}$; ApoE$^{-/-}$ mice (% area of atherosclerotic lesions: 11.8% in MRTF-A$^{+/+}$; ApoE$^{-/-}$ vs 2.5% in MRTF-A$^{-/-}$; ApoE$^{-/-}$, $P<0.05$). Expression of vinculin, MMP-9 and integrin beta1, three SRF targets and key regulators of cell migration, in injured arteries was significantly weaker in Mkl1$^{-/-}$ mice than in wild-type mice. Knocking down MRTF-A using siRNA in cultured rat aortic vascular smooth muscle cells reduced expression of these genes, resulting in a significant impairment in PDGF-BB-induced migration. Underlying the increased MRTF-A expression in dedifferentiated vascular smooth muscle cells was the down-regulation of microRNA-1 concomitant with a decrease in myocardin expression. Collectively, these results demonstrate that MRTF-A plays a critical role in vascular remodeling by regulating vascular smooth muscle cells migration.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P308

**Association of PON-1 activity and other anti-oxidative enzymes with long-chain fatty acids profile in T2DM**

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Background
Serum paraoxonase-1 (PON-1) activity is decreased in clinical conditions associated with low high density-cholesterol (HDL-C), increased lipid peroxidation and low-grade chronic inflammation such as in type 2 diabetes mellitus (T2DM). Although many studies have shown that some poly unsaturated fatty acids (PUFA) modulate cardiovascular risk in T2DM, until now there is no data about the association of any fatty acid (FA) with PON-1 activity. This study aims to investigate this field.
Materials and methods
The 20 consenting adult T2DM patients, and 16 healthy controls were included in this cross-sectional study. Serum PON-1 activity, was measured together with other anti-oxidative enzymes activity, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), as well as plasma glucose level, HbA1c, lipids, high sensitive C-reactive protein (hs-CRP) and insulin resistance, homeostasis model assessment (HOMA-IR). The extraction and preparation of FA methyl esters and their GC analysis were performed too.
Results
HbA1c, plasma insulin, HOMA-IR and triglycerides were significantly higher in T2DM patients, whereas HDL-C was lower in those subjects. The levels of pro-oxidative enzyme malonildialdehyde (MDA), and the marker of chronic inflammation hs-CRP, were significantly higher in plasma of T2DM patients, and on contrary anti-oxidative enzymes SOD, and PON-1 activity were decreased in T2DM patients. Total PUFA and *n*-6 PUFA were significantly higher in T2DM patients, particularly linoleic acid (LA, 18:2 *n*-6) and arachidonic acid (AA, 20:4 *n*-6), whereas *n*-3 PUFA, particularly docosahexaenoic acid (DHA, 22:6 *n*-3) were significantly lower in T2DM patients. Stepwise multiple regression analysis have shown that only LA and DHA, independently predicted PON-1 activity of our subjects. LA, DHA and the presence of T2DM explained 70% of variance in PON-1 activity.
Conclusions
We have shown that decreased serum PON-1 activity may be in part influenced by higher level of LA and lower level of DHA in T2DM patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P309

**Risk factors of metabolic syndrome in adolescent with classical congenital adrenal hyperplasia**

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Background
Patients with classical congenital adrenal hyperplasia (CAH) require life-long therapy with glucocorticoids in order to suppress the androgen production through the inhibition of CRH and consequently ACTH. The therapy must be balanced to avoid either iatrogen hypercortisolism or hyperandrogenism both potentially associated with adverse cardiovascular risk profile.
Objective
Aim of this cross-sectional controlled study was to investigate the metabolic risk profile in children and adolescents with classical CAH.
Subjects and methods
Twenty CAH patients (ten males and ten females, age range 9–19 years) and 20 age- and sex-matched healthy controls underwent clinical examination and lipids, blood pressure, fasting glucose concentrations, serum insulin levels and HOMA evaluation. Adiposity was expressed as BMI SDS. Waist circumference (WC) and waist-to-hip ratio (WHR) were used to evaluate visceral adiposity.
Results
BMI (0.9$\pm$0.9 vs $-$0.13$\pm$1.53; $P=0.008$), WC (82.9$\pm$13.7 vs 72.77$\pm$13.6; $P=0.01$), fasting insulin levels (12.0$\pm$7.6 vs 5.1$\pm$5.08; $P=0.003$) and HOMA index (2$\pm$1.34 vs 0.98$\pm$1.03; $P=0.01$) were significantly higher in CAH patients compared to controls. A significant relationship was observed between WC and BMI SDS ($r=0.78$, $P<0.0001$), fasting insulin levels ($r=0.4525$, $P=0.04$) and HOMA ($r=0.45$, $P=0.04$). No differences between the two groups were observed for lipid profile and blood pressure.
Conclusions
Our data suggest that children and adolescents with classical CAH may have a cluster of metabolic abnormalities that may place them at an increased risk of metabolic syndrome and cardiovascular disease. WC seems to be a good predictor of these metabolic abnormalities and therefore it should be routinely measured in the follow-up of patients with classical CAH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P310

**Alterations of protein expression profiles in 3T3-L1 adipocytes by interleukin 4**

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Obesity, characterized by excess intra-abdominal adipose tissue, is closely associated with a cluster of metabolic disorders such as type 2 diabetes mellitus (T2DM). Our previous study revealed significant associations between interleukin 4 (IL4) genotypes and T2DM, as well as IL4 genotypes and high density lipoprotein-cholesterol levels. Our most recent study results reveal that IL4 has anti-hyperglycemic function by improving insulin sensitivity and glucose tolerance. In addition, IL4 is also involved in lipid metabolism by regulating serum levels of adipokines and free fatty acids. Taking the above results together, it strongly suggests that IL4 involves in the regulation of lipid and glucose metabolism. The aim of the present study is to identify differentially expressed proteins in 3T3-L1 adipocytes under IL4 treatment for further elucidating roles of IL4 in adipocyte metabolism. Differentially expressed proteins in mature 3T3-L1 adipocytes under IL4 treatment were identified by proteomic strategy. Levels of ATP synthase δ chain, cytochrome c reductase, pyrophsphatase and vimentin are increased, whereas levels of alpha-enolase, gelsolin, vinculin and valosin are down-regulated in the presence of IL4 stimulus. IL4 tends to potentiate the elevation of intracellular ATP levels and promote metabolism in adipocytes by up-regulating expression levels of proteins accelerating ATP synthesis. Our results suggest that metabolism of adipocytes is deviated to catabolism with an unfavorable condition for lipid storage by IL4. The lipids in adipocytes might either be released into periphery or metabolized intracellularlly, which in turn influence the systemic energy metabolism of other insulin-targeted organs.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P311

**Anti-Müllerian hormone may be a hormonal regulator of the cardiovascular system**

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Anti-Müllerian hormone (AMH, Müllerian inhibiting substance) is a protein hormone expressed by the Sertoli cells of the testes and the granulosa cells of the ovaries. AMH is best known for its paracrine functions in reproductive organ development and function. The hormonal roles of AMH are largely unexplored, but functions are emerging in growth and brain development. To date, there is no putative function for AMH in the blood of adults. AMH belongs to the TGF-β superfamily, and shares signalling components with the BMP sub-family, which contributes to the regulation of blood pressure and cardiovascular morphology. We therefore postulated that AMH may be a hormonal regulator of the vasculature.

The potential function of AMH was examined in a cross-sectional study, which correlated the levels of AMH in 153 healthy mature men to their cardiovascular status. This included measurement of the exterior diameter of the abdominal aorta by ultrasound (4–7 MHz).

The men's level of serum AMH negatively correlated with the external diameter of their aortas at both the mid-infrarenal and distal-infrarenal sites. The suprarenal aorta, in contrast, has limited capacity for remodelling and its diameter did not associate with the men's level of serum AMH ($r=-0.10$). In regression models, the association of AMH to aortic diameter ($r=-0.37$, $P<0.001$) was equal but opposite to the men's body surface area (BSA), which is the strongest known determinant of aortic diameter. The association between AMH and aorta was independent of the men's lipid profile, history of smoking and intimal thickness of their carotid artery, indicating that AMH may affect aortic diameter independent of atherosclerosis.

In conclusion, the results are consistent with AMH being a hormone in adults, with one of its functions relating to an aspect(s) of cardiovascular physiology, which directly or indirectly regulates the remodeling of the aorta.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P312

**Anti-oxidataive actions of urocortin on HL-1 cardiomyocytes**

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Cardioprotective actions of urocortin (Ucn) I have been proposed by several studies and may play a critical role on stress adaptation. And one of the beneficial actions on cardiovascular cells may be anti-oxidative actions. In addition, Ucn I may be regulated by oxidative stress, therefore, the present study is designed to reveal anti-oxidative actions of Ucns on cardiomyocytes. Mouse atrial cell line, HL-1 cardiomyocytes, was cultured in standard Claycomb medium supplemented with 10% fetal bovine serum and norepinephrine. Thereafter, HL-1 cardiomyocytes were further incubated in serum- and norepinephrine-free Claycomb medium containing 0.1% BSA with or without Ucn I, Ucn II, and astressin 2B, an antagonist of corticotroipin-releasing factor type 2 receptors (CRFR2) and oxidative stress was evaluated by measurement of conversion of 2′,7′-dichlorodihydrofluorescin diacetate to 2′,7′-dichlorodihydrofluorescein. Interestingly, Ucn I, but not Ucn II, suppressed oxidative stress of HL-1 cardiomyocytes without other stimulatory agents of oxidative stress. In addition, astressin 2B (10–6 mol/l) did not abolish the anti-oxidative action of Ucn I. It have been already reported that Ucn I exert anti-oxidative actions against angiotensin II-induced oxidative stress in human umbilical vein endothelial cells. Taken together with the present results, Ucn I may also exert cardioprotective actions via the anti-oxidative stress in cardiomyocytes, not via the CRFR2 signaling pathway.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P313

**Adipokine expression profile in pericoronary and periaortic adipose tissue in correlation with coronary and aortic atherosclerosis**

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Introduction
Adipose tissue is a source of peptides with paracrine and endocrine actions, called adipokines. Adipokines produced by periadventitial fat have been implicated in vascular pathology, including atherosclerosis. We investigated the expression of novel adipokines chemerin and vaspin, as well as visfatin, apelin and adiponectin in human pericoronary and periaortic fat in correlation with coronary and aortic atherosclerosis.
Methods
Paraffin embedded samples of human left coronary arteries ($n=40$) and abdominal aortas ($n=40$), including periadventitial fat, were evaluated for chemerin, vaspin, visfatin, apelin and adiponectin expression using immunohistochemistry. AHA classification was used for atherosclerosis assessment. PASW Statistics was used for statistical analysis.

Results
Chemerin, vaspin, visfatin, apelin and adiponectin expression of varied degree was detected in 40/40, 40/40, 40/40, 40/40 and 39/40 pericoronary fat samples and in 37/40, 40/40, 39/40, 40/40 and 37/40 periaortic fat samples respectively. Differences in expression of adipokines were observed between pericoronary and periaortic adipose tissue; visfatin showed higher expression in pericoronary fat ($P=0.001$), as did apelin ($P<0.001$). Atherosclerosis was detected in 37/40 coronary arteries and 34/40 aortas. Coronary atherosclerosis was negatively correlated with pericoronary fat adiponectin expression ($r=-0.496$, $P=0.001$) and positively correlated with pericoronary fat chemerin ($r=0.333$, $P=0.036$) expression. Aortic atherosclerosis was negatively correlated with periaortic adiponectin expression ($r=-0.701$, $P<0.001$) and positively correlated with periaortic chemerin ($r=0.824$, $P<0.001$), vaspin ($r=0.498$, $P=0.001$) and visfatin ($r=0.562$, $P<0.001$) expression. Pericoronary fat vaspin and visfatin expression was not associated with the severity of coronary atherosclerotic lesions. Finally, periadventitial apelin expression was not associated with atherosclerosis at any site.
Conclusions
Our results lend support to the concept of differential expression of adipokines at distinct sites of adipose tissue. Moreover, periadventitial fat may affect vascular function through local adipokine production and, more interestingly, might differently affect the atherosclerotic process in different locations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P314

**Fatty liver index and lipid accumulation product index as markers of liver steatosis in premenopausal women with nonalcoholic fatty liver disease**

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Background
Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognised disorder with an estimated prevalence of 20–30% in the general population and is considered as the hepatic manifestation of the metabolic syndrome. Fatty liver index (FLI) has been developed as an indicator of hepatic steatosis (HS). Another marker, the lipid accumulation product (LAP) index, initially developed to assess cardiovascular risk, has been recently shown to be a reliable marker of HS.
Objective
To evaluate the use of FLI and LAP markers for the identification of HS in premenopausal women with NAFLD.
Patients and methods
Forty apparently healthy overweight and obese (BMI: 26.0–46.9 kg/m$^2$) premenopausal women (age: 18–45 years) with a history of none or minimal alcohol consumption, were evaluated prospectively for the presence of NAFLD with abdominal ultrasonography and biochemical testing, after excluding causes of secondary liver disease. FLI was calculated using body mass index, waist circumference, serum triglycerides and gamma-glutamyl transferase levels and log-transformed LAP (lnLAP) was calculated using waist circumference and serum triglycerides levels. The diagnostic performance of FLI and lnLAP were assessed with receiver operating characteristic analysis.
Results
HS was detected in 22/40 women (55%) by ultrasonography. Metabolic syndrome was diagnosed in 9/22 HS(+) women (40.9%) and in 1/18 (5.6%) HS(−) women, $P=0.01$. FLI and lnLAP were higher in HS(+) compared to HS(−) women ($71.7\pm28.5$ vs $47.3\pm24.5$ ($P<0.01$) and $4.0\pm0.8$ vs $3.5\pm0.4$ ($P<0.01$) respectively). The best cut off for FLI to detect HS was $\geq$ 47 (sensitivity 77%, specificity 67%) and for lnLAP was $>3.5$ (sensitivity 82%, specificity 67%).
Conclusion
Calculation of FLI and lnLAP are useful for detecting patients at high risk for NAFLD. In our cohort of premenopausal women with NAFLD a cut-off level $\geq$ 47 for FLI and $\geq 3.5$ for lnLAP were more discriminative for HS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P315

**Effects of long-term treatment of hypogonadal men with testosterone undecanoate on blood pressure, fasting glucose, HbA1c and C-reactive protein**

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Introduction
Hypogonadism is associated with metabolic syndrome and all its individual components. This study analysed effects of normalization of serum testosterone in mainly elderly, hypogonadal men.
Methods
Prospective registry study of 252 men (mean age $60.6\pm8.0$ years), with testosterone levels between $\leq 3.5$ ng/ml. They received parenteral testosterone undecanoate 1000 mg at day 1, week 6 and every 12 weeks thereafter for up to 66 months.
Results
After 60 months the following changes were observed: systolic blood pressure declined from $153.74\pm17.61$ to $137.74\pm10.92$ mm Hg, diastolic blood pressure from $93.67\pm11.26$ to $79.61\pm7.35$ mm Hg. The changes were statistically significant vs baseline ($P<0.0001$). Both systolic and diastolic blood pressure reached a plateau within the second year of treatment.
Fasting glucose declined from $103.46\pm14.51$ to $97.54\pm2.34$ mg/dl. This decline was statistically significant vs baseline ($P<0.0001$), and a plateau was reached after $\sim$2 years. HbA1c was only measured in a subset of 123 patients at baseline and declined from $6.97\pm1.55$ to $6.01\pm1.41\%$ after 60 months which was statistically significant vs baseline ($P<0.0001$).
C-reactive protein (CRP) decreased from $6.3\pm8.01$ to $1.03\pm1.87$ mg/dl ($P<0.0001$ vs baseline).
Conclusions
Raising serum testosterone to normal for up to 66 months resulted in improvements in blood pressure, glycemic control, and CRP.
Declaration of interest
I fully declare a conflict of interest. Details below:
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## P316

**Dysregulation of tissue renin–angiotensin–aldosterone system in obese hypertensive rats**

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The activation of local renin–angiotensin–aldosterone system (RAAS) plays a pivotal role in the overall pathophysiology of the cardiovascular and renal diseases. Aldosterone can activate local renin and angiotensin converting enzyme (ACE). However, the effects of blockade of aldosterone on tissue RAAS including (pro)renin receptor and ACE2 are unclear in obese hypertensive rats induced by high-salt diet. Obese Zucker rats (ZOR) were fed with normal or high-salt diet for 8 weeks, and treated with eplerenone (100 mg/kg per day). ZOR fed a high-salt diet increased blood pressure (BP), kidney weight, urinary albumin excretion and urinary angiotensinogen excretion. Under these conditions, the rats exhibited decreased PRA and plasma aldosterone concentration and concomitant with increased expression of renal (pro)renin receptor protein and mRNA levels of angiotensinogen and ACE in the kidney. Treatment with eplerenone in ZOR fed a high-salt diet was associated with significant improvements in BP, kidney weight, urinary albumin excretion, urinary angiotensinogen excretion and decreased renal (pro)renin receptor protein expression and angiotensinogen and ACE mRNA levels. These results suggest that a high-salt diet increased renal RAAS, while blockade of aldosterone attenuated renal injuries by decreasing the activity of tissue RAAS in obese hypertension.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P317

### The relationship between 25(OH)D levels and markers of atherosclerosis and inflammation in type 2 diabetic patients

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Vitamin D deficiency has a leading role in endothelial functions and pathogenesis of type two diabetes and is supposed to take part in atherosclerosis. In this study we aimed to investigate the correlation between markers of atherosclerosis and inflammation with 25(OH)D levels in patients with type two diabetes. Forty patients with type two diabetes and macrovascular complication and 40 patients without macrovascular complication were included in the study. Thirty healthy subjects served as the control group. As markers of inflammation hsCRP, homocysteine, erythrocyte sedimentation rate, fibrinogen and adiponectin levels were studied. Carotid artery intima-media thickness (IMT) that is supposed to be a marker of atherosclerotic process was measured and 25(OH)D levels were studied in all subjects.
Levels of hsCRP, parathormone and body mass indices(BMI) were significantly higher in patients with 25(OH)D levels below 30 nmol/l than patients with 25(OH)D levels above 30 nmol/l. HsCRP, fibrinogen, erythrocyte sedimentation rate and homocysteine levels showed a negative correlation with 25(OH)D levels in all cases. Also, there was a negative correlation between waist circumference, BMI and 25(OH)D levels among diabetics. No significant difference between patients with 25(OH)D levels above or below 30 nmol/l was found for IMT, however IMT was significantly higher in diabetic patients. No significant difference was observed between patients with 25(OH)D levels above or below 30 nmol/l for levels of adiponectin, but there was a positive correlation betweeen adiponectin and 25(OH)D levels. There was no significant difference between diabetics with and without macrovascular complications regarding 25(OH)D levels
Levels of 25(OH)D is closely associated with markers of inflammation. Vitamin D deficiency also correlates with a predisposition to obesity and inflammation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P318

### Plasma total homocysteine levels in transsexuals after cross-sex hormonal treatment

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Introduction
The transsexuals are persons that having born with a biological sex feel to belong to the opposite sex. To obtain the sexual characters opposite to his (her) biological sex they need treatment with cross-sex hormones.
This treatment with sexual steroids can produce changes in cardiovascular risk factors (CRF). There has been described that hyperhomocysteinemia is an independent CRF that is modifiable by cross-sex hormonal treatment (CHT) in transsexuals but there is no agreement on the sense of these changes. We investigate the effects of CHT on plasma total homocysteine (Hcy) levels.
Material and methods
We measured at baseline and after 12 and 36 months of treatment with conjugated oral estrogens (2.4–3.6 mg/day), in combination with the antiandrogen, cyproterone acetate (100 mg/day), in 63 male-to-female transsexuals (MFT), aged 32.4 ± 8.5 years, and with testosterone gel (50 mg/day) in 79 female-to-male transsexuals (FMT), aged 29.0 ± 7.9 years. None had done gonadectomy.
Results
At baseline the levels of Hcy were significantly higher in the MFT group. In FMT, the plasma Hcy level increased from geometric mean 10.9 to 11.2 μmol/l ($P=0.032$) after 12 months, and to 11.8 μmol/l ($P=0.003$) after 36 months. In MFT, the plasma Hcy level decreased from geometric mean 11.7 to 10.6 μmol/l ($P=0.006$) after 12 months, on the contrary these levels increased to 12.5 μmol/l ($P=0.003$) after 36 months.
Conclusion
Hcy levels increase after androgen administration to female (transsexual) subjects, resulting in worsening of the cardiovascular risk in androgen-treated FMT. On the contrary after estrogens administration to male (transsexual) subjects, those decrease after 12 months but increase after 36 months. These changes may be due to the long-term different effects of testosterone and estrogens on metabolism of Hcy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P319

### Body composition and association to NT-proBNP and myostatin levels in heart failure patients

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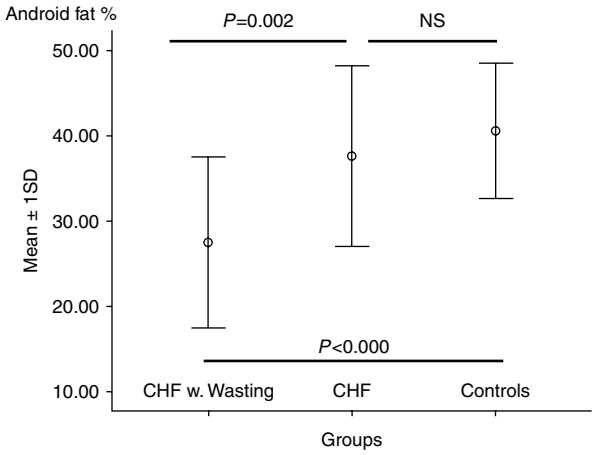
Objectivs
Plasma NT-proBNP is inversely associated with BMI in patients with heart failure (HF). A possible mechanism may be the lipolytic effect of natriuretic peptides on abdominal fat.
Myostatin is previously described as a negative regulator of skeletal muscle mass in cachexia.
We assessed the hypothesis that body composition is related to plasma NT-proBNP and that myostatin levels are associated with loss of fat free mass (FFM) in HF.
Methods
We included 57 patients (19 with systolic HF and cardiac cachexia, 19 HF patients without cachexia and 19 patients with prior MI). The groups were matched by age and gender, mean (S.E.M.) age 78.3 (1.0) years and 79% males. Body composition was assessed by DEXA, measuring FFM and fat mass as well as fat distribution.
Results
In univariate linear regression analyses abdominal fat % was inversely associated with NT-proBNP ($\beta=-0.30$; $P=0.025$) no association with gynoid fat % ($P=0.51$), FFM % ($P=0.20$) nor total body fat % ($P=0.12$) was observed. Multivariable linear regression analysis adjusted for well-established predictors of NT-proBNP (LVEF, kidney function, NYHA functional class and age) demonstrated no attenuation ($\beta=-0.28$; $P=0.018$) in this association.
No correlation between serum myostatin and fat free mass ($\beta=-0.058$; $P=0.67$) was observed. Mean (S.E.M.) myostatin was significant lower in the cachexia group (5.2 (0.3) ng/ml vs 6.2 (0.5) ng/ml and 6.7 (0.5) ng/ml, respectively; $P=0.036$).
Conclusions
Reduced abdominal fat mass was independently associated with high NT-proBNP, suggesting NT-proBNP could be related to the body composition alterations observed in HF patients with cachexia. The lack of association between myostatin and FFM was unexpected. However, the fact that myostatin was reduced among HF with cachexia raises the possibility that myostatin plays a secondary protective role in muscle loss in cachexia and has no primary involvement in pathogenesis.




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P320

### Subclinical atherosclerosis and arterial stiffness are associated with levels of circulating androgens in healthy recently menopausal women

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Introduction
Endogenous sex hormones may affect the cardiovascular risk profile in postmenopausal women indirectly by inducing metabolic, hemodynamic and immunological changes as well as directly through steroid receptors on the arterial wall. The results however, remain controversial.
Objectives
This cross-sectional study aims to examine associations between circulating sex hormone levels and indices of vascular function and structure.
Methods
One hundred and twenty healthy postmenopausal women, aged 41–60 years, were recruited. We performed biochemical and hormonal evaluation, including levels of FSH, LH, estradiol, testosterone, sex hormone-binding globulin, DHEAS, and Δ4-androstenedione. Indices of arterial structure and function included carotid and femoral intima-media thickness (IMT) and atheromatous plaques presence as well as flow-mediated dilation of brachial artery, carotid-femoral pulse wave velocity (PWV) and augmentation index (A.I.). Possible associations between circulating sex hormones and surrogate markers of vascular function and structure were investigated.
Results
Free androgen index predicted significantly mean levels of common carotid artery IMT ($\beta = 0.236$, $P$ value $= 0.014$) as well as levels of PWV ($\beta = 0.254$, $P$ value $= 0.027$) in multivariate models that included other confounders (e.g. age, smoking, BMI, HOMA-IR and blood lipids). Free estrogen index had a significant positive effect on PWV, but not independently of HOMA-IR and blood lipids. Age-adjusted levels of DHEAS exhibited a significant independent negative association with measures of A.I. ($\beta = -0.267$, $P$ value $= 0.029$). None of the remaining hormones associated with any of the vascular parameters independently of traditional cardiovascular risk factors.
Conclusion
Circulating testosterone is associated with both subclinical atherosclerosis and arterial stiffness in healthy recently menopausal women, independently of traditional cardiovascular risk factors or insulin resistance indicating a possible direct association. On the contrary, serum DHEAS exhibits a negative association with arterial stiffness. The documentation of elevated androgens as a risk factor for cardiovascular disease will have important implications with regard to primary prevention policies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P321

### Metabolic syndrome, hormone levels and inflammation in patients with erectile dysfunction

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Introduction
Chronic inflammation has been found to play an important role in the development of cardiovascular risk factors and erectile dysfunction (ED). Metabolic syndrome (MS) is associated with endothelial dysfunction and higher cardiovascular risk. The end point of this study was to investigate the prevalence of metabolic syndrome in patients with ED in comparison with control subjects and to analyse the association with acute phase reactants (CRP, ESR) and hormone levels.
Methods/Design
This case-control study included 65 patients, 37 with erectile dysfunction, according to the International Index of Erectile Function (IIEF) from the Urology Department of San Cecilio University Hospital, Granada (Spain), and 28 healthy controls. The prevalence of metabolic syndrome was calculated according to ATP-III criteria. Hormone levels and acute phase parameters were studied in samples drawn between 0800 and 0900 h after a rest period of $\geq 30$ min.
Results
The mean age of patients with ED was $55.8 \pm 7.7$ years vs. $52.5 \pm 4.8$ years in the control group ($P = 0.06$). The ATP-III criteria for MS were met by 64.9% of the patients with ED and only 9.5% of the controls ($P < 0.0001$, OR $= 17.53$, 95% CI: 3.52–87.37). Binary logistic regression analysis showed a strong association between patients with ED and MS, even after additional adjustment for confounding factors (OR $= 20.05$, 95% CI: 1.24–32.82, $P < 0.034$). Patients with hypogonadism presented a significantly higher prevalence of metabolic syndrome. Multiple linear regression analysis showed that systolic BP and CRP predicted 0.46 (model R2) of IIEF changes.
Conclusions
Chronic inflammation found in patients with ED might explain the association between ED and metabolic syndrome. Cardiovascular screening by MS criteria assessment in patients with ED may be useful to detect at-risk individuals and start preventive treatment against the development of cardiovascular disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P322

### Duration effect of statin therapy on bone metabolism in dyslipidemic patients

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Statins are cholesterol-lowering drugs decreasing bone resorption by inhibition of the farnesyl diphosphate synthase step in the mevalonic acid pathway and therefore are believed to have beneficial effects on bone status. Objective is to examine the relationship between lipid status and duration of statin therapy on the bone metabolism in dyslipidemic patients. A hundred and sixty subjects were divided into five groups depending on duration of statin therapy: (controls 0 years); (0.1–1.5 years); (2–5 years); (6–10 years); (11–30 years). ELISA method was performed on fasting serums using bone formation markers: osteoprotegerin (pmol/l) and osteocalcin (ng/ml) and bone resorption markers: s RANKL (pmol/l) and CrossLaps (ng/ml). For each bone marker and bone densitometry parametar, differences between statin groups were analyzed by repeated measures analysis of variance. Scheffe *post hoc* was used to identify specific differences between groups. Bone markers showed significant variation by duration of statin therapy ($F(16,268) = 2.49$; $P < 0.01$). The trend showed increase of bone formation markers and decrese of bone resorption markers with increasing duration of statin therapy. Bone mineral density (BMD g/cm$^2$) did not show significant changes due to statin therapy ($F(16,260) = 0.98$; $P = 0.47$). Clearly, statin therapy influences bone metabolism, which is observed by dynamics of bone markers changes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P323

### Coronary flow reserve is inversely related to urinary cortisol in Cushing's syndrome

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There is evidence for a strong association between Cushing's syndrome, characterized by a cluster of systemic complications, and increased

cardiovascular risk. To our knowledge, coronary circulatory function has not been explored in Cushing's syndrome. The aim of the study was to evaluate coronary flow reserve (CFR), an index of coronary microvascular function, in patients with Cushing's syndrome. Thirteen newly diagnosed patients with Cushing's syndrome (12 F/1M; mean age 42.3±9.9 years), were selected for having no clinical evidence of ischemic heart disease. There were 11 cases of pituitary-dependent Cushing's disease and two of cortisol-producing adrenal adenoma. Thirteen subjects matched for age, sex, and major cardiovascular risk factors were used as controls. Coronary flow velocity in the left anterior descending coronary artery was investigated by transthoracic Doppler echocardiography at rest and during adenosine infusion. CFR was obtained as the ratio hyperaemic/resting diastolic flow velocity. Mean CFR was similar in patients with Cushing's syndrome and controls (2.9±0.4 vs 3.1±1.4, *P* NS). A reduced coronary reserve (hyperaemic/resting ratio ≤2.5) was found in 4/12 (30.7%) Cushing patients and in 4/12 controls. In all patients with abnormal CFR epicardial coronary stenosis was excluded by multi-slice CT coronary angiography. CFR was inversely related to urinary cortisol levels in patients with endogenous hypercortisolism (Spearman's $\rho = -0.63$, $P = 0.02$) while no correlation was found in control subjects.
Conclusions
Coronary microvascular function, as assessed by CFR, is reduced in a considerable proportion of patients with Cushing's syndrome without clinical evidence of ischemic heart disease. The relationship between CFR and cortisol may contribute to explain the increased risk of cardiovascular mortality in Cushing's syndrome. Whether exposure to excess cortisol (possibly linked to duration of disease) may have per se a pathogenic role on coronary dysfunction, requires further studies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P324

**Myeloperoxidase is not associated with applications of coronary artery disease and type 2 diabetic patients**

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Myeloperoxidase (MPO), an enzyme of the innate immune system, exhibits pro-atherogenic effects and includes influence on oxidative damage to LDL- and HDL-cholesterol, and promotion of endothelial dysfunction.
The aim
To investigate the disturbance of MPO in type 2 diabetic (T2D) patients depending on intensity of atherosclerosis
Material and methods
We investigated 244 patients aged from 45 to 60 years (M±SD 54.0±4.63) including 67 T2D patients treated with oral antidiabetic drugs with coronary artery disease; 88 patients with applications of coronary artery disease only and 89 healthy persons. The activity of MPO in plasma were estimated with special spectrophotometric method using *O*-dianisidine and MPO inhibitor hydraside 4-aminobenzoic acid. MPO concentration estimated with original IFA method with MPO antibodies from immunizated rats and rabbits.
Results
We revealed standart lipid abnormalities in examined patients. The MPO concentration in T2D were 46.9 (18.05–73.00) ng/ml to compare patients with control group 35.31 (19.30–84.40) ng/ml and patients without diabetes 40.20 (40.20–40.20) ng/ml. There were no difference of MPO activity: in T2D 0.0041 (0.008–0.010) EU; in healthy persons 0.0034 (0.001–0.007) EU and 0.0012 (0.001–0.001) EU in patients with applications of coronary artery disease.
Conclusions
T2D is not associated with higher concentration or activity of MPO.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P325

**Analysis of hyponatremia in a tertiary hospital during a 5 year period**

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Aims
Hiponatremia is the most frequent electrolyte disturbance. Our aim is to retrospectively analyze the diagnosis of hyponatremia in the discharge sheets of a tertiary hospital during 5 years
Method
Data from the MBDS (Minimum Basic Data Set) of the discharged patients from the Hospital Clínico San Carlos (Madrid) between the years 2005 and 2009 was analysed. The 5th Edition of the ICD-9-CM was used for the codification of the diagnoses and procedures. The system of patient classification of diagnosis related groups – AP-DRG (version 21.0) – was used for the grouping of discharge processes. The Charlson index was use as a measure of comorbidity. Patients with a main or secondary diagnosis of hyponatremia were analyzed. Age, sex, length of stay, comorbidity, procedures and mortality were taken into account.
Resultados
Concerning destiny after discharge, 81.6% go home, 1.4% to another hospital and 2.8% to a nursing home. Mortality was 13.1 vs 4.3% among the general population. We found a statistically significant difference between hyponatremic patients and the rest concerning destiny to nursing homes (0.8 vs 1.6%) and in the univariate analysis for the mortality rate between hyponatremic patients and the rest: 13.1 vs 4.3%.
Discusion
The first important observation of our study is the extremely low prevalence of hyponatremia in our series (1.5%). In some series, mild hyponatremia has been reported to be 30 and 2.5% of patients show sodium levels below 125 mmol/l. We have observe an increased in mortality for hyponatremic patients; alter correcting for other confusing variables, patients with hyponatremia have 23% more possibilities of dying than dose with normal sodium levels. Our observations showed the importance of treating this frequently underdiagnosed disturbance and its negative prognostic implications on hospital admittances.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P326

**Effect of melatonin supplementation on lipid peroxidation and on the enzymes of antioxidative system in patients with chronic coronary artery disease.**

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Background
Melatonin as strong antioxidant and free radical scavenger can play important, protective role in disease in which oxidative damage is a significant component e.g.: in coronary heart disease, neoplastic disease, neurodegenerative disease. The aim of our study was determination of the effect of melatonin supplementation on malonyldialdehyde (MDA) concentration and activity of: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in patients with chronic coronary artery disease.
Material and methods
The study included 20 patients with CAD at the mean age of 63.9±8.7 years. Patients were divided into subgroups (diabetics, nondiabetics, smokers, nonsmokers). Each patient received 5 mg melatonin for the mean of 96.3 ±14.2 days. The material of study was blood, taken at 08.00 am at the beginning of the study and after 3 months of the melatonin administration. MDA concentration was determined in plasma and SOD, CAT, GSH-Px activity were determined in hemolysate.
Results
There were found statistically significant differences in SOD activity in patients with diabetes mellitus comparison to non- diabetes mellitus patients (6.06±3.05 i 3.82±1.19 IU/mg protein; $P = 0.026$) and higher MDA concentration in smokers comparison to non-smokers (3.23±2.9 i 2.32±0.36 nmol/ml $P = 0.006$). After 3 months of melatonin treatment increase GSH-Px from 53.06±15.85 IU/mg protein to 92.06±78.55 IU/mg protein ($P = 0.036$), increase SOD activity in non-diabetes mellitus group from 3.82±1.19 to 5.28±1.31 IU/mg Protein ($P = 0.002$) and in non-smokers group from 3.99±1.18 to 5.06±1.20 IU/mg Protein

($P=0.014$) were found. In addition, decrease of MDA concentration were observed, from $2.85\pm0.98$ to $1.18\pm0.29$ nmol/ml ($P=0.017$), in Patients with diabetes mellitus.
Conclusions
The antioxidative Properties of melatonin were confirmed in the study. The supplementation of melatonin may be useful as a supportive therapy in Patients with chronic coronary artery disease.
Key words: melatonin, oxidative stress, coronary disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P327

**N-terminal pro-brain natriuretic peptide (NT-proBNP) in patients with non-ST-elevation myocardial infarction (NSTEMI) compared to patients with unstable angina (UA) in a lot of patients admitted for non-ST-elevation acute coronary syndrome (NSTE-ACS)**

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Introduction
The study aimed to evaluate the differences in secretion of NT-proBNP and cardiac troponin T (cTnT), in patients with NSTE-ACS for the early detection of myocardial ischemia in NSTE-ACS.
Methods
Hundred and sixteen patients admitted for non-ST-elevation ACS (NSTE-ACS), 71(61.2%) men, mean age $68.7\pm10.2$ were prospectively investigated. Plasma NT-proBNP and cTnT were measured by electrochemiluminescence (Elecsys/-Cobas e, Roche Diagnostics) on admission. Study population was divided into two groups, based on clinical findings, 12-lead electrocardiography (ECG), 2D echocardiograpy (ECHO) and the plasma cut-off level of cTnT $=0.1$ ng/ml, as follows: group 1, consisiting of $n=34$ (29.3)% patients with NSTEMI (cTnT $\geq$ 0.1 ng/ml) and group 2, including $n=82$ (70.7)% patients with UA (cTnT $<0.1$ ng/ml). Study protocol was approved by the local Ethics Committee and each patient enrolled signed an informed consent. Statistics: SPSS 16.0.; MedCalc 11.4.4.
Results
Plasma levels of NT-proBNP were significantly higher in patients with NSTEMI (group 1) compared to patients with UA (group 2): 1115 (235.7; 2289.5) vs 155.6 (60.4; 313.6) pg/ml, $P<0.0001$ values expressed as median (25th; 75th percentile). NT-proBNP did not significantly correlate to cTnT ($R=0.3$, $P=0.1$). The area under the receiver operating characteristic curve (AUC) for NT-proBNP in discriminating patients with NSTEMI from those with UA was 0.68 (95% Confidence Interval C.I. 0.55 - 0.81), $P < 0.001$.
Conclusion
Plasma levels of NT-proBNP was significantly higher in patients with non-ST-elevation acute myocardial infarction compared to patients with unstable angina, but did not significantly correlate with plasma levels of cTnT in our study group of patients with non-ST-elevation acute coronary syndrome.
This work is part of the Scientific Research Project No. 17678/2010-2011, supported by Roche Diagnostics.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P328

**Effect of medical therapy on 24-h blood pressure profile in acromegaly**

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To investigate the blood pressure profile (BP) in active acromegaly and the effect of medical treatment of acromegaly on BP circadian rhythm, we studied 21 acromegalics before and after 3–60 months of treatment with somatostatin analogs (15/21) or pegvisomant (5/21) or both (5/21). Ambulatory 24-h BP was recorded. The 24-h mean BP (MBP), the mean systolic BP (SBP) and diastolic BP (DBP), the day-time (day) and night-time (night) MBP, SBP and DBP and the 24-h mean heart rate (HR) were evaluated. At baseline, 11 patients were normotensive (NT) and ten were treated with antihypertensive drugs. In hypertensive (HT), mean night BP was higher than in NT ($P\leq0.01$). The BP circadian rhythm was abnormal in 62% of active acromegalics with no difference between the two groups. No correlation between BP and GH or IGF1 was found in HT acromegalics. After therapy, acromegaly was controlled (C) in 12 patients (6 NT and 6 HT) whereas in nine (5 NT and 4 HT) was not controlled (NC). After therapy, 24-h and night MBP and 24-h, day- and night DBP were lower ($P\leq0.05$, $P\leq0.01$) in HT and NC patients whereas SBP, MBP and DBP were increased ($P\leq0.05$, $P\leq0.01$) in NT and NC acromegalics. No difference before and after treatment was found in the C group although three patients reduced or discontinued the antihypertensive drugs after IGF1 normalization. After therapy, the BP circadian rhythm was disturbed in 47% of all patients and in 60% of NC. The 24-h mean HR was lower either in HT ($P=$NS) or NT ($P\leq0.05$) after treatment regardless of disease control. Active acromegaly is associated with alterations of 24-h BP profile; control of acromegaly may reduce the risk to develop hypertension and is associated with improvement of several BP parameters.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P329

**Polycystic ovary syndrome induces leukocyte/endothelial cell interactions**

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Insulin resistance is a feature of polycystic ovary syndrome (PCOS) and is related to mitochondrial and endothelial function.We tested whether hyperandrogenic insulin-resistant women with PCOS, who have an increased risk of vascular disease, display impaired leukocyte-endothelium interactions, and mitochondrial dysfunction. This was a prospective controlled study conducted in an academic medical center.
The study population consisted of 43 lean reproductive-age women with PCOS and 39 controls subjects.
We evaluated anthropometric and metabolic parameters, adhesion molecules, and interactions between leukocytes and human umbilical vein endothelial cells. Mitochondrial function was studied by assessing mitochondrial oxygen consumption, membrane potential, reactive oxygen species production, glutathione levels (GSH), and the oxidized glutathione (GSSG)/GSH ratio in polymorphonuclear cells.
Impairment of mitochondrial function was observed in the PCOS patients, evident in a decrease in oxygen consumption, an increase in reactive oxygen species production, a decrease in the GSH/GSSG ratio and GSH levels, and an undermining of the membrane potential. PCOS was related to a decrease in polymorphonuclear cell rolling velocity and an increase in rolling flux and adhesion. Increases in IL6 and TNF$\alpha$ and adhesion molecules (vascular cell adhesion molecule-1 and E-selectin) were also observed.
This study supports the hypothesis of an association between insulin resistance and an impaired endothelial and mitochondrial oxidative metabolism. The evidence obtained shows that the inflammatory state related to insulin resistance in PCOS induces a leukocyte-endothelium interaction. These findings may explain the increased risk of vascular disease in women with PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P330

**Effect of weight loss on C3 and C4 components of complement in obesity**

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Circulating C3 levels are elevated in obese patients, but how this factor is affected after weight loss through diet is a question that is yet unanswered. Therefore, the aim of this study was to evaluate the effects of weight loss on lipid and hydrocarbonated metabolism parameters and on the levels of C3 and C4 components of complement in obese patients. This is a longitudinal intervention study based on a 6 week very low calorie diet (VLCD), a liquid formula of 603 kcal/day. A total of 131 middle-aged patients were distributed among grades II, III and IV of obesity. Anthropometric parameters, total cholesterol, triglycerides, high-density lipoprotein cholesterol, LDLc, apolipoproteins A-I and B-100, glucose, insulin, HOMA-IR and C3 and C4 levels were evaluated at baseline and after 6 weeks of intervention. After VLCD, the moderate weight loss was accompanied by a significant reduction in C3 levels in grade III and grade IV patients (10.2 and 15.4%, respectively; $P<0.001$). C4 levels were not altered. Adherence to the diet improved anthropometric parameters and was accompanied by a significant decrease in all lipid profile parameters ($P<0.001$). In addition, weight loss was associated with an improvement in hydrocarbonated metabolism as shown by the decrease in glucose levels and HOMA-IR ($P<0.01$). Our findings show that in severely obese patients following a VLCD for 6 weeks produces reductions in factor C3, a biomarker of cardiovascular disease, and a significant improvement in some features of metabolic syndrome. In this way, the abovementioned diet may represent an effective strategy for treating obesity and related cardiovascular risk factors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P331

**Vitamin D serum levels and cardiovascular risk factors in postmenopausal women**

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Results of several clinical studies have suggested that there is an excess risk of cardiovascular disease in persons with vitamin D deficiency.

We examined association between serum levels of vitamin D - 25(OH)D and select cardiovascular risk factors in 254 postmenopausal women at a mean age of $55.38 \pm 205$ years.

Vitamin D levels, lipoproteins, TNF-$\alpha$, Interleukin 6, adiponectin, insulin (using RIA methods) and glucose were assessed in fasting blood samples of healthy postmenopausal women. Total body fat mass was measured by dual energy X-ray absorptiometry.

The mean vitamin D level was $24. \pm 11.5$ ng/ml. The group with vitamin D level $<15$ ng/ml vs group with vitamin D level $\geq 40$ ng/ml demonstrated significant elevated percent of total body fat ($P<0.033$), higher levels of Interleuki $n$ 6 ($16.12 \pm 5.3$ vs $14.03 \pm 3.16$ pg/ml, $P< 0.08$) and decrease level of adiponectin ($12.93 \pm 7.4$ vs $15.83 \pm 6.8$ µg/ml, $P<0.036$). An inverse correlations between vitamin D and % of total body fat ($P<0.0004$, $r=-0.198$, $n=254$) and interleukin 6 ($P<0.017$, $r=-0.147$, $n=254$) were observed in total group.

Vitamin D deficit is associated with cardiovascular risk factors in postmenopausal women.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P332

**Sympathetic over-activity and low adrenocortical responses to hypoglycemia in patients in early stage of hypertension**

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Increased activity of sympathetic nervous system (SNS) was observed in early stage of hypertension onset. The aim of our present study was to estimate catecholamines response to stress stimulus (insulin induced hypoglycaemia) in young males with early diagnosed non-treated hypertension grade 1 (HT) and normotensive controls (NT).

Methods

Insulin tolerance test (ITT, 0.1 IU/kg body weight, Actrapid HM, i.v.) was performed in 21 HT male subjects aged $20.0 \pm 0.6$ (mean $\pm$ S.E.M.) years with BMI $22.0 \pm 0.5$ kg/m$^2$ and in 19 NT males aged $23.1 \pm 1.0$ years with BMI $22.8 \pm 0.4$ kg/m$^2$. Concentrations of glucose, epinephrine, norepinephrine, plasma renin activity (PRA), growth hormone (GH), prolactin (PRL), adrenocorticotropic hormone (ACTH) and cortisol were determined in venous plasma.

Results

Increased baseline levels of norepinephrine ($P<0.05$), increased response of norepinephrine ($P<0.001$) and decreased response of GH ($P<0.001$), PRL ($P<0.001$), ACTH ($P<0.05$) and cortisol ($P<0.001$) to hypoglycemia were found in hypertensive patients when compared to normotensive controls. We did not observe differences in PRA baseline concentration or response to insulin induced hypoglycemia.

Conclusions: The early stage of onset of hypertension (in normal weight and young patients) is associated with sympathetic overactivity and decreased pituitary response to metabolic stress stimulus. These changes may also contribute to metabolic cardiovascular risk factors.

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P333

**The effect of thyroxine replacement on reduction of small dense low-density lipoprotein (sdLDL) in patients with primary hypothyroidism.**

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Background

Dyslipidemia is a well-known manifestation of thyroid dysfunction and there is evidence of an association between hypothyroidism and coronary heart disease. Recently, small dense low-density lipoprotein (sdLDL) has been linked with development of cardiovascular disease. But relations between thyroid function and sdLDL remain incompletely understood.

Objective

Our objective was to determine whether replacement therapy with thyroxine for patients with hypothyroidism is associated with improvement of lipid profiles including sdLDL.

Design

We conducted a prospective clinical study. Nine patients with primary hypothyroidism were enrolled in this study and treated by replacement with thyroxine. Lipid profiles, sdLDL, TSH, thyroid hormone were measured in pre- and post-treatment blood samples.

Results

Seven patients (3 males and 4 females with Hashimoto's disease) of the patients completed this study and mean duration of treatment that normalized TSH was 126 days (85–153 days). The pre-/post-treatment TSH, free T4 (fT4), LDL cholesterol (LDL), sdLDL and sdLDL/LDL ratio were $181.0 \pm 219.2/7.3 \pm 7.5$ µIU/ml, $0.4 \pm 0.2/1.3 \pm 0.3$ ng/dl, $160.7 \pm 61.1/121.8 \pm 25.6$ mg/dl, $26.1 \pm 16.8/14.7 \pm 4.7$ mg/dl, $0.157 \pm 0.047/0.138 \pm 0.043$, respectively. TSH and sdLDL were significantly decreased and fT4 was significantly increased after treatment.

Conclusion

The present study demonstrated a reduction of sdLDL levels of patients with hypothyroidism after treatment associated with an increase in thyroid hormone,

which may contribute to some cardiovascular alterations. Even though we found a reduction in sdLDL levels after treatment, the mechanism of this effect was still not well understood.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P334

**Metabolic consequences of obesity in children and adolescents**

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Introduction

The prevalence of obesity in children and adolescents has dramatically increased over the last 20–30 years.

Childhood overweight is associated with higher risk of obesity into adulthood and a higher incidence of metabolic consequences.

Objectives

To identify the prevalence of metabolic complications in obese children and adolescents.

Methods

One thirty-two obese children and adolescents (age over 10) admitted to our clinic over last two years were considered for participation in a transversal observational study.

There were performed auxological measurements (weight, height, abdominal circumference), clinical evaluations (blood pressure), bone age, blood samples for morning glucose, a 2 h glucose tolerance test, insulinemia, glicated haemoglobin, total cholesterol and its fractions, serum triglycerides.

Results

There were 65 girls and 67 boys, aged 10 to 18 years.

The metabolic syndrome (IDF consensus, Lancet 2007) was found in 31.8% of the obese children and adolescents.

Independent of their age, the independent risk factors for metabolic and cardiovascular disease had a high prevalence among the study' s subjects: impaired fasting glucose in three cases, an altered glucose tolerance in another four cases; 35.6% of the patients had biochemical criteria of insulinresistance (basal insulin levels $>24$ μUI/ml or HOMA-IR $>5.6$); 27% of patients had dyslipidemia; 8% of patients had serum levels of TGP slightly altered, suggesting steatosis; 30% of patients had high blood pressure.

The glycated haemoglobin values were not altered in any of our patients, suggesting a week correlation to the metabolic complications.

Conclusions

Metabolic consequences have a high rate of prevalence in obese children and adolescents (minimum 30% of cases).

Key words: obesity, children, metabolic syndrome

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P335

**Study on the DHEA-S secretion dynamics in elderly pacients with cardiovascular diseases**

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Background

The general population trend shows an increase in the elderly. The most affected systems in the aging process are the endocrine and cardiovascular systems. The cardiovascular conditions are very important among the diseases of the elderly, the mortality due to cardiovascular diseases holding the first place in geriatrics.

The aim of the study is to determine the peculiar aspect of dehydroepiandrosterone sulfate secretion dynamics in the elderly and correlate this hormonal parameter with the cardiovascular diseases, in order to realize ways to improve the early diagnosis of these patients.

Material and methods

The study group consists of 135 subjects aged over 65 years old divided in two subsets: the control subset comprising 66 healthy elderly and the subset of elderly diagnosed with various cardiovascular diseases (69 patients). The cases were investigated by clinical and paraclinic examination and the results were statistically processed.

Results

Serum dehydroepiandrosterone sulfate values in the subjects aged over 65 years old were normal, situated in the lower of the normal range. The patients with cardiovascular diseases showed a less DHEA-s average by about 50% compared to the one in the healthy subjects.

Conclusions

Healthy subjects have shown a decreasing trend in the DHEA-S levels with their aging. Diseases such as arterial hypertension, coronary ischemic heart disease, heart failure and atrial fibrillation might influence the DHEA-S secretion. The altered serum DHEA-S levels could become predictive factors for cardiovascular diseases.

Key words:DHEA-S, elderly, cardiovascular diseases.

Declaration of interest

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## P336

**Testosterone and androstane-3α, 17β-diol glucuronide correlate with coronary disease**

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The relationship between endogenous testosterone concentrations and coronary disease in men is controversial. Recently, it was demonstrated that the glucuronide metabolite of dihydrotestosterone (DHT), androstane-3α, 17β-diol (3-DIOL-G), but not testosterone (T), is strongly associated to several metabolic risk factors (fat mass, insulin, HOMA index, HDL, triglycerides, ApoA1, etc.) in young and elderly men. The object of the present trial was to evaluate the plasma levels of androgens and 3-DIOL-G in middle-aged men with coronary disease in comparison with a control group, independently of some risk factors.

Twenty-five patients (40–65 years old) with a history of cardiovascular disease (infarction, angina, etc.) were matched according to age, lipid status, diabetes and BMI with 33 men (42–63 years old) with no cardiovascular pathology.

Plasma levels of testosterone, 3-DIOL-G, estradiol, glucose and insulin, as well as the 3-DIOL-G/T ratio and HOMA index were determined.

The coronary group showed lower testosterone levels than the control group ($3.1\pm1.0$ vs $4.0\pm1.3$ ng/ml, $P<0.02$) but 3-DIOL-G level and 3-DIOL-G/T ratio were significantly higher ($6.7\pm3.1$ vs $4.8\pm2.8$ ng/ml, $P<0.02$ $\pm$and 2.5 $\pm1.4$ vs $1.3\pm0.8$, $P<0.001$, respectively). Besides, the insulin level and HOMA index were higher in the coronary group ($10.7\pm6.1$ vs $6.5\pm3.2$ mUI/ml, $P<0.002$ and $3.2\pm2.1$ vs $1.6\pm0.8$, $P<0.004$, respectively). Finally, there was an inverse correlation between insulin and testosterone ($r=-0.39$, $P<0.04$) while there was a direct correlation between insulin and the HOMA index with 3-DIOL-G/T ratio ($r=-0.43$, $P<0.01$, $r=0.39$, $P<0.04$, respectively).

The present results indicate that patients with history of coronary disease have higher plasma levels of 3-DIOL-G than controls, independently of their BMI. Unlike testosterone, the metabolite of the potent DHT androgen, is a steroid that correlates with parameters of insulin-resistance. Future studies are required to determine its participation on the development of coronary disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P337

### Cardiovascular function and IGF1 deficit (IGF1-D) in β-thalassemia major

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Introduction
Both GH and IGF1 have important effects on the heart mass and contractility and deficiency of either hormone is associated with increased cardiovascular morbidity and mortality. GH/IGF1 axis abnormalities (mostly primary or secondary IGF1-D) are frequent in adult patients with β-thalassemia major (β-Thal). IGF1-D could therefore contribute with iron overload to the heart's damage of β-Thal patients, but this question has been little investigated so far.
Purpose
Retrospective evalutation of cardiovascular function by echocardiographic parameters in a cohort of 80 patients with β-Thal, 55 F and 25 M, aged 31–52 years, subdivided according to blood levels IGF1 using a cutoff of 70 ng/ml, indicative of marked IGF1-D for the entire age range.
Results
Patients with IGF1 $<70$ ng/ml showed significantly larger left atrium (LA), left ventricle (LV) and, to a lesser extent, right ventricle (RV) diameters (Table). No significant difference was found between the two groups in aorta diameter, ejection fraction (EF) and ferritin levels. The cumulative prevalence of several cardiac complications (atrial fibrillation, atrioventricular block, right or left bundle branch block, heart failure and cor pulmonale) was significantly ($P<0.01$) higher (25 in 53 patients) in patients with IGF1 $<70$ ng/ml, when compared to those with IGF1 $>$ 70 ng/ml (4 in 28 patients).
Conclusions
Our data provide evidence for a negative effect of IGF1-D on heart of β-Thal patients. These data provide the basis for further studies and suggest a potential indication for treatment with rhGH and/or IGF1 in β-Thal patients with low serum IGF1.

**Table 1**

| Patients ($n=80$) | Ferritin (ng/ml: m±s.d.) | EF (%: m±s.d.) | LA (mm: m±s.d.) | LV (mm: m±s.d.) | RV (mm: m±s.d.) | AORTA (mm: m±s.d.) |
|---|---|---|---|---|---|---|
| IGF1 $<70$ ng/ml ($n=53$) | 1004.1± 784.4 | 64.3±6.7 | 38.1±5.1 | 49.0±5.2 | 19.8±7.0 | 30.0±4.1 |
| IGF1 $>70$ ng/ml ($n=27$) | 769.7± 521.8 | 66.4±5.0 | 33.9±5.3 | 46.1±5.0 | 16.6±6.3 | 28.8±3.0 |
| $P$ | 0.15 | 0.15 | 0.0004 | 0.014 | 0.032 | 0.18 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P338

### Psoriasis is associated with decreased adiponectin levels beyond cardiovascular and metabolic risk factors

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Introduction
Psoriasis is a systemic inflammatory skin disease, which is associated with increased cardiovascular disease (CVD) potentially through metabolic derangements due to chronic inflammation. Serum adiponectin appears to decrease the risk of CVD and has been shown to negatively associate with waist size and insulin resistance (IR), both of which are increased in psoriasis. It is unknown, however, if psoriasis is associated with decreased adiponectin levels beyond traditional CVD and metabolic risk factors.
Methods
We prospectively enrolled a consecutive sample of patients with psoriasis ($n=122$) and compared cardiometabolic risk factors with an asymptomatic sample without psoriasis from our practice ($n=129$). Fasting lipids, insulin, glucose, and total plasma adiponectin were measured by commercial assays. HOMA-IR was used to estimate IR. We performed stepwise multivariable linear regression adjusting for CVD (age, gender, smoking status, hypertension, lipids) and metabolic (HOMA-IR, waist size, triglycerides) risk factors using STATA12 software.
Results
Psoriasis patients were more insulin resistant (HOMA-IR: 3.5 (2.3–6.6) vs 1.4 (0.94–2.1), $P<0.001$) and had higher waist sizes (40 (35–44) vs 35.5 (33–39.5), $P<0.001$) than patients without psoriasis. Total serum adiponectin was lower in psoriasis than controls (7.1 (4.9–11.3) vs 14.5 (8.4–24.2), $\beta=-0.59$, $P<0.001$), even after adjustment for CVD ($\beta=-0.56$, $P<0.001$) and metabolic variables ($\beta=-0.49$, $P<0.001$).
Conclusion
Total serum adiponectin levels are lower in psoriasis patients compared to healthy controls, even after adjustment for CVD and metabolic risk factors. These results suggest that decreased adiponectin levels are a unique feature of psoriasis pathophysiology, which may play a role in the increased risk of CVD. Larger prospective studies looking at CVD outcomes and effects of psoriasis treatment on adiponectin are warranted.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P339

### The importance of HDL cholesterol to predict adiponectin and retinol binding protein 4 concentration after one session resistance exercise

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Introduction
The capability of exercise to induce useful effect on cardiovascular health could be partly attributed to its concomitant effect on adipokines and HDL cholesterol. Little information is available on whether post exercise concentration of these adipokines could be influenced by HDL cholesterol. Thus this study was designed to examine the possible independent effect of HDL cholesterol on serum level of adiponectin and retinol binding protein 4 following one session resistance exercise in healthy young men.
Method
Thirty-four healthy young BMI- matched male students (age, 20–26 years) were recruited. The study participants were allocated to experimental ($n=18$) and control ($n=16$) groups by using black balanced randomization method. The experimental group underwent a 120 minutes intensive resistance exercise session. Blood samples were taken at baseline and after 4 hours of training program to measure RBP4, adiponectin, HDL cholesterol and other cardio metabolic markers.
Results
The serum level of adiponectin, RBP4 and HDL cholesterol was not significantly different between two groups at baseline. We found that, after exercise concentration of adiponectin were negatively correlated with basal RBP4 and TG and positively correlated with basic level of adiponectin and HDL cholesterol.
Post exercise concentrations of RBP4 were negatively correlated with adiponectin and positively correlated with basal RBP4, age and BMI. Subsequent adjusted regression model showed HDL cholesterol ($\beta=0.28$, $P=0.03$) were the strong predictor of post exercise adiponectin concentration, explaining 28% of its variability. Whereas the basic level of RBP4 appeared to be the only predictor of after exercise RBP4 concentration ($\beta=0.46$, $P=0.02$) and HDL cholesterol has no role in this regard.
Conclusion
This study showed, HDL cholesterol, which was responsible for noticeable proportion of post exercise adiponectin variation, appeared to be a strong predictor of adiponectin but not for RBP4 following one session intensive resistance exercise in healthy young men.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P340

### Intrinsic myocardial disease in adults with GH deficiency is characterised by subclinical left ventricular longitudinal dysfunction revealed by tissue Doppler

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Purpose
GH deficiency (GHD) is associated with increased cardiovascular events, however, the detailed mechanisms have not been assessed yet extensively. We set up this study in order to evaluate cardiac, arterial, and endothelial function, by conventional

echo, TDI, and biomarkers (proBNP and troponin I), in GHD patients by comparison with normal individuals with similar cardiovascular risk factors profile.
Methods
Thirty GHD patients (46±14 years, 18 males) with low cardiovascular risk factors were compared with 30 matched N. Global LV systolic and diastolic functions were assessed from ejection fraction (EF), indexed cardiac output (COi), E/Vp and E/Ea ratios; longitudinal function from global longitudinal strain (GLS), and the sum of all times from the AVC to peak strain (SumTAVC); radial function from global radial strain (GRS); circumferential function from global circumferential strain (GCS); and LV torsion from peak basal (RotB) and apical rotation (RotA), and derived times (time to RotB/RotA), LV torsion (LVtor), twist rate (TR), untwist rate (UTR), time-to-peak twist (TT), and time from the AVC to UTR (AVC-UTR). Arterial function was assessed from intima-media thickening (IMT) , local wave speed (LWS), and stiffness index (β); endothelial function from flow mediated dilation (FMD).
Results
GHD patients had all global, longitudinal, and circumferential systolic parameters significantly decreased, but with similar GRS (52±16 vs 52±15%) (Table). They had also higher dyssynchrony, end-diastolic LV pressure, and lower torsion with prolonged AVC-UTR. RotB correlated with GLS ($r=0.4$, $P<0.05$). Arterial and endothelial functions were similar to N.

**Table 1**

| | EF | COi | E/Vp | E/Ea | GLS | Sum-TAVC | GCS | RotB | LVtor | TR |
|---|---|---|---|---|---|---|---|---|---|---|
| GHD | 52±9 | 1.8±0.5 | 1.7±0.4 | 8±1 | −16.8±2.5 | 64±5.0 | −16.5±3.3 | −5.2±2.5 | 0.18±0.05 | 100±32 |
| N | 68±7 | 2.2±0.6 | 1.5±0.3 | 6±2 | −19.2±2.5 | 7±5 | −19.4±4.0 | −6.7±2.0 | 0.23±0.08 | 122±42 |
| *P* value | <0.001 | 0.03 | 0.033 | 0.002 | 0.001 | 0.003 | 0.004 | 0.02 | 0.03 | 0.027 |

Conclusions
GHD patients have subclinical longitudinal and circumferential LV dysfunction, with maintained radial function; these were correlated with impaired LV torsion. Since arterial and endothelial functions were not affected, our findings suggest that patients with GHD have intrinsic myocardial disease, due probably to insufficient development of the myocardial fibers.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P341

### Paraoxonase, malondialdehyde and lipids in subjects with asymptomatic gallstones

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Aim
Gallstones are associated with excess body fat. Our aim was to evaluate lipid levels and markers of oxidative stress in subjects with asymptomatic gallstones.
Material and method
Eighty subjects (55 females, 25 males) with a mean age±S.D. of 51±14 years with asymptomatic gallstones were admitted to the study. Forty subjects (25 females, 15 males) with a mean age±S.D. of 57±12 years ($P<0.05$) without gallstones were enrolled as controls. Lipid levels were determined by routine methods. Serum paraoxonase (PON1) activity was measured spectrophotometrically. Malondialdehyde (MDA) level was determined by thiobarbituric acid method.
Results
Subjects with cholelithiasis was younger than control subjects, but there was no difference in body mass index (BMI) (27±2 kg/m$^2$ in both groups, $P=0.97$). Total cholesterol (TC) and MDA levels were significantly higher and HDL-C, PON1 ($P<0.01$) and LDL-C ($P<0.05$) levels were significantly lower in subjects with asymptomatic cholelithiasis than in subjects without cholelithiasis. Subjects with cholelithiasis who had impaired fasting glucose (glucose>100 mg/dl, $n=39$) had significantly higher serum triglyceride (TG), TC ($P<0.05$) and MDA levels ($P<0.01$) than subjects with cholelithiasis who had normal fasting glucose (glucose<100 mg/dl, $n=41$). PON1 activity was significantly lower in cholelithiasis patients with impaired fasting glucose ($P<0.01$). Subjects with cholelithiasis who had hypertriglyceridemia (TG>150 mg/dl, $n=32$) were older ($P<0.05$), had higher BMI, glucose, TC and MDA levels and lower HDL-C and PON1 activity ($P<0.01$) than subjects with cholelithiasis who had normal TG levels (TG< 150 mg/dl, $n=48$).
Conclusions
Patients with asymptomatic cholelithiasis have higher levels of total cholesterol and lower levels of HDL-C compared to subjects without cholelithiasis and have increased lipid peroxidation and decreased antioxidant capacity. Patients with asymptomatic cholelithiasis who have impaired fasting glucose or hypertriglyceridemia have more lipid peroxidation and less antioxidant capacity than subjects with asymptomatic cholelithiasis who do not have impaired fasting glucose or hypertriglyceridemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P341.1

### Cardiac hormones are dual inhibitors of vascular endothelial growth factor and the VEGFR2 receptor

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Introduction
The growth of human tumors depends on the formation of new blood vessels and vascular endothelial growth factor (VEGF) helps control this process via inducing new capillaries to sprout from pre-existing blood vessels. Four cardiac hormones which eliminate up to 80% of human pancreatic cancers and up to 86% of human small-cell lung cancers growing in mice were investigated for their effects on VEGF and the VEGFR2/KDR/Flk-1 receptor. The VEGFR2 receptor is the main receptor mediating VEGF's cancer enhancing effects.
Methods
The cardiac hormones' effects on VEGF/VEGFR2 levels in three human cancer cell lines were measured by ELISAs.
Results
These four cardiac hormones, i.e. vessel dilator, long-acting natriuretic peptide (LANP), kaliuretic peptide, and atrial natriuretic peptide (ANP) over a concentration range of 100 pM to 10 μM maximally decreased the VEGFR2 receptor in human pancreatic adenocarcinoma cells by 48, 49, 74, and 83%. Vessel dilator, LANP, kaliuretic peptide, and ANP decreased the VEGFR2 receptor by 77, 89, 88, and 67%, respectively in human small-cell lung cancer cells. Maximal decrease of VEGFR2 receptor in human prostate cancer cells by vessel dilator, LANP, kaliuretic peptide, and ANP was 48, 92, 64, and 71%. VEGF itself in human pancreatic carcinoma cells was decreased by 42, 58, 36, and 40% respectively, by vessel dilator, LANP, kaliuretic peptide and ANP. In small-cell lung cancer cells, the maximal decrease in VEGF levels was 25, 23, 17 and 23% secondary to vessel dilator, LANP, kaliuretic peptide, and ANP. In human prostate cancer cells, VEGF was maximally decreased 24, 20, 23, and 24% secondary to vessel dilator, LANP, kaliuretic peptide, and ANP respectively.
Conclusion
Four cardiac hormones are the first dual inhibitors of VEGF and the VEGFR2/KDR/Flk-1 receptor.
COI Details
Dr Vesely has assigned the patent to treat cancer with these cardiac hormones to the University of South Florida, which has not licensed this patent to any commercial entity. There has been no pharmaceutical company funding or input into the studies described herein. The contents of this publication do not represent the views of the Department of Veterans Affairs or the United States Government. None of the other authors has any potential conflict of interest.
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## Clinical case reports - Pituitary/Adrenal

### P342

**Localization of occult adrenal tissue with cosyntropin-stimulated 18F-FDG-PET/CT in a patient with metachronous adrenocortical tumor who presented with persistently elevated SDHEA after bilateral adrenalectomy**

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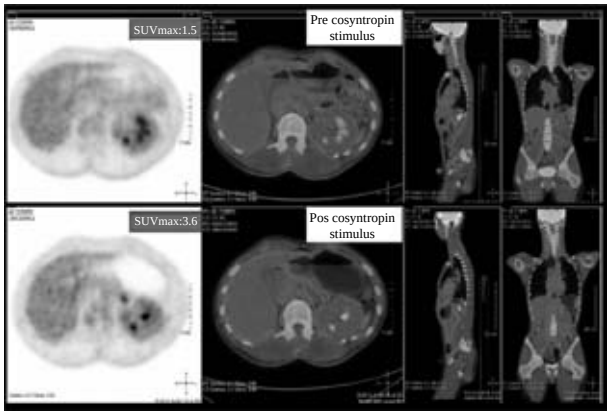
Introduction
Adrenocortical carcinoma (ACC) is a rare and potentially fatal disease in childhood. Complete resection of the tumor and metastasis can improve survival. Case: A 2.6 year-old boy presented isosexual pseudo-precocious puberty at 2.1 years. CT revealed a single nodule in the left adrenal (2 cm) which was resected by adrenalectomy. Histology revealed an adrenocortical tumor (2 cm, 3.0 g, Weiss score 4), ENSAT stage I. Partial regression of the puberty features and normalization of hormonal levels were observed. At 4.8 years, recurrence was observed, with high androgen levels and an 8 cm right adrenal mass on CT. Right adrenalectomy and nephrectomy was performed, which revealed an adrenocortical tumor (8 cm, 30 g, Weiss score 7) with no apparent metastasis at that time. At 6 years, DHEAS levels became increasingly high and CT showed a pulmonary nodule that was surgically removed. After 8 months of partial lung resection, abnormally high DHEAS was present with no obvious source on conventional imaging techniques. 18F-FDG-PET/CT showed a mediastinal lesion that was resected and histologically confirmed as metastatic ACC. DHEAS levels remained undetectable until he was 13 years-old, with a progressive increase not suppressible by high doses of dexamethasone (0.5 mg qid for 7 days). Conventional radiological techniques did not identify any metastatic lesions. At 18.5 years of age, the patient underwent 18F-FDG-PET/CT under stimulation with intravenous cosyntropin 250 mgc, which identified a 1.5 cm nodular lesion in left adrenal topography. FDG hypermetabolism was shown in that area pre-stimulus (SUV = 1.5), with an increase 60 min after stimulation to 3.6, suggesting remaining tumorous tissue.
Conclusions
We report a long-term evolution of a pediatric patient with an indolent ACC and suspected local recurrence identified by cosyntropin-stimulated $^{18}$F-FDG-PET/CT. This new diagnostic modality may be used in clinical practice to locate occult metastasis of ACC.




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### P343

**A case of pheochromocytoma which represented multiple myeloma like change**

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A 48-year-old female was admitted to our hospital because of prolonged fever of unknown origin for three months, anemia, renal dysfunction, and hypercalcemia. There were no abnormal parameters of physical examination except fever of 37.6 °C, BMI of 26 5 kg/m$^2$ after 11 kg of body weight loss in 2 months, and her blood pressure was 138/87 mmHg. C-reactive protein and WBC were 24.6 mg/dl and 10 900/μl, respectively. A/G ratio was elevated with total protein of 8.7 g/dl and albumin of 1.6 g/dl, platelet count was 858×104, serum creatinine was 0.89 mg/dl. Plasma glucose and HbA1C level were elevated to 150 mg/dl and 7.4% respectively. As her bone marrow biopsy revealed hyper cellular bone marrow with an increased number of plasma cells, we diagnosed her multiple myeloma with Durie-Salmon stage III.
At the same time, abdominal CT scan revealed left adrenal mass with a diameter of 35 mm, which was strongly enhanced in enhance CT, and hyper-intense and heterogeneous on T2-weighted images in MRI. Urine adrenalin, urine noradrenalin, and urine dopamine were 14.5, 1470, and 3100 μg/day respectively. Addition with that, as I-131 MIBG adrenal scintigraphy demonstrated high uptake of left adrenal area, we diagnosed her pheochromocytoma.
To avoid the risk of inducing adrenal crisis by administrating steroid, we gave priority to the treatment for left adrenal pheochromocytoma and performed adrenalectomy. Serum and urine catecholamine levels were normalized after surgery. Anemia and hypercalcemia is also normalized, and the hyperglycemia which required 68 units of insulin per day before surgery had stabilized only with dietary cure. As the multiple myeloma like changes disappeared in bone marrow biopsy, we are following her carefully only with the oral medication for hypertension.
Though there are several reports of the IL6 producing pheochromocytoma with systemic inflammatory responses, we could not find any case representing the multiple myeloma like changes as we experienced. On the other hand, IL6 is the cytokine reported to be an autocrine growth factor in myeloma and plasmacytoma. There are possibilities that cytokine secretion from pheochromocytoma itself or from the necrotic lesion of pheochromocytoma led the plasma cells over expression and represented multiple myeloma like changes, which are all normalized after adrenalectomy for pheochromocytoma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### P344

**Reactivation of Takayasu arteritis in two patients with Cushing's disease after normalization of cortisol secretion.**

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Introduction
The reactivation of autoimmune disorders has been described in patients with hypercortisolism after normalization of cortisol secretion. This phenomenon is probably related to the loss of immunosuppressive effect of endogenous glucocorticoids. This is a report of two patients with Takayasu arteritis (TA) and Cushing's disease (CD), a novel association never described in literature, in whom TA was exacerbated after normalization of cortisol secretion.
Case report
Two women, 55 and 53 years old, presented with a clinical syndrome suggestive of hypercortisolism, in whom a diagnosis of CD was performed (urinary free cortisol: 188 and 264 mcg/day respectively), reported a history of TA, complicated by acute coronary syndrome, peripheral vascular disease and chronic renal failure. Not considered candidates for pituitary surgery, they were treated with inhibitors of adrenal steroidogenesis. Six months later, they had a normalization of daily cortisol excretion (urinary free cortisol: 98 and 118 mcg/day) but complained typical symptoms of a systemic vascular inflammatory process: fatigue, weight loss, fever and arthromyalgia; in one of the two patients, the strong inflammatory factor, inhibiting erythropoiesis, in association with iron and vitamin B12 deficiency, caused severe anemia (Hb 4 g/dl), requiring blood transfusions. The clinical picture, the strong increase of inflammatory markers such as VES, C-reactive protein and fibrinogen, and signs of vascular insufficiency such anisosphygmia in femoral and tibial pulses, in bilateral carotid axes and absence of right brachial and radial pulses, have suggested a process of reactivation of the TA. Treatment with corticosteroids was necessary to relieve the clinical symptoms of TA and to reduce the inflammatory markers.
Conclusions
These cases, particularly challenging because of the two concomitant disorders, CD and TA, confirm the crucial role of cortisol secretion reduction in the reactivation of the autoimmune process, supporting the hypothesis that TA activity was suppressed by endogenous glucocorticoids excess. This evidence suggests that it is mandatory to

investigate on the possible development or reactivation of autoimmune diseases after normalization of cortisol secretion in patients with a history of Cushing's syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P345

**Delayed presentation of late onset CSF rhinorrhoea following dopamine agonist therapy for giant prolactinoma**

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Background
CSF rhinorrhoea is a rare but recognised complication of dopamine agonist therapy for macroprolactinoma. In the majority of cases, onset of CSF rhinorrhoea is within 4 months of commencing therapy.
Case report: A 23-year-old man presented to the Emergency Department in April 2010 with acute weakness in his left arm and leg associated with intermittent headaches. Examination revealed mild right-sided ptosis and inadequate androgenisation. Visual fields were full to confrontation.
MRI pituitary demonstrated a 5 cm lobular/cystic mass invading the right cavernous sinus, displacing and compressing the midbrain, with destruction of the bony sella. Serum prolactin was $>150\,000$ mIU/l.
Cabergoline was commenced (initially 250 μg twice/week). Neurological symptoms resolved with dramatic reduction in tumour size. Serum testosterone levels normalised. Prolactin level plateaued at 20 000 mIU/l despite repeated increments in the dose of cabergoline to 500 μg three times/week over the course of 12 months. There was still significant residual sellar and right parasellar tumour. Consequently, the cabergoline dose was increased to 500 μg/day following which he developed continuous daily rhinorrhoea (May 2011).
The patient presented to his general practitioner who referred him to an otolaryngology clinic. When next seen in the routine regional endocrine clinic (November 2011) he was admitted for urgent endoscopic transnasal transphenoidal repair of CSF leak. CT pituitary confirmed the likely site of the leak was in the left basisphenoid where there was marked thinning of the bone. Preoperative serum prolactin was 3240 mIU/l on cabergoline 500 μg/day. Histology confirmed a prolactinoma with proliferation index of 2%.
Discussion
In this case, CSF rhinorrhoea occurred 13 months after initiation of cabergoline suggesting a need for vigilance throughout therapy.
There was significant delay before this complication was brought to the attention of the regional pituitary team. There is a need for patients and healthcare professionals to be educated about early recognition and management of this complication.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P346

**ACTH-dependent Cushing's syndrome secondary to an ectopic source of ACTH/CRH: three clinical cases with different prognosis and outcome**

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Introduction
Ectopic ACTH-dependent Cushing's syndrome (ACS) may present with different clinical pictures and it may represent a diagnostic challenge.
Methods
We describe the clinical presentation and the laboratory, imaging and pathologic findings of three patients with ectopic ACS.
Results
Case 1. A 31-year-old man with a 1-year history of spontaneous rib and vertebral fractures was admitted to our hospital for ACS evaluation. He displayed typical cushingoid features. Pituitary magnetic resonance imaging (MRI), chest/abdomen computed tomography (CT) and whole body Octreoscan (WBO) were unremarkable. Petrosal sinus sampling with corticotrophin-releasing hormone (CRH) stimulation was negative. A (68Ga)DOTATOC-PET/CT showed a 7 mm-pulmonary nodule that was excised and diagnosed as a typical carcinoid. Six months later, he showed nearly complete biochemical and clinical recovery.
Case 2. A 72-year-old man was referred to endocrinological evaluation for hyponatremia. Biochemical analysis revealed ACS, thrombocytopenia and monoclonal gammopathy. Chest CT showed a widespread mass in the left lung. Both the cytology on bronchoalveolar lavage and the osteomedullary biopsy revealed neuroendocrine-differentiated cells. He started chemotherapy, but died after few weeks.
Case 3. A 66-year-old man with a 2-year history of diabetes mellitus and hypogonadotropic hypogonadism and recent leg swelling was referred to our center for ACS. He didn't show cushingoid features except for muscle hypotrophy. Melanodermia, marked hypokalemia and psychosis were present. Chest/abdomen CT and WBO were negative. Pituitary MRI showed a microadenoma and petrosal sinus sampling with CRH stimulation was inconclusive. Despite ketokonazole and mifepristone treatment, he became hemodynamically unstable for a massive bilateral pleural effusion and sepsis, and rapidly died. The post-mortem result of cytologic analysis on pleural liquid was positive for adenocarcinoma.
Conclusions
Ectopic ACS can be caused by tumors with different degrees of malignancy, thus conditioning different prognosis. The disease course can also be complicated by the difficulty in tumor localization with conventional imaging techniques.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P347

**Hyponatraemia assessment and outcomes in acute medically ill patients**

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Hyponatraemia is the most common electrolyte abnormality, encountered in up to 30% of inpatients. Inappropriate management can have serious implications for patients; including demyelinating disease, coma, and death.
Methods
Patients ($n=100$) admitted to the medical admission unit of a district general hospital with a serum sodium (Na) $<130$ were selected for the study. All details including patient demographics, blood biochemistry, date of admission and date of death were taken from the case notes and hospital computerised system. Details on assessement of hyponatraemia including thyroid, adrenal and renal function were also recorded.
Results
Mean age was $69.7\pm18.1$ years; 41 males. Mean serum Na on admission was $125.8\pm4.1$ mmol/l. Of the 100 patients 32 died in hospital. Admission serum Na in survivors vs died was $126.5\pm3.8$ vs $124.3\pm4.5$ mmol/l ($P<0.01$). Patients investigated for hyponatraemia were as follows: serum cortisol ($n=6$), plasma osmolality ($n=9$), urine osmolality ($n=9$), short synacthen test ($n=0$), urine sodium ($n=3$), thyroid function tests ($n=19$).
Conclusions
Patients admitted with acute medical conditions with severe hyponatraemia have a high mortality and those with lower serum sodium have greater risk of death. Patients were also inadequately worked up for assessment of cause of the hyponatraemia and further education of medical specialists is urgently required to improve management and outcome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P348

**A clinical case of a 21 years old man with inborn pituitary dystopia**

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A 21 years old man had growth failure since 11 y.o., micropenis, high-pitched voice, the absence of secondary sex characteristics and male sexual behavior,

constant fatigue and weakness. The boy was born in time after 1st normal pregnancy, birth weight 3000 g, length 57 cm, had unilateral cryptorchidism (surgical treatment in 2002). At the age of 21 the height of the patient was 144 cm (SDS - 4.62), BMI 15 kg/m$^2$ (BMI SDS −3.89), Tanner G1P1, bone age 13 y.o. Inborn hypopituitarism as a result of inborn pituitary dystopia was diagnosed: GH deficiency (GH 0.38 ng/ml (0.5–5.0), IGF1 <25 ng/m (94–252)), ACTH deficiency (ACTH <1,1 pmol/l (0–10.2), cortisol (in blood) 55,5 nmol/l (119–618), daily urine free cortisol excretion - 10 nmol/day (20–200)), TSH deficiency (free T4 - 9,9 pmol/l (10–23.2), TSH - 0.63 μIU/l (0.4–4.0)), gonadotropin deficiency (LH - 2.3 IU/l (0.6–12), FSH - 2.3 IU/l (1.0 - 12.0), total testosterone 0.07 ng/ml (3–12)). MRI showed neurohypophysis dystopia in the floor of third ventricle, total absence of the infundibulum, small adenohypophysis (9x10x3 mm). Replacement therapy was started with recombinant GH 0.033 mg/kg per day s.c., hydrocortisone 7,5 mg/day. After establishing adequate adrenal function thyroid-replacement therapy was given: Levothyroxine 50 μg per day. Testosterone replacement therapy was started with Sustanon 0.2 ml by intramuscular injections every 4 weeks. Perspectives: The GH replacement therapy will be effective until the epiphyseal plates closure. Testosterone replacement therapy will help to maintain androgenic anabolic effects, although its efficacy in development of the external genitalia is questionable due to the age of the patient. Hypocorticism and hypothyrosis replacement therapy can show clinical efficacy and significantly improve the quality of life. Performing genetic test in patient and relatives is planned.

Conclusion

This case shows the importance of strict growth curves monitoring in children, highlights necessity of early diagnosing and treatment of hypopituitarism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P349

### A case of acromegaly without clear evidence of pituitary adenoma or ectopic GH/GHRH secreting tumors

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Introduction

Acromegaly secondary to an extrapituitary source of GH or GHRH is rare, usually associated with bronchial/thymic carcinoids, neuroendocrine enteropancreatic tumors or extrasellar somatotropinomas. Few series of acromegalic patients with negative pituitary imaging and lack of an ectopic source are reported in the literature.

Methods

We report the clinical history and the laboratory and imaging results of an acromegalic woman with no clear evidence of pituitary somatotropinoma or ectopic GH/GHRH source.

Results

A 66-year-old woman was referred to our outpatient clinic for the follow-up of a multifocal differentiated thyroid carcinoma treated by total thyroidectomy 7 years before. The thyroid oncologic follow-up was negative but physical examination revealed acromegalic features. She also complained frontal headache, amaurosis, fatigue, paraesthesias, muscular pains and sleep apnea syndrome. Basal IGF1 levels resulted elevated for age (312 ng/ml; r.v. 78-212), and a nadir GH of 1.45 ng/ml during oral glucose tolerance test (OGTT) was diagnostic for acromegaly. Magnetic resonance imaging of the pituitary was unremarkable but an abnormal contrast-enhancement of the right cavernous sinus (CS) was present. Computed tomography of chest and abdomen and a whole body Octreoscan didn't show any evidence of ectopic GH/GHRH-releasing tumor. Short-acting octreotide formulation treatment was started as once-daily administration because of gastrointestinal intolerance, with a partial biochemical remission of acromegaly. Nine months later, octreotide was discontinued and OGTT and imaging scans were repeated, confirming previous observations.

Conclusions

The described patient did not show an obvious source of GH or GHRH secretion. So far, only one case of ectopic somatotropinoma has been described in the CS. A trans-sphenoidal exploration could be crucial in our patient in order to exclude a micro-adenoma of the pituitary and explore the possibility of an CS ectopic somatotropinoma, thus avoiding further invasive and expensive imaging tests.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P350

### Evaluation on clinical application and long term outcomes of microwave endometrial ablation

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Study objective

To evaluate the long term outcomes of menorrhagia treated by microwave endometrial ablation(MEA) as well as the factors which influence the outcomes.

Design

Prospective, single-arm study.

Setting

GuangDong Women's and Children's Hospital, GuangDong, China.

Patients

Three thirty four women with menorrhagia were selected for MEA, the age of these patients ranged from 29 to 59 years.

Interventions

Endometrial ablation using microwave endometial ablation.

Measurements and main results

All the patients were followed up, the change of menstrual cycle, the amount of flow, and complication of the procedure were recorded. 53 women underwent outpatient diagnostic hysteroscopy, the biopsy tissue was taken from the endometrium of these patients for histopathological examination. The mean duration of follow-up was 64.7 months (3–115 months). Overall effective rate was 91.3% (305/334), in which amenorrhea rate was 49.7% (166/334),menstruation reduction rate was 41.6% (139/334); 71.1% (140/197)of the cases who previously had dysmenorrheal had relieved their pain and the satisfaction rate was 91.9% (307/334); 42 cases required subsequent treatment as a result of recurrence, of which 9 cases were given repeat MEA and 33 cases underwent hysterectomy. A completely destroyed endometrium was seen after MEA by hysteroscopy, pathologic effects of MEA showed two zones of necrotic tissue.

Conclusions

MEA is characterized by simplicity of its performance, marked effectiveness and fewer complications, holding especially for patients complicated with severe diseases. Incomplete removal of endometrium was the important factor in reducing the effiency, young age and adenomyosis showed significant increased risk of treatment failure.

Declaration of interest

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## P351

### Familial central diabetes insipidus due to a novel mutation in exon 3 of the arginine vasopressin gene

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Neurohypophyseal diabetes insipidus (DI) is said to be caused by familial forms in about 5% of cases[1]. Hereditary transmission is autosomal dominant in most families and is caused by a mutation in the arginine vasopressin (AVP) gene on chromosome 20p13, which encodes for a large precursor hormone.

A 19-years old otherwise healthy patient seeked endocrine care for an inadequately treated DI. He reported about an undoubtful disease history with about 10 l of diluted urine without therapy since his second year of life. His father and sister were affected as well. Adjustment of the patient's therapy resulted in a well-treated DI.

After informed consent, a genetic analysis was performed on the patient and his sister by sequence analysis of the AVP gene.

A hitherto undescribed heterozygous mutation p.Cys110 Arg in codon 110, exon 3 of the AVP gene was found, both in the patient and his sister. Since a stop mutation causing DI in the same codon has been described before[2] and since the mutation results in a non-conservative amino acid substitution, we conclude that this mutation is the cause for DI in this family.

Our findings add a new mutation to a list of 66 mutations so far described for the AVP gene (Biological Database HGMD Professional). Interestingly, the disease manifests not immediately after birth but later during the first and second year of life. This is in contrast to mutations in two other genes involved in familial DI (namely the arginine vasopressin receptor 2 gene and the vasopressin-sensitive water channel gene). Recent studies hypothesized that the disease is caused by formation of inclusion bodies hampering cell function[3] and that there is a

genotype–phenotype correlation[4]. Therefore, new mutations can add important information to the elucidation of the mechanisms that are involved in DI.

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## P352

### Successful pregnancy during use of octreotide and pegvisomant in an acromegalic patient

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Introduction

Acromegaly is preferentially treated by surgery and/or radiotherapy before pregnancy, in order to avoid use of medication during pregnancy, and prevent GH excess related complications for mother and child. Only two cases of use of pegvisomant during pregnancy have been described; in one patient medication was discontinued after the first trimester, and in the other pegvisomant was used as monotherapy.

Case report

A 29-years old female had been treated for acromegaly with transsphenoidal surgery. Before surgery she started with octreotide. Surgery was incomplete, and pegvisomant was added because of insufficient control. Radiotherapy was proposed, but denied by the patient. We advised against pregnancy while using pegvisomant and octreotide, but she was adamant and became pregnant at the age of 32, and was under strict medical control during pregnancy. Pregnancy was uneventful. During pregnancy, her IGF1 levels declined from 39.8 to 19.4 nmol/l with unchanged doses of 30 mg octreotide LAR once every 3 weeks, and pegvisomant 40 mg twice weekly, as before pregnancy. A healthy child was born after a full term uneventful pregnancy. After pregnancy, IGF1 levels rose to values consistent with those before pregnancy. The levels of octreotide and pegvisomant were measured in peripheral blood samples from the mother and in umbilical cord blood samples taken immediately after birth. Octreotide levels in the mother's blood: 2142.790 and in umbilical cord blood: 807.106 pg/ml, pegvisomant levels in the mother's blood: 1543 ng/ml, and in umbilical cord blood: undetectable.

Conclusion

We report the first successful pregnancy in an acromegalic patient using the combination of pegvisomant and octreotide during complete pregnancy. There were no congenital malformations. Pegvisomant (80 mg weekly), was not detectable in umbilical cord blood, while detectable in the mother's blood. Octreotide was detectable, albeit in lower levels in umbilical cord blood than in the mother's blood.

Declaration of interest

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## P353

### Long-term efficacy and safety of pasireotide in Cushing's disease: a 36-month case report

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Background

A recent, large phase III study ($n=162$) showed that treatment with pasireotide rapidly decreased urinary free cortisol (UFC) levels and improved signs and symptoms in patients with Cushing's disease. Here, we report the experience of a patient enrolled in this trial who received pasireotide for 36 months.

Results

A 31-year-old male presented in June 2008 with persistent Cushing's disease despite two previous pituitary surgical procedures. Examination revealed he was overweight (79 kg; BMI 28.3 kg/m$^2$), had mild hypertension (139/83 mmHg), and elevated UFC (112.5 μg/24 h; ULN, 52.6) and ACTH (55 ng/l; ULN, 46); his serum cortisol level was 10.5 μg/dl (ULN, 22.4). In July 2008, he began twice-daily injections of pasireotide 600 μg s.c. After 3 months, UFC had decreased to 72.0 μg/24 h. In January 2009 (UFC 73.8 μg/24 h), the pasireotide dose was increased to 900 μg s.c. bid. By July 2009 (end of 12-month core study), an improvement in all key biochemical parameters was observed: UFC (36.4 μg/24 h) and ACTH (31 ng/l) normalized, and serum cortisol decreased (7.8 μg/dl). Reductions in weight (69 kg), BMI (24.7 kg/m$^2$) and blood pressure (127/70 mmHg) were also reported. Pasireotide 900 μg bid was continued and at 18 months, although UFC was slightly elevated (58.0 μg/24 h), clinical improvements were maintained, including blood pressure (116/80 mmHg), weight (72 kg) and BMI (25.8 kg/m$^2$). After 36 months' treatment with pasireotide, UFC was normalized (33.7 μg/24 h), and improvements from baseline in weight (74 kg), BMI (26.2 kg/m$^2$) and blood pressure (125/82 mmHg) were sustained. The patient's health-related quality of life also improved, as evaluated by the CushingQoL questionnaire. Several adverse events occurred that were suspected to be related to the study drug: mild (grade 1) hyperglycemia was noted at 6 and 9 months, while at 18 months, moderate (grade 2) hyperglycemia occurred, necessitating initiation of metformin, then glimepiride due to gastrointestinal complaints. Mild hyperglycemia was reported again at 27 months. Grade 2 cholelithiasis was also reported at month 12.

Conclusions

This case report illustrates the long-term efficacy and safety of pasireotide in Cushing's disease, supporting its potential use as a targeted treatment for ACTH-secreting pituitary adenomas.

Declaration of interest

I fully declare a conflict of interest. Details below:

Funding

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## P354

### Control of acromegaly in an era where it is technically feasible in almost every patient, is by no means invariable even in a specialist unit

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We have audited control of acromegaly in a specialist endocrine unit in Oxford now that this should usually obtainable. We have 240 patients under active surveillance, seen annually since diagnosis. Data from 115 of these seen in the last year have been used for this analysis.

Methods

We grouped patients according to whether they were controlled in either or both IGF1 and GH or uncontrolled. Controlled IGF1 was defined according to age and sex and GH was defined as basal $<5$ mU/l ($<2$ μg/l). We analysed the most recent IGF1 and GH values and compared these to initial GH levels, initial tumour size and the types of treatment given.

Results

Control of GH 72% ($n=83$), control of IGF1 62% ($n=71$), control of both 49% ($n=56$), uncontrolled of both 13% ($n=15$).

Control of IGF1- vs initial tumour size; extrasellar macro-, intrasellar macro- and microadenoma (71%, 58%, 56%) and for control of GH- (71%, 54%, 88%)

High GH ($>50$ mU/l) vs low ($<50$ mU/l) GH at presentation (74 vs 96% - controlled).

Analysis of the factors at presentation of uncontrolled patients were tumour size at diagnosis macroadenoma- 79% and initial GH 'high' at diagnosis 79%.

Conclusions

1. Even in a specialised unit, control of acromegaly is not invariable after treatment. This may relate in some patients to large tumour size and high initial GH levels at presentation but in some it relates to the inability to obtain funding for expensive drugs.
2. There is a considerable but previously described discordance between GH control (72%) and IGF1 control (62%).
3. Initial tumour size predicates GH control.
4. High levels of GH at commencement of treatment predicate a poorer outcome.
5. Uncontrolled patients almost invariably have a large tumour or initially high GH levels at diagnosis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P355

### A case of adrenocortical carcinoma with fever of unknown origin and elevated serum IL6 level

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A 31-years-old female was admitted to our hospital because of prolonged fever of unknown origin for 2 months. As she was originally diagnosed to have upper respiratory infection, antibiotics and antipyretic analgesics were prescribed. However, there is a recurrence of fever up to 39 °C without antipyretic analgesics. There were no changes in body weight or the size of clothes, and she did not notice any clinical features of Cushing's syndrome. Her height and weight was 152 cm and 58 kg, with BMI of 24.9 kg/m$^2$, and the blood pressure was 118/78 mmHg. Except light moon face like change and conjunctive anemia, she had no abnormal physical features including Cushing's syndrome such as buffalo hump, truncal obesity, or purple striae. C-reactive protein and WBC were 15.7 mg/dl and 8900 /μl, respectively. Hemoglobin level was decreased to 9.2 g/dl, and platelet count was 609×103. ACTH was 12.3 pg/ml while serum and urine cortisol levels were 26.3 μg/dl and 246 μg/day, respectively. A normal circadian rhythm was not present. Dexamethasone suppression testing (0.5, 1.0, and 8.0 mg) did not decrease serum cortisol levels to the reference levels. Abdominal CT scan revealed left adrenal mass with a diameter of 55 mm, which was weakly enhanced, and was also consisted of the poorly enhanced region, suggesting the necrosis inside the mass. While there were no high uptakes in I-131 MIBG, adosterol, or gallium scintigraphys, (18)-FDG–PET/ CT scan showed high uptake only in left adrenal mass. As we diagnosed her adrenocortical carcinoma (ACC) with stage 1 and Cushing's syndrome, adrenalectomy was performed. Histopathologic analysis revealed at least four parameters of the Weiss system, including atypical mitoses, high mitotic rate, or capsular invasion, and MIB-1 labeling index was over 10%. Adjuvant treatment with mitotane (o,p,-DDD) was initiated following surgery.

Not only the serum IL6 level, which showed elevated to 126 pg/ml before adrenalectomy, immediately decreased to 4.7 pg/ml, but also the fever and systemic inflammatory responses improved. IL6 is known to have various functions such as inducing B cell proliferation or systemic inflammatory responses, including elevations in ESR or CRP. We hypothesized the possibility of IL6 producing ACC or that IL6 elevation was part of the response for necrotic area of ACC, produced from endothelial.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P356

### Desmopressin and non steroidal anti-inflammatory drugs: a case report of severe water intoxication during replacement therapy and review of the literature

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Context

Most of the clinical data on safety profile of desmopressin (DDAVP), i.e. an effective treatment of both polyuric conditions and bleeding disorders, originates from studies on tailoring of drug treatment, whereas few reports describe severe side effects secondary to drug–drug interaction.

Objective

To describe a case of severe hyponatremia complicated with seizure and coma due to the intake of non steroidal anti-inflammatory drugs (NSAIDs) in a patient on DDAVP replacement therapy for central diabetes insipidus (DI).

Patient and methods

We report a 50-year-old Caucasian man, with congenital central DI, who developed an episode of generalized tonic-clonic seizure, resulting in coma immediately after being admitted to the Emergency Unit for weakness and emesis. Based on his medical history and clinical findings, water intoxication secondary to ketoprofen intake (200 mg/day for the last 3 days) concomitant with DDAVP replacement therapy (Minirin 60 μg four tablets a day) was hypothesized as cause of the severe euvolemic hypotonic hyponatremia (natremia 113 mEq/l, plasma osmolality 238 mOsm/kg). After emergency procedures, the aquaretic tolvaptan (Samsca 7.5 mg) was administered and hydratation was maintained according to water excretion. He completely recovered in 72 h. We discuss this case report in the context of the published literature.

Conclusions

Contrary to the several cases of hyponatremia reported in patients on DDAVP for different indications, no study has highlighted the potentially life-threatening side effects associated with over-the-counter NSAIDs during DDAVP replacement therapy for central diabetes insipidus so far. Risks and benefits of co-treatment should be carefully considered and therapeutic alternatives to NSAIDs should be recommended to patients with central DI, in order to improve DDAVP safety.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P357

### Germ cell tumor of the pituitary–hypothalamic region: case series and clinical experience in the Hospital Universitario Virgen del Rocío

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Background

Germ cell tumors (germinoma, teratoma, embryonal carcinoma, yolk sac tumor and choriocarcinoma) constitute 0.3–3.4% of all primary intracraneal tumors. Germinoma is the most common and of better prognosis.

Aim

Describing the behaviour of germ cell tumors of the pituitary–hypothalamic region: location, clinical characteristics, diagnosis and follow-up.

Material and methods

Review of medical records of patients with germ cell tumors of the pituitary–hypothalamic region diagnosed between 1998 and 2011 attended at Hospital Universitario Virgen del Rocío in Seville. Were determined the clinical presentation, location, process followed to achieve diagnosis, treatment and evolution.

Results

Thirteen patients were diagnosed: four male (30.7%) and nine female (69.23%). Nine of the 13 patients (69.2%) had an age of 14 or younger.

Twelve patients (92.3%) displayed diabetes insipidus, being the first symptom in 11 of them (84.6%). All subjects displayed panhypopituitarism at the beginning of the treatment.

In four patients (30.7%) with diabetes insipidus, the initial MRI was normal or only displayed thickened pituitary stalk; these patients were finally diagnosed with a radiologic follow-up and/or biopsy.

Nine of the 13 patients (69%) were diagnosed after biopsy or surgery and in the four remainders (31%) by the elevation of the serum and/or cerebrospinal fluid (CSF) tumor markers.

In nine of which pathologic anatomy records were available, eight were germinomas and one was yolk sac tumor.

The average time of evolution since diagnostic is 4.67 years. Nowadays, 12 of the 13 patients are alive.

Conclusion

Diabetes insipidus is the most frequent symptom in the germinomas of the pituitary-hypothalamic region. When diabetes insipidus is diagnosed a close clinic and radiologic follow-up is compulsory as the patient might suffer a germinal tumor or develop other hormonal deficits.

Declaration of interest

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## P358

**Adrenal venous sampling for two cases with primary pigmented nodular adrenocortical disease**

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Introduction
Primary pigmented nodular adrenocortical disease (PPNAD) is a rare cause of Cushing's syndrome. Total bilateral adrenalectomy is mostly chosen for treatment because pathological changes usually grow out in both adrenal glands among PPNAD cases. Meanwhile total adrenalectomy may result in life-threatening acute adrenal insufficiency. In this report, two PPNAD patients were examined to check the laterality of hormone secretion from both glands by adrenal venous sampling (AVS) ahead of surgical intervention, with a view to clarify the distribution of adrenal regions which could be spared during surgery.
Case report
Two females, aged 24 and 25 years, showed typical clinical features and laboratory findings of ACTH independent Cushing's syndrome. Imaging tests showed bilateral adrenal nodules and bilateral radioiodine uptake. Besides, other examinations revealed the history of atrial myxoma or the existence of breast tumor, which were characteristics of Carney complex. Although these findings validated the diagnosis of PPNAD, we investigated hormonal laterality to know whether there would be any way to avoid total adrenalectomy and symptoms due to cortisol deficiency. Cortisol, aldosterone, epinephrine, norepinephrine levels were measured by AVS including ACTH stimulation. The laterality of cortisol concentration varied from 1.3–1.5 times to 1.2–1.6 times, respectively before and after ACTH administration. The predominance of aldosterone and others were ipsilateral in the first case, but contralateral in the second case. The results seemed inadequate to prove the significant laterality of hormone secretion. Taking all these findings into consideration, both patients underwent laparoscopic bilateral complete adrenalectomy. Histopathological analyses diagnosed both as PPNAD and also revealed that some normal steroid synthetase expressions still remained in non-nodular area.
Conclusion
We have shown that AVS gave us meaningful information to elucidate the pathophysiologic mechanism of PPNAD. Further studies are waited to determine the optimum treatment for bilateral functional adrenal tumor.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P359

**Cyclic Cushing's syndrome due to occult ectopic ACTH secretion**

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Introduction
Cyclic Cushing's syndrome (CCS) is extremely rare. It's defined by periodic hypercortisolism with at least three proven peaks and two drops in cortisol secretion.
Case report
A 35-year-old woman was reffered for evaluation of endogenous hypercortisolism after a diagnosis of central serous coriorethinopathy. Initially she had mild symptoms. Urinary free cortisol (UFC) was 2.881 μg/24 h; ACTH 17.45 pg/ml and night salivary cortisol 7.08 mmol/l. Administration of 1 mg of dexametasone (DXM) failed to suppress cortisol secretion but adequate suppression was observed after 8 mg. Pituitary MRI was normal. Two hospital admissions over a period of 3 months showed normal UFC and night salivary cortisol and adequate suppression with 1 mg of DXM. Basal cortisol was about 7 μg/dl and response to i.v. ACTH (250 μg) was normal. During a third hospital admission due to clinical worsening UFC was >705 μg/24 h, night salivary cortisol 1.05 μg/dl, cortisol cyrcadian rhythm was absent and ACTH concentrations were 44 pg/ml. A desmopressin test showed an ACTH increase of 400%. Inferior petrosal sinus catheterization during hypercortisolism (UFC > 540 μg/dl) showed no gradient between petrosal sinuses and periphery. Diagnosis of CCS due to ectopic ACTH secretion was made. Cervical, chest and abdomen CT, PET and octreoscan were normal. Eighteen months later, the patient had gained 20 kg and had developed frontal alopecia, hirsutism, ankle edema and osteopenia. A laparoscopic bilateral adrenalectomy was performed. Annual imaging and ectopic co-secretion markers remain negative 3 years after surgery.

**Table 1** Inferior petrosal sinus catheterization during hypercortisolism (UFC > 540 μg/dl)

| | Cortisol (μg/dl) | ACTH Rps (pg/ml) | ACTH Lps (pg/ml) | ACTH p |
|---|---|---|---|---|
| −15′ | 24.5 | 84 | 80 | 83 |
| 0′ | 22.4 | 81 | 83 | 50 |
| 3′ | 21 | 81 | 81 | 85 |
| 5′ | 22.5 | 89 | 91 | 87 |
| 10′ | 22.2 | 44 | 99 | 96 |
| 30′ | 29.3 | | | 97 |
| 60′ | 29.4 | | | 51 |

Rps, right petrosal sinus; Lps, left petrosal sinus; p, periphery. Before and after surgery.

Discussion
Of the 66 cases of CCS described until now, only two are due to ectopic occult ACTH secretion. For a correct diagnosis, frequent determination of UFC and night salivary cortisol are necessary and diagnostic tests should be performed during hypercortisolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P360

**Use of tolvaptan for delayed hyponatremia after transsphenoidal surgery for pituitary adenoma: a case report**

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Introduction
Disorder of water and electrolyte balance may develop after transsphenoidal (TNS) surgery for pituitary adenomas. In particular, delayed hyponatremia (Na < 135 mEq/l) due to SIADH is reported in 8–35% of patients and usually resolves with fluid-intake restriction within 6 days. The possible role of vasopressin-2 receptor antagonists in this clinical setting has not been elucidated.

Case report
We report a case of a 57-years-old Caucasian woman who underwent TNS surgery for nonfunctioning pituitary macroadenoma. Postoperative period was uncomplicated and serum sodium levels in the normal range were recorded. On postoperative day 5, sodium concentrations rapidly decreased to 127 mEq/l and symptoms related to hyponatremia (haedhache, weakness) occurred. Treatment by water restriction (<1000 ml/day) and salt rich diet was started. Thirty-six hours after the beginning of fluid restriction, the serum sodium concentration was 129 mEq/l and the patient remained symptomatic. Given the lack of efficacy, water restriction was stopped and tolvaptan (Samsca 15 mg) was administered. Four hours after vasopressin-2 receptor antagonist intake, sodium levels increased to 131 mEq/l and rose to 142 mEq/l in 24 h, with disappearance of symptoms. Tolvaptan was discontinued and clinical status and serum sodium concentration was monitored. The patient was discharged two days after with a sodium value of 140 mEq/l and in good clinical condition.
Conclusions
Although in many cases of delayed acute hyponatremia after transsphenoidal surgery fluid-intake restriction is effective, in our patient this treatment resulted insufficient to reverse hyponatremia and required a significant prolongation of hospitalization. Conversely, administration of a single dose of tolvaptan rapidly and safely resolved hyponatremia. In conclusion, treatment with tolvaptan may represent a useful alternative to fluid restriction in mild symptomatic delayed hyponatremia after TNS surgery and may possibly shorten hospitalization of these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P361

**Familial isolated pituitary adenoma cases in Hungary**

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Familial isolated pituitary adenoma (FIPA) occurs if two or more members of a family develop pituitary adenoma with no features of multiple endocrine neoplasia type 1 or Carney complex. FIPA is an autosomal dominant disease with incomplete penetrance. FIPA families can be divided into two distinct groups based on genetic and phenotypic features. In 20% of FIPA families mutations have been identified in the aryl hydrocarbon receptor interacting protein (AIP) gene. The AIP-positive families have a distinct phenotype with younger age at diagnosis and a predominance of somatotroph and lactotroph adenomas. In the AIP-negative group patients present with wider age-range at diagnosis and a more varied adenoma types. No causative gene has been identified.
We have identified four FIPA families in Hungary to date. Family 1 has two adult-onset (77 years and 54 years) acromegaly in the proband and her first cousin, twice removed. The proband responded well to primary medical treatment with somatostatin analogues. In Family 2 acromegaly was diagnosed in the proband at the age of 68 years and prolactinoma in his 34-years-old nephew. Families 3 and 4 have prolactinoma in two brothers and in a grandmother–mother–granddaughter triplet. None of these families harbour an AIP mutation. Harvey Cushing in 1910 has described a family with gigantism from Hungary, but their family members, if alive, have not been identified yet.
Current data suggest that familial pituitary adenomas are diagnosed at higher frequency than previously thought. Detailed family history helps to identify candidates for genetic and clinical screening in order to achieve early diagnosis and treatment at an earlier stage of the pituitary tumor.
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## P362

**Hypopituitarism in an adult patient as the first sign of langerhans cell histiocytosis**

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Langerhans cell histiocytosis (LCH) is a rare entity characterized by clonal proliferation and accumulation of cells resembling the epidermal dendritic cells called Langerhans cells (distinct margins, pink granular cytoplasm, Birbeck granules at electron microscopy and CD1 positivity by immunocytochemistry). These cells in combination with lymphocytes, eosinophils ad normal hystiocytes form the typical LCH lesions. About half of affected patients have extraskeletal manifestations, including involvement of the hypothalamus–pituitary axis, lung, heart, retroperitoneum, skin, liver, kidneys and spleen. There is an ongoing debate on the exact pathogenesis of this disorder and on its classification as reactive versus neoplastic.
This report describes the case of a 54-years-old man who presented with hypogonadism, central hypothyroidism, diabetes insipidus and GH deficiency ten years ago. At the time of diagnosis a thickening of the pituitary stalk, with a slight compression of the posterior region of the optic chiasma, was recorded at MRI images. A moderate hyperprolactinemia was also present. At that time a work-up for granulomatous or infectious diseases was negative. Nearly one year ago, the patient experienced skin involvement (skin biopsy of the perianal region positive for LCH) and massive bone involvement (sclerotic infiltration of vertebral bodies, femoral and tibial metaphysis and diaphysis). At the present time the patient is doing well after two cycles of chemotherapy. MRI imaging is still stable.
Hypopituitarism may be the first sign of a so far undiagnosed extracranial disease; mainly inflammatory or granulomatous disease should be carefully considered in differential diagnosis.
Declaration of interest
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## P363

**A case of cyclic Cushing's syndrome which was induced remission dramatically by dopamine agonist**

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Cyclic Cushing's syndrome (CysCS) is one of the peculiar types of Cushing's syndrome, characterized by the iteration between remission and recurrence. The precise mechanism in which the hormonal activity is changed suddenly has not been determined, while several hypotheses have been presented. Here, we report a case of CysCS which was induced remission dramatically by dopamine agonist. A 67-years-old woman was pointed out increasing ACTH and cortisol (F) levels during the examination for hyperglycemia and hypokalemia. Her ACTH and F were not decreased in midnight, and not suppressed by high dose dexamethasone. Then she was diagnosed as ACTH dependent Cushing's syndrome. Although cavernous sinus sampling suggested ectopic producing of ACTH, general whole-trunk CT, MRI, and FDG–PET could not detect any ACTH producing tumor. Interestingly excess ACTH and F were normalized dramatically and completely by the short-term treatment with cabergoline (Cab), potent dopamine receptor type 2 (D2R) agonist, where after they were kept in normal range for several months without any treatment. Similar episodes were recurred three times for recent 3 years, suggesting CycCS. Every active phase was terminated by D2R agonists, while abnormal secretion of ACTH was induced by domperidone (Dpm), D2R antagonist, even in the remission phase.
The clinical course of this case suggests D2R agonists are effective in the treatment of ACTH dependent Cushing's syndrome. In addition, this case suggests D2R might be involved in the conversion between remission and recurrence of CycCS, although the mechanism is unclear.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P364

### Antinociceptive effect of pasireotide on octreotide-resistant acromegaly-related headache

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Background
Headache often occurs as incapacitating symptom among the patients with acromegaly. GH involvement in the pathophysiologic mechanism and an analgesic effect of somatostatin analogues has been described, but the exact mechanism is not clear. Whether the pan somatostatine (sst)-receptor agonists are superior as concern the antinociceptive effect than the more selective ones is not evidenced.
Case report
A 21-year old woman, diagnosed with acromegaly, presented with visual disturbances and incapacitating headaches lasting for 8 months (MRI: pituitary macroadenoma with supra- and parasellar propagation). Several daily attacks of headache were resistant to high doses of pain-killers. Preoperative neuroendocrine values on oral contraceptives: elevated mean spontaneously GH and insulin-like growth factor (IGF1) (Table 1. apr-09); low-normal free $T_4$ (13.4 pmol/l (14–23 pmol/l)); low levels of FSH $<0.2$ IU/l and LH $<0.1$ IU/l; low-normal response to Synacthen test (peak cortisol: 904 nmol/l). After two surgeries in 2009, tumour size was significantly reduced. Different combinations and dose concepts of octreotide, sandostatin LAR and somavert were applied but IGF1 remained high and the headaches worsened. Pasireotide–pegvisomant combination was introduced with prompt marked headache-relief, persisting until few days before next injection. IGF1 normalized for the first time (Table 1. apr-11).
Conclusion
Pasireotide may have a superior antinociceptive effect, as compared to other somatostatin analogues. Very possibly pan-sst receptor agonists may be superior to selective sst2 ones regarding their antinociceptive effect, but further studies are needed to clarify this.

**Table 1** Overview of disease activity and medical therapy

| Date | Therapy for acromegaly | GH (mIU/l) | IGF1 (ng/ml) | IGF1 SD |
|---|---|---|---|---|
| Apr-09 | None | >120 (S) | 1249 | +9 |
| Sep-09 | None | >120 (S) | 1600 | +10.68 |
| Jan-10 | Octreotide 100 μgx2 Sandostatin LAR 20 mg/month | 72.9 (OGTT) | 1399 | +9.69 |
| Apr-10 | Octreotide 100 μg at onset of headache Sandostatin LAR 30 mg/month | 36.3 (OGTT) | 1418 | +9.90 |
| Aug-10 | Octreotide same Sandostatin LAR 30 mg/2 weeks Somavert 15 mg/day | 71.1 (S) | 1259 | +9.06 |
| Oct-10 | Octreotide 100 μg up to 12/day Somavert 15 mgx2 Ipstyl 120 μg/2 weeks | 47.7 (OGTT) | 583 | +4.34 |
| Apr-11 | SOM230 40 mg/month (introduced in Jan-11) Somavert same | 33.6 (OGTT) | 278 | +0.89 |
| July-11 | SOM230 40 mg/26 days Somavert same | 39.06 (S) | 246 | +0.45 |
| Dec-11 | SOM230 same Somavert same | 21.2 (OGTT) | 342 | +1.91 |

(S): mean spontaneous GH concentration; (OGTT): lowest GH concentration during oral glucose tolerance test.

Declaration of interest
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## P365

### Giant cystic pheochromocytoma in an asymptomatic man

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Introduction
Cystic adrenal incidentalomas are relatively rare. Large cystic pheochromocytomas are extremely rare and there are few reports of them being clinically silent.
Case presentation
We report the case of a 57-years-old man, who was admitted to our clinic for evaluation of a large cystic lesion that was found ultrasonographicaly in a typical check-up. We ordered a CT that revealed a giant right adrenal cyst ($91.3\times96.8\times92.1$ mm) with thick wall (2–5.5 mm) and no enhancement after i.v. contrast agent infusion. Before admitting the patient for surgery he was evaluated for pheochromocytoma even if he was totally asymptomatic and his blood pressure was normal in multiple measurements. The results from two 24 h urine collection specimens were a surprise: metanephrine 1800–1132 μg/24 h (normal range 20–350), epinephrine 168–130 μg/24 h (<20), norepinephrine 163–239 μg/24 h (<110), normetanephrine 1697–2171 μg/24 h (30–600), dopamine 351 μg/24 h (<600). Evaluation for multiple endocrine neoplasia type 2 syndrome was negative. The patient was preoperatively prepared with very low dose of phenoxybenzamine and propranolol. Right adrenalectomy was performed and intra- and postoperative course was uncomplicated. The surgical specimen revealed pure cystic pheochromocytoma.
Conclusion
Large cystic pheochromocytomas are very rare and often asymptomatic. Pheochromocytoma should always be ruled out in patients presenting with cystic adrenal incidentalomas, even in the absence of hypertension or other symptoms.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P366

### Unusual presentation of hypopituitarism

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A 37-years-old man was referred to rheumatology with a 3 month history of widespread joint pains, worse on rising in the mornings with early morning stiffness lasting about 15 min. Hands were affected first followed by knees and hips. On further questioning, he also complained of generalised fatigue, loss of libido and thinning of the hair on his chest, as well as 15 kg weight loss over 6 months. He recalled a febrile illness with headache prior to the onset of his symptoms, but had no ongoing headache or visual symptoms.
On examination there was no evidence of joint synovitis. Blood testing done by his GP 2 months prior to referral showed: TSH 0.07 μU/ml (0.35–6.9), free $T_4$ 6.0 pmol/l (5.6–21) and free $T_3$ 5.1 pmol/l (3.7–7.0). ESR was mildly elevated at 12 mm/h and CRP was 8.0 mg/l. There was a mild normocytic anaemia (Hb 12.1 g/dl). On repeat testing 1 month later TSH was 0.1 μU/ml, free $T_4$ 3.0 pmol/l, free $T_3$ 3.0 pmol/l. 9 am blood tests were organised for the following day which showed the following: cortisol 16 nmol/l (138–690), free $T_4$ 3.0 pmol/l, testosterone <0.3 nmol/l (10–35), LH 0.4 IU/l (2–18), FSH 1.3 IU/l (1–16), prolactin 185 mIU/l (50–500), glucose 5.2 and IGF1 25 μg/l (75–344). X-ray of the joints were normal.
MRI pituitary confirmed a pituitary macroadenoma. Formal visual perimetry was normal. He was subsequently commenced on Hydrocortisone therapy and two weeks later on thyroxine replacement with resolution of joint symptoms. He is awaiting transphenoidal adenomectomy.
Discussion
There are reports of hypopituitarism presenting with musculoskeletal symptoms. These have been attributed mainly to hypothyroidism and hypoadrenalism. Hypothyroidism, as in this case, can present with polyarthralgia/polyarthritis or myalgia/myositis which may be associated with elevated muscle enzymes. Hypothyroidism is also associated with calcium pyrophosphate crystal deposition in joints (presenting as acute pseudogout or a chronic arthropathy) and carpal tunnel syndrome. Hypoadrenalism can produce various nonspecific symptoms including fatigue and myalgias which could lead to patients being referred for rheumatological assessment.
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## P367

### Rhabdomyolysis and myopathy in Addison's disease: is there a connection?

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Introduction
Rhabdomyolysis coexisting with Addison's disease is a rare condition and the mechanism is not clearly defined. We report a patient presenting with sever rhabdomyolysis which resolved after steroid replacement therapy.

Case report
A 33-year-old man without accompanying comorbidity, presented with progressive myopathy and fatigue. He was hypotensive (RR 90/70), hypovolemic with hyponatremia (125 mmol/l, range 137–146), elevated serum CPK (12 560 U/l, range <177) and high serum (3003 μg/l, range 28–72) and urinary myoglobin (384 μg/l, range <30). The muscle biopsy and tests of humoral and cellular immunity were normal. The hormonal findings confirmed adrenal insufficiency: serum cortisol (33 nmol/l (0800 h) <20 nmol/l (0500 h), range 138–800), ACTH 415.7 pmol/l (range 2.0–13.3), serum aldosterone 28 pmol/l (range 105–868), urinary aldosterone <1.0 nmol/24 h (range 6.23–59.3), renin >300 pg/ml (range 3.5–65.5) (upright posture). The absence of neurological symptoms and a prompt remission following steroid administration were against adult-onset form of adrenoleukodystrophy. The PPD probe, chest X-ray and CT scan of adrenal glands were normal. The etiology of Addison's disease was problably autoimmune; this hypothesis is supported by a positive anti 21-OH autoantibody. Normal values of testosterone, gonadotropins and blood glucose suggested that no other glands were involved in the autoimmune disease. The patient was treated with a high (200 mg), gradually decreasing dose of hydrocortisone i.v., then with 20+10 mg p.o. Finally, two months later, all laboratory signs of rhabdomyolysis disappeared with complete clinical recovery.
Conclusion
The association between Addison's disease and rhabdomyolysis is not clearly understood. Hyponatremia has been blamed for myopathy due to maladaptive cellular mechanism in response to fluid hypoosmolality. Myopathy was described in hyponatremia resulting from water intoxication, but, in spite of hyponatremia we were unable to detect a syndrome of inappropriate ADH secretion. Possibly, a glucocorticoid deficiency per se, by a mechanism unknown, could be involved in rhabdomyolysis. This case suggests that, in patients with rhabdomyolysis, an adrenal failure should be considered. It also raises the question of potential benefit of a glucocorticoid administration in other forms of rhabdomyolysis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P368

**Efficacy of tolvaptan treatment in a patient with syndrome of inappropriate antidiuresis (SIAD) after severe traumatic brain injury**

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Tolvaptan, an oral antagonist of the vasopressin V2 receptor, has been found to improve hyponatremia in patients with SIAD. We report the case of a 65-years-old male, who developed recurrent episodes of hyponatremia after severe traumatic brain injury, TBI, with polytrauma (CGS=8, decompressive craniectomy, spontaneous breathing by percutaneous tracheotomy). During hospitalization in intensive care unit he resumed alertness and received i.v. hypertonic saline infusion and oral fludrocortisone therapy for hyponatremia. At admission to the intensive rehabilitation unit (1 month after TBI) he was on fludrocortisone therapy and i.v. sodium supplementation, and presented an extremely severe disability (Functional Independence Measure, FIM=18/126; Disability Rating Scale, DRS=21/30; Levels of Cognitive Functioning, LCF=3/8), needing enteral nutrition. After discontinuing sodium supplementation, hyponatremia recurred (125 mEq/l); at the same time the patient underwent ventriculo-peritoneal shunt for hydrocephalus and presented seizures requiring antiepileptic drug (levetiracetam). Serum sodium continued to fluctuate for 2 months, despite fludrocortisone therapy, prolonged sodium supplementation (i.v. or enteral) and/or water restriction. Hypoadrenalism and hypothyroidism were excluded and SIAD was diagnosed, after endocrinologist consultation. Sodium returned to normal values and remained stable for 3 months. A sudden drop in sodium levels again required hypertonic saline infusion. Diagnosis of SIAD was confirmed and therapy with oral tolvaptan was started (SAMSCA 15 mg/die for one month, then 7.5 mg/die). Serum sodium rose to >135 mEq/l in three days and remained stable throughout treatment for five months until now. No side effects were observed, seizures disappeared, antihypertensive drugs were discontinued due to normal blood pressure levels. At discharge from inpatient rehabilitation, cognitive and motor functioning scales improved (FIM=35, DRS=16, LCF=4), but the patient still presented severe disability. This case demonstrates that tolvaptan is safe and effective in the treatment of hyponatremia due to SIAD after severe TBI.

Declaration of interest
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## P369

**A rare case of primary tubercular pituitary abscess in a diabetic patient**

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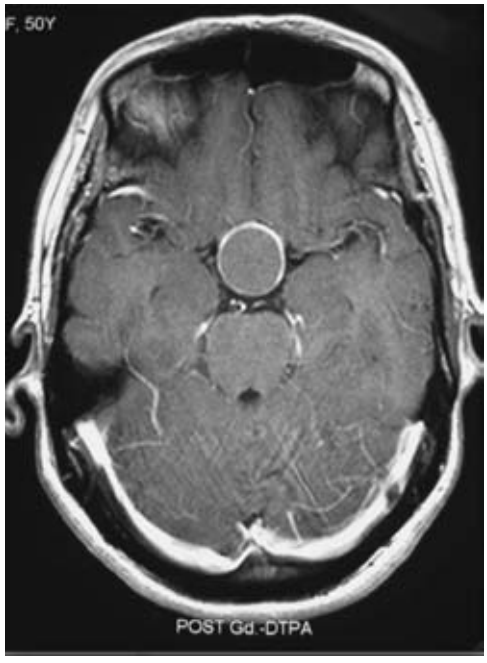
Introduction
Primary tubercular pituitary abscess is an extremely rare disease. Only few cases had been reported and the diagnosis was reached only after surgery. There is a need to distinguish between a tuberculoma, pituitary abscess and other functioning and nonfunctioning pituitary adenoma to direct our management into either medical or surgical management, especially here in the Philippines where tuberculosis is an endemic disease.
Case
We present a case of a 50-years-old diabetic Filipina who came in for complains of gradual loss of vision, bitemporal, since four months prior to admission. This was associated with frequent headaches, polydipsia and polyuria. No previous history of pulmonary tuberculosis. Physical examination and vital signs were normal except for hemianopsia. Chest X-ray was normal. Perimetry exam revealed temporal hemianopsia on the right, and almost complete temporal anopsia, left. MRI showed a relatively large 2.8 cm cystic-appearing mass involving the sellar and suprasellar compartments compatible with a cystic adenoma or a craniopharyngioma. Serum $FT_4$ was 10.5 pmol/l (11–24), TSH 1.4 mIU/l, serum prolactin 2404.2 mIU/l (90–500) and serum cortisol at 409.8 nmol/l (154–638). The patient underwent transphenoidal pituitary surgery and intraoperatively the sellar floor was noted to be thinned out with noted egress of a purulent material during opening of the dura. Staining of the purulent material was positive for acid fast bacilli. Anti Kochs medication was started, together with vancomycin and cefepime. Postoperatively, patient developed diabetes insipidus, but there was significant improvement in vision.
Learning points
Always consider tubercular pituitary abscess in a diabetic patient living in a tuberculosis endemic area and presenting with a sellar suprasellar mass.
MRI of the brain showing a 2.8 cm cystic mass involving the sellar and suprasellar compartments causing severe compression of the overlying optic apparatus.



Declaration of interest
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## P370

### Central diabetes insipidus as first sign of progression to accelerated phase in a chronic myeloid leukemia patient

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Introduction
Pituitary involvement has been rarely reported during the course of hematological diseases like thalessemia, Langerhans-cell histiocytosis and lymphoma. We here present a case of chronic myeloid leukemia (CML) with pituitary involvement, in which central diabetes insipidus (DI) was the first sign of progression to accelerated phase.
Case report
A 61-year-old man was consultated by our clinic due to polyuria with 6000 ml daily urine output. His past medical history revealed CML which was in complete remission for 2 years. He had signs of dehydration, orthostatic hypotension and splenomegaly. Laboratory tests demonstrated severe hypernatremia, decreased urinary and increased serum osmolarities (Table 1). Water deprivation test was not held due to severe signs of dehydration. Desmopressin was started with a presumptive diagnosis of DI after which urine output and biochemical tests returned to normal. Pituitary MRI revealed thickened enhancing pituitary stalk and loss of the bright spot of the neurohypophysis (Figure 1). Patient was referred for hematological reevaluation. Reappearance of the Philadelphia chromosome and increased blast ratio in bone marrow was documented which showed progression from chronic remission phase into accelerated phase.
Conclusion
Diabetes Insipidus has been reported in patients with acute leukemia or myelodysplastic syndrome and almost always been associated with gravid course. Monosomy 7 and/or inv(3) have been linked with DI in some cases. Direct infiltration by the leukemic cells, hemorrhage, thrombosis and infection have been suggested as the possible factors underlying the pituitary damage. Appearance of DI during the course of hematological malignancies should alert the physician for acceleration or blastic transformation of the disease.

**Table 1** Laboratory tests

| Test | Result | Reference range (unit) |
|---|---|---|
| Sodium | 167 | 136–145 (mEq/l) |
| Potassium | 3.8 | 3.5–5.1 (mEq/l) |
| BUN | 20.8 | 8.4–25.7 (mg/dl) |
| Creatinine | 1.03 | 0.1.3 (mg/dl) |
| Urine osmolarity | 146 | (mOsm/kg) |
| Serum osmolarity | 299 | (mOsm/kg) |
| WBC | 10.5 | 4–10.3 (μl) |

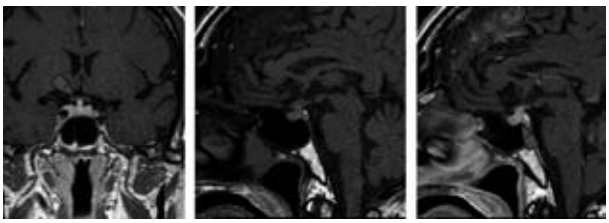

**Figure 1** Pituitary MRI.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P371

### Contrast-enhanced ultrasound supports diagnosis in a patient with paravesical paraganglioma

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Introduction
We report the unusual case of a young patient with micturition induced headache, in which contrast-enhanced ultrasound helped to assure diagnosis of paravesical paraganglioma.
Case
An 18-year-old male presented with attacks of severe headache lasting several minutes and occurring strictly after micturition. Hypertensive values up to 200/105 mmHg were detected subsequent to nearly every micturition while values before and some minutes after it were normal.
A paravesical tumor with regressive changes, measuring 3.7×3 cm, was seen by B-mode ultrasound and confirmed by MRI. Contrast-enhanced ultrasound was performed. After i.v. injection of 2.4 ml SonoVue, the tumor showed an early arterial contrast enhancement with peripheral to central contrast filling and a distinct, irregular hypervascularisation – signs which have been described as typical for pheochromocytomas before.
Plasmametanephrines revealed a distinct elevation of normetanephrine (1934 pg/ml, norm $<180$), so we diagnosed a functional paravesical paraganglioma. The patient had no family history for phaeochromocytoma or other endocrine tumors. A Fluorodopamine (F-Dopa)-PET CT was performed with uptake of F-Dopa confined to the paravesical tumor but not in further locations. After adrenergic blockage with Urapidil the tumor was successfully removed. Mutational analysis of SDHB, VHL, SDHD and RET was performed without any pathologies.
Conclusions
There are only few reports of functional paravesical paragangliomas which are very rare tumors (less than 1% of all pheochromocytomas). However, to avoid serious complications including hypertensive crisis during operation, it is important to consider these tumors in the differential diagnosis of paravesical tumors. Contrast-enhanced ultrasound can be a cost-effective, less-invasive method to support diagnosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P372

### Pituicytoma and meningeoma presenting as suprasellar tumor: case report

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Pituicytoms is a very rare low-grade glioma of the neurohypophysis and can affect both the sellar and suprasellar regions. We describe a case of a suprasellar pituicytoma and mengioma of a patient admitted to our Clinic as a suprasellar tumor patient.
Clinical presentation
A 55-years-old man with a history of persistent headache in the right frontal area for a period of 10 years prior to the admission as well as visual disturbances and decreased libido for 1 year period prior to the admission. The hormonal profile revealed low FSH (0.368 mU/l), LH (0.1 mU/l), testosterone, cortisol (6.34 nmol/l) and PRL (19.39). Magnetic resonance imaging showed a solid homogeneously enhancing suprasellar mass 2.5 cm compressing the optic chiasm. A craniotomia pteryonal l. dex. was performed as well as a subtotal resection of the tumor. The hystopathological finding indicated meningeoma fibroblasticum. Due to the deterioration of the sight a new MRI was performed after 9 months that showed an identical finding of suprasellar macroadenoma. A recraniotomia reg, frontalis l. dex. and reextirpatio tumoris were performed and the hystopathological finding indicated pituicytoma (infundibuloma) Ki 67 $<3\%$.
After
The surgery was followed by a radiation therapy, after which the patient developed symptoms of frontal lobi, diabetes mellitus and diabetes insipidus. The patient died of a heavy adrenalin crisis.
Conclusion
Pituicytoma are rare tumors of the neurohypophysis. The clinical presentation is similar to other non-functional pituitary adenomas and imaging exams may suggest pituitary adenomas. The diagnostic is based on histopatological analysis.
Key words: Hypophysis, Pituicytoma, pituitary tumors, neurohypophyseal tumor.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P373

### A clinical case report of an successful spontaneous pregnancy in acromegaly

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Introduction
Acromegaly is a clinical condition results from GH hypersecretion which is usually elaborated by a somatotroph adenoma. Mass effect of adenoma and resulting hormonal changes impair fertility during pregnancy, estrogen-mediated pituitary enlargement along with enhanced pituitary vascularity is a true risk for tumor hemorrhage.
Case presentation
The 28-years-old patient first visited by the endocrinologist in 2004 for secondary amenorrhea and increased GH level. She was married in 1999 and had a successful spontaneous pregnancy in 2000. She stayed amenorrheic for 5 years after delivery until she made an appointment with the gynecologist. The gynecologist noticed the early clinical and para-clinical features of acromegaly and referred her to the endocrinologist. Radiographic and lab results indicated an active pituitary adenoma. Prednisolone and levothyroxine started and 3 months later trans-sphenoidal surgery performed for tumor resection. Eight months later after surgery neither IGF1 nor GH decreased, so octreotide was prescribed and patient was sent for gamma knife therapy on November 2006. Four months after gamma therapy while she was still taking octreotide, she was became amenorrheic again. Work up started and revealed the pregnancy. Octerotide discontinued. Visual field assessments were normal and MRI did not show any changes in the tumor size. Treatment continued with bromocriptine and levothyroxine. Prednisolone replaced by hydrocortisone stayed under close observation till term. GH, IGF1, and prolactine levels were monitored every 3 months during pregnancy. Suppression test with 100 g glucose were performed. Results were in normal range. MRI in 6th month of pregnancy, revealed slight decrease in the size of tumor. Blood pressure was normal. At the end of 8th month of pregnancy, IGF1 returned to its normal level. Serial sonographies did not reveal any abnormalities. Lipid profile and BS were normal. In 39th week of pregnancy, C/S performed for delivery. The female newborn was healthy.
Discussion
Today with advent of advanced surgical and medical management of these patients, increasing numbers could have successful pregnancy and child bearing.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P374

### Childhood onset hypopituitarism and central apnea: looking beyond hormonal replacement

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Introduction
Childhood-onset hypopituitarism is an uncommon yet increasingly diagnosed disorder. As endocrinologists, we generally deal with the complexities of hormonal replacement and, when required, follow these patients to detect tumor recurrences. However, some causes of childhood- onset-hypopituitarism have comorbidities which can significantly increase morbi-mortality. We present the case of a 21-year-old girl who was diagnosed with ROHHAD syndrome.
Case report
A 3-year-old female child presented to a Pediatric Clinic in Madrid because of rapid-onset-hyperphagia and increased-body-weight. The patient did not receive hormonal assessment at that stage and was discharged with dietary advice. One year later the patient developed episodes of unprovoked respiratory arrest but could be resuscitated. Respiratory assessment revealed central apnea that required ongoing ventilatory support and nocturnal-oxygen airflow. The patient also showed impaired language skills and mild mental retardation, and continued surveillance detected a mediastinal neuroblastoma (successfully resected), and autonomic-dysfunction-related chronic diaphoresis and intermittent diarrhea.
The hyperphagic behaviour persisted and, on reporting growth arrest, the patient, aged 8, was finally referred to a Pediatric Endocrinology Department (PED). Hormonal profile confirmed GH deficiency, hypogonadotropic hypogonadism and secondary hypothyroidism, which were duly replaced. Genetic study did not find PHOX2B mutations, but the patient was nonetheless diagnosed with ROHHAD syndrome on clinical grounds.
The patient is now a 23-years-old adult female who follows daily outdoor activities at a specialised educational centre. She still receives estrogen–progestin replacement, levothyroxine, and she is about to restart GH after her transition from a PED to our Adult Endocrinology clinic was delayed for 2 years.
Conclusion
We present a case of ROHHAD syndrome, an uncommon cause of early-onset-hypopituitarism whose cardinal features include hyperphagia, neuroblastomas, autonomic dysfunction and life-threatening central apnea. Early recognition dramatically reduces morbimortality. These patients also need transition schedules to ensure that proper surveillance strategies continue through adult life.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P375

### Cystic pituitary macroadenoma complicated by recurrent mucocoeles: a rare case report

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Introduction
Non-functioning pituitary adenomas, usually macroadenomas, represent around 30% of pituitary tumors. Patients often present with signs of mass effect and symptoms of pituitary insufficiency. We report a complicated case of a cystic pituitary 'macroadenoma' presenting with recurrent mucocoeles likely due to underlying sinusitis.
Case report
A 42-year-old Caucasian female presented with chronic headaches and unilateral temporal visual field defect. She also reported secondary amenorrhoea, intermittent galactorrhoea, polydipsia with polyuria. Investigations confirmed a cystic pituitary macroadenoma with chaismal compression associated with hyperprolactenaemia (adjusted PRL 1245, normal range $<400$). Rest of the baseline pituitary profile was normal. Histology following transsphenoidal surgery was suggestive of a necrotic tumour of unknown aetiology. Postoperative MRI scan showed a thickened stalk with crescentric residual tissue. She later developed sphenoid sinusitis requiring ENT intervention. Postoperatively, she presented with two further episodes of severe headaches with recurrent pituitary mucocoeles warranting further neurosurgery. On at least one occasion sterile purulent fluid was aspirated. Given the suspicion of sphenoid sinusitis causing recurrent mucocoeles, she received a prolonged course of intravenous antibiotics. She recently underwent radical excision of the inflamed sinusoidal mucosa with obliteration of the sinus with a fat graft. Since her last presentation with a pituitary mucocoele, she has had no further recurrence over past 22 months.
Conclusion
Recurrent pituitary cysts are usually craniopharyngiomas or remnants of Rathke's cleft. Infected pituitary cysts or abscesses are rare, characterized by systemic signs of toxaemia, mass effects due to enlarging pituitary and/or associated endocrine dysfunctions. Diabetes insipidus on presentation, has also been reported in the literature.The infection usually extends from paranasal sinuses, whereas involvement of the pituitary gland in systemic sepsis is quite rare because of effective blood brain barrier.
Hence we highlight the role of both medical and surgical intervention in the management of a rare and complicated case of a pituitary adenoma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P376

**A patient with TSH-secreting pituitary macroadenoma, after previous thyroid ablation, successfully treated with long-acting octreotide formulation (octreotide-LAR) and transsphenoidal surgery**

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Introduction
Thyrotropin (TSH)-secreting pituitary adenomas are rare (<1% of all pituitary tumors) and cause secondary hyperthyroidism.
Case report
A 33-year old woman with type 1 diabetes mellitus presented in 2009 with palpitations. She had a past medical history of radioiodine therapy for thyrotoxicosis in 1998. After radioiodine treatment, she received an increasing daily dose of thyroxine (from 50 to 175 μg) because of her gradually rising serum TSH concentration.
Initial findings were: elevated serum TSH of 14.6 mIU/l (normal range: 0.27–4.2), elevated free-triiodothyronine ($fT_3$) concentration of 4.3 pg/ml (normal range: 1.64–3.45) and elevated free-thyroxine ($fT_4$) concentration of 1.87 ng/dl (normal range: 0.71–1.85). Thyroid antibodies were within normal ranges. After administration of 200 μg of thyrotropin-releasing hormone, TSH rose slightly from 24.9 to 31.26 mIU/l at 30 min. and to 29.14 mIU/l at 60 min. Alpha-subunit was 18 mIU/ml (normal range: 0.0–1.0). Other pituitary hormones and IGF1 were within normal ranges.
Magnetic resonance imaging (MRI) revealed a pituitary macroadenoma (13×12.5×10 mm) infiltrating the left cavernous sinus.
The patient was initially treated with octreotide-LAR (20 mg i.m. every 28 days) for ten months. During therapy serum TSH levels were reduced by more than 50%. No significant change in pituitary tumor volume was observed in MRI performed after octreotide-LAR treatment. In January 2011 the patient underwent uncomplicated transsphenoidal surgery, and immunohistochemical staining showed positive reactivity for TSH. Early postoperative TSH concentration was low (0.072 mIU/l) and became normal in 5 weeks. In subsequent months the patient remained euthyroid on 125 μg thyroxine daily. Pituitary MRI performed during one year follow-up did not reveal tumor recurrence.
Conclusions
We report for the first time the data on preoperative somatostatin analogue treatment of thyrotropinoma in a patient who previously underwent thyroid ablation by radioiodine. Since aggressive and invasive macroadenomas are more frequently observed in these patients, we suggest that preoperative treatment with somatostatin analogues should be mandatory in these patients.
Declaration of interest
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## P377

**Synchronous papillary and medullary thyroid carcinoma in an acromegaly patient**

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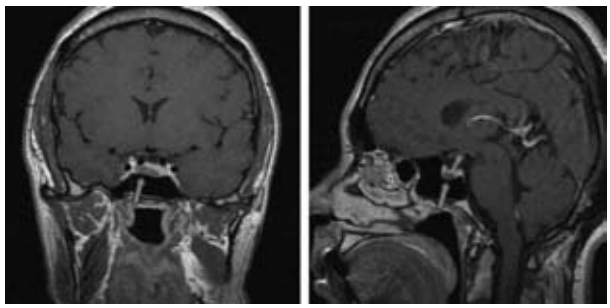
Introduction
The increased mortality in acromegaly is mainly related to cardiovascular, respiratory and neoplastic complications; neoplasms being responsible in 15%. Although most of the thyroid nodules in acromegaly are benign, there are studies revealing an increased ratio of differentiated thyroid cancer in this disease. Medullary thyroid carcinoma (MTC) has not been related with acromegaly so far. In this report, we aimed to present a patient with acromegaly whose thyroid pathology revealed coexisting medullary and papillary thyroid carcinoma (PTC).
Case report
Fourty nine year old woman had been operated for suspicios thyroid cytology after being evaluated for euthyroid multinodular goitre. Total thyroidectomy had been performed and her pathology revealed medullary carcinoma (2.3×2.3 cm) and multifocal papillary carcinoma. After ablative radioactive iodine therapy, she had been referred to our institution for thyroid hormone supression therapy. During the follow up, she complained of acral enlargement and weight gain. She had progressive coarsening of the facial features which reminded us acromegaly. Diagnostic tests consisting of basal and post-glucose load growth hormon, age adjusted insulin like growth factor 1 (IGF1) confirmed the diagnosis of acromegaly. Pituitary magnetic resonance imaging (MRG) showed a macro-adenoma (19×9×7 mm) in the right part of the pituitary. The patient has been referred to neurosurgery clinic for pituitary surgery.
Conclusion
MTC may be seen as a part of multiple endocrine neoplasia (MEN) type 2. Acromegaly may seldomly appear as a MEN1 component. Rarely, components of MEN1 and MEN2 coexist in a single patient simultaneously. Coexistance of acromegaly, MTC and PTC has not been reported in literature so far. Further analysis is needed to explain pathogenetic mechanism of this coexistence.
Pituitary MRI revealing adenoma



Declaration of interest
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## P378

**Pregnancy in an acromegalic woman: a case report**

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Introduction
Acromegaly is a rare disease caused by an increased secretion of GH, usually derived from a benign pituitary tumor. Women with acromegaly are usually infertile, because of tumoral hypersecretion, compression or surgical sequelae. We can prevent infertility with an adequate treatment. There are some reports describing pregnacies among acromegalic women. It is believed that there is an increased risk of devellopping diabetes mellitus and hypertension, especially when there is an active or uncontrolled acromegaly, and a symptomatic tumor size increase during pregnancy.
Case Report
Thirty five year-old woman, with acromegaly diagnosed when she was 24: IGF1 = 1305 ng/ml (*n*: 75–780); GH = 78 ng/ml (*n*: 0–18); prolactin = 38.8 ng/ml (*n*: 0–25). The sellar magnetic resonance imaging (MRI) showed an expansive rounded sellar lesion (20×20×20 mm), with supra-sellar expansion, optic chiasm stretch and left stalk deviation. She underwent transphenoidal adenomectomy on 20/09/2001 (9 months after diagnosis). The histology confirmed a GH producing adenoma. She wasn't cured by surgery (GH = 8.8 ng/ml (*n*: 0–8.6); IGF1 = 1355 ng/ml (*n*: 150–780)), so she started on medical treatment with octreotide-LAR (20 mg), once a month, on 25/01/2002, becoming assymptomatic. Seven and nine years after surgery, she had two term pregnancies, both delivered by elective caesarean, normossomic newborns, without malformations or neonatal complications. Octreotide was stopped once the pregnancy diagnosis was made. The patient had neither gestational diabetes nor hypertension. There was no sellar changes on MRI and no changes in visual fields. The levels of GH ranged between 1.47 and 8.68 ng/ml and the levels of IGF1 between 188 and 804 ng/ml throughout pregnancies.
Conclusion
As described in the literature, in this patient, acromegaly treatment kept the patient fertile, allowing two pregancies without complications, with normal neonates. Even with the use of octreotide-LAR until the knowledge of the pregnancy diagnosis, there weren't malformations in both neonates or other complications.
Declaration of interest
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## P379

**Acromegaly, primary hyperparathyroidism and renal cell carcinoma**

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Introduction
Acromegaly is a rare disorder and sporadic cases are the most frequent. It can also occur in association with genetic syndromes, such as Carney complex, multiple endocrine neoplasia type 1 (MEN-1), MEN-1 like syndrome, isolated familial acromegaly and familial isolated pituitary adenomas.
Case report
Thirty five -year-old man was observed in 1994 for gynecomastia. Acromegaly was diagnosed based on clinical, biochemical (IGF1 1094.7 ng/ml, baseline GH 48.0 ng/ml) and imaging criteria. He was submitted to complete transsphenoidal resection of a macroadenoma in 7/6/1995 (staining for GH and prolactin) and reached surgical cure. In 2001 primary hyperparathyroidism was diagnosed and subtotal parathyroidectomy was performed. Biochemical and imaging screening of MEN-1 was negative, as well as the genetic test. Acromegaly biochemical recurrence was detected in 2003 but MRI showed no tumor mass. He was started on octreotide LAR (10 mg s.c. monthly) and IGF1 and GH levels normalized. Octreotide was stopped in 2008. In 2010 a kidney mass was detected and the cytology showed a papillary renal cell carcinoma. He underwent left nephrectomy in 8/9/2010. Currently, the patient maintains acromegaly cure criteria and no evidence of hyperparathyroidism or renal cell carcinoma recurrence.
Discussion
This is an unusual case of acromegaly associated with hyperparathyroidism and renal cell carcinoma. Although there is no family history of pituitary tumors and the search for MEN-1 mutation was negative we can't exclude a genetic syndrome. MEN-1 like syndrome remains a possibility but the search for the CDKN1B was not performed. Renal cell carcinoma was described in 0.5% of patients with acromegaly and its screening in these patients is not recommended. This case highlights the need for constant monitoring of acromegalic patients even when apparently cured. The search for GH and IGF1 receptor expression in neoplastic tissues of acromegalic patients could improve our understanding of cancer development mechanisms in these patients.
Declaration of interest
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## P380

**Late diagnosis of adrenal tuberculosis**

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Introduction
Tuberculosis is an insidious disease and may be diagnosed after it afected many organs.
Case presentation
We present the case of a 56 years old female, who followed antituberculosis treatment in 2003 for pulmonary tuberculosis and during the treatment developed acute renal failure and for this reason the treatment was stopped. During the evaluations a left kidney tumor was detected. Left nephrectomy was performed in 2003 (HP examination – angiomyolipoma), and left suprarenalectomy in 2003 for CSR non-secretive adenoma. In March 2007 she was diagnosed with a non-secretive adrenal tumor of 3/2/2.5 cm at the level of right adrenal gland. The patient was evaluated by PET with FDG imaging in August 2008, which raised suspicion of malignancy, and afterwards a transcutaneous biopsy was performed which showed areas of necrosis. In January 2010 the patient developed adrenal insufficiency. Because the adrenal tumor formation was in dimensional evolution, the PET examination and biopsy raised suspicion of malignancy and the patient developed adrenal insufficiency, surgery was decided. Intervention by laparoscopy was performed and an intensive process of left supra and inframezocolic perivisceritis was outlined. The adrenal gland with a volume enlarged to 10/8 cm presented an 8/2 cm tumor, hard, whitish, heterogeneous across the section, without invasion of adjacent structures. Histopatologic examination was suggestive for adrenal tuberculosis. Consequently, the previous histological pieces were reassessed and the conclusion was of tuberculosis with multiple determinations: adrenal (bilateral), peritoneal and retroperitoneal. Antituberculosis treatment was decided, the patient having a favorable evolution. Unfortunately, the diagnostis was performed after many years of tuberculosis evolution.
Conclusions
Tuberculosis remains a common cause of adrenal failure especially in patients who have not followed full treatment. Histopathological evaluation is very important for correct diagnosis in order to determine therapeutical aproach and the subsequent evolution and prognosis of these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P381

**Spontaneous remission of acromegaly due to pituitary apoplexy: case report**

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Introduction
Pituitary apoplexy is a rare acute complication in patients with functioning pituitary adenomas (including acromegaly) often followed by hypopituitarism and occasionally may show remission of symptoms after an apoplectic episode. We report this in an acromegalic patient who developed remission following pituitary apoplexy.
Case report
A 24 years old woman is diagnosed with acromegaly after presenting for a period of 6 months moderate headache, subtle increase in her shoe size, mild soft tissue swelling, secondary amenorrhea, bilateral galactorrhea, no visual disturbances. Pituitary MRI revealed a macroadenoma with suprasellar extension. Laboratory tests sustain the diagnosis: nonsupressive GH level in glucose tolerance test (23.4 –19–18.1–15.7–13.9 ng/ml), high IGF1 929 ng/ml, hyperprolactinemia 126 ng/ml but no hypopituitarism. Within 2 weeks from the diagnosis, just before her schedule for surgery that was no longer performed, she presented an episode of severe headache not associated with other neurological signs, subsequently she noticed a reduction of swelling, with progressive remission of the headache and of galactorrhea, restoring of her menses and developed preprandial hypoglycemia (up to 33 mg/dl). Pituitary MRI 2 weeks after the acute episode, revealed reduction of the macroadenoma with peripheral hemorrhage areas. GH values in GTT were suppressed $<1$ ng/ml, IGF1 was low 87.11 ng/ml, normal prolactin, no gonadotrope, thyreotrope and corticotrope insufficiency; same results at 3 months reassessment.
Conclusion
We conclude the results as pituitary apoplexy in a patient with GH secreting pituitary macroadenoma subsequently only with somatotrope insufficiency (explaining the hypoglycemia too). Although apoplexy may occur with apparent precipitating factors, in our case, spontaneous apoplexy due to hemorrhage was diagnosed; no precipitating factors were determined.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P382

**Oligosymptomatic paraganglioma in the setting of neurofibromatosis type 1**

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Neurofibromatosis type 1 is an autosomal dominant genetic disorder that results from loss of function of the neurofibromin gene, leading to failure of a tumor suppressor mechanism. Usually characterized by 'café-au-lait' spots, cutaneous neurofibromas, and a predisposition for tumorigenesis occurring in childhood,

NF1 can have variable clinical expression, with 'mildly symptomatic' cases eluding diagnosis until adulthood.
A 53-year-old woman with mild clinical stigmata but no previous diagnosis of NF1, presented for endocrine assessment after the surgical excision of an oligosymptomatic pheochromocytoma. The voluminous (10.8 cm) cystic mass, located above the left kidney, was discovered incidentally on abdominal ultrasonography, confirmed by computed tomography and then surgically excised. The pathology report established pheochomocytoma, and serial imagining over the next year showed an expanding adrenal remnant (3.3–4.6 cm). At initial endocrine evaluation, the patient was hypertensive (150/90 mmHg) despite medication, but stable and without the 'adrenergic spells' of a pheochromocytoma. She presented with multiple 'café-au-lait' spots, freckling in the axillary and inframammary areas. Her daughter was diagnosed in childhood with NF type 1. Blood assays documented the normetanephrine hypersecretion (metanephrines = 14–44 pg/ml, normetanephrines = 436 pg/ml, chromogranin A = 40 ng/ml), which suggested a paraganglioma. Normal PTH and calcitonin levels excluded MEN type 2. After adequate alpha followed by beta blocade the patient undewent complete left adrenalectomy, with biochemical and morphological remission of the pheochromocytoma for 1.5 years post-operatively. She remained hypertensive but with more easily controlled BP levels (probably essential hypertension). As screening for the endocrine features of NF1, she was tested for carcinoid tumors and hypothalamic-pituitary dysfunction but had normal serotonin levels and pituitary function.
Atypical in the context of a disorder involving predominantly the skin, skeleton and nervous system, and unusual in an undiagnosed adult patient, our case illustrates the rare, clinically mild, but possibly severe endocrine complications of NF1.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P383

**Histiocytosis X-manifested with insipid diabetes clinical case**

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Histiocytosis is a group of syndromes /synonyms: eosinophilicgranulomatosis, pulmonary Langerhans cell histiocytosis, fibrous lung disease, disease of smokers, non-lipid reticuloendotheliosis; disease of Hand–Schuller–Christian; disease of Letterer–Siwe/. It is a rare disease and its real frequency is unknown. In a reference center in the U.S. the disease is found in 5% in patients with lung biopsy carried out in connection with the diagnosis of interstitial lung disease.
In adults inflammation leads to a loss of elasticity (fibrosis) and destruction of the walls of pulmonary alveoli. In some patients spontaneous improvement (remissions) can be observed, while others progress to an end-stage fibrotic lung disease.
A woman, 32, was born prematurely with a congenital atresia of the esophagus, with operational adjustment and plastic of the esophagus at an early age. Her development was according to the age. From the summer of 2009 complaints of polydipsia and polyuriaappeared – up to 7 l/day, headaches and dizziness, impaired memory, amenorrhea. MRI showed a tumor in chiasmaopticum and pressing on the third ventricle. Operation was madeon 05.02.2010 – right-sided craniotomy and partial extirpation of the tumor. Histological result-Langerhans histiocytic tumor cell / tests: plasma cortisol −54.6 in the morning at 0800 and 2200 h in the evening, 75.9 (normal 70–240 IU/l; prolactin −54 IU/l; LH −0.048 IU/l; FSH −0.06 IU/l). Concentration of sample Zimnitski-diuresis with relative weight -1000.
In the course of treatment there was a sequential adjustment of hipocorticismand hypothyroidism, electrolyte abnormalities. PET/CT did not reveal any dissemination process.
We present a clinical case of isolated hypothalamic–pituitary disease a form of insipid diabetes, panhypopituitarism, foudroyant stroke and death.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P384

**A case history as example for diagnostics and treatment of acromegaly: started and ended with pregnancy**

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The history of a female patient is demonstrated as an example of diagnostic and therapeutical pitfalls in acromegaly. The patient was first operated on a pituitary macroadenoma at her age of 21. Prior to surgery, serum prolactin was found moderately elevated and the patient was raromenorrhoic, without galactorrhea. Soon recurrence of the tumour was trialed with bromocriptine medication, however, without any inhibitory effect on the process. The patient underwent a second neurosurgery at the age of 26. Serum prolactin was found normal without bromocriptine thereafter. Her primary sterility was treated with FSH and hCG injections resulting in a healthy trigeminal pregancy. After these preceding events, she first visited the present authors with amenorrhea and unequivocal signs of acromegaly. Diurnal variation of serum hGH and OGTT proved a GH-excess. Chronic administration of somatostatin octreotid LAR was ineffective. A third surgical intervention was performed on the re-recurred macroadenoma at the age of 30. Hormonal supression with lanreotide was not successful either. Further growing of the tumoral remnant was tried to be influenced by gamma knife at the age of 34 followed by pegvisomant administration in order to blunt the clinical effect of the still existing GH excess and in the hope of a GH-decrease as aimed consequence of the latter irradiation therapy. One year after irradiaton and pegvisomant administration, the IGF1 level was in the normal range, and the tumour remnant showed some regression. While continuing the pegvisomant treatment and after more then a decade amenorrhea, the patient conceived. Then, we stopped the pegvisomant administration; during pregnancy no tumour growing was detected with MRI, and the patient gave birth to a healthy boy. An update therapeutical regime may involve neurosurgery, dopamin agonists, somatostatin analogs, irradiation and GH-receptor antagonist alike; an individualized therapy may be sometimes be astonishingly complex.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P385

**Adrenal neurilemoma**

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Introduction
Adrenal neurilemoma (or schwannoma) are vary rare tumors. They usually present as incidental finding in asymptomatic patients, which makes them dificult to diagnose preoperatively.
Case report
We present a 60 year old woman with incidentally discovered adrenal mass of 5 cm on the right side. The tumor was detected by ultrasound and then it was confirmed by MSCT scan. We performed careful endocrine workup. Results showed normal levels of urinary catecholamines that excluded active pheochromocytoma. Midnight cortisol level was in the normal reference range with adequate suppression of cortisol in 1 mg overnight dexamethason suppression test and normal basal level of ACTH. Aldosteron was also in the normal reference range with normal aldosteron to PRA ratio. According to findings of MSCT scan, vena cava and diaphragm were infiltrated by tumor. Surgery was performed. Intraoperative findings showed adrenal tumor of about 6 cm in size. There was no evidence of the abdominal lymphadenopathy or vascular involvement. Tumor was completely and successfully removed with adrenalectomy. Pathohistology and immunohystochemistry of the tumor showed neurilemoma with perineural differentiation (diffusely positive on Vimentine, S100, Ki-67, and focally positive for EMA perineurium). Our patient is in good health recovering from surgery.
Conclusion
Although schwannomas are rare and usually benign, as it was in the case of our patient, malignant potency of these tumors raises complex questions regarding further follow up.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P386

**A case of autoimmune polyglandular syndrome type II presenting with adrenal crisis**

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Introduction
Autoimmune polyglandular syndrome type II is defined by the occurrence of Addison's disease with thyroid autoimmune disease and/or type I diabetes mellitus.
Case presentation
A seventy-four-years old woman came to the hospital due to fatigue, nausea, vomiting and skin hyperpigmentation that had begun two months ago. On clinical examination, the patient had hyperpigmentation throughout the skin, large dark brown areas on the lips and the buccal mucosa and her blood pressure was 90/60 mm Hg. She had recently been diagnosed with hypothyroidism and had been receiving levothyroxine for two days before admission to the hospital. Her past medical history was otherwise unremarkable.
Laboratory tests revealed hyponatremia (serum sodium: 130 mEq/l), hyperkalemia (serum potassium: 5.56 mEq/l and anemia (hemoglobin: 11.8 g/dl).
Morning blood cortisol level was $<0.2$ μg/dl and ACTH levels were 1248.0 pg/ml. Addison's disease was confirmed and antibodies directed against 21-hydroxylase (OH) were positive, a finding suggestive of autoimmune adrenalitis. TSH levels were 6.62 μU/l, $T_3$: 204.5 ng/dl, $FT_4$: 1.02 ng/dl, anti-TPO: 314.6 IU/ml, anti-Tg: 613.6 IU/ml.
Autoimmune adrenalitis together with autoimmune thyroid disease was diagnosed, a combination known as autoimmune polyglandular syndrome type II or Schmidt's syndrome. It seems likely that the prescription of levothyroxine prior to adrenal steroid hormone replacement in this patient with concurrent Addison's disease precipitated an adrenal crisis.
Conclusions
In hypothyroid patients, careful history taking, thorough clinical examination and laboratory tests should be performed to exclude concurrent Addison's disease before the initiation of levothyroxine replacement as prescription of levothyroxine prior to adrenal steroid hormone replacement may be hazardous.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P387

**Elephantiasis nostras verrucosa as a presenting cause of Cushings' disease**

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Introduction
Elephantiasis nostras verrucosa (ENV) is a rare, chronic, deforming disorder characterized by hyperkeratosis and papillomatosis of the epidermis with underlying woody fibrosis of the dermis and subcutaneous tissue resulting from chronic nonfilarial lymphoedema. Lymphatic obstruction can be primary or due to various causes such as surgery, tumour, radiation, congestive heart failure or obesity. However, to the best of our knowledge, it has not been reported as a presenting sign of Cushing's disease.
Case
A 28 years old male patient presented with obesity and left lower extremity enlargement and deformity which was present since 6 years. Physical findings revealed central obesity, purple stria on abdomen, buffalo hump and moon face. Non-pitting edema with lichenification, hyperkeratotic papules, nodules, and verrucous cobblestone-like plaques was found on left lower extremity. Histopathological examination of the tissue obtained by biopsy revealed dense dermal fibrosis, edema of the papillary dermis and extensive pseudo-epitheliomatous changes consistent with ENV. His laboratory findings demonstrated ACTH: 60 pg/ml (0–46 pg/ml), baseline cortisol level 26.8 μg/dl (6.2–19 μg/dl), midnight cortisol level: 17.17 μg/dl, urine cortisol level: 530 μg/24 h. Serum cortisol levels failed to supress after low dose dexamethasone supression test (DST) and supressed after high dose DST (1 mg: 9 μg/dl, 8 mg: 4.2 μg/dl respectively). A combined CRH-DST was consistent with Cushing's disease (3.6 μg/dl). His pituitary MRI was normal. Inferior Petrosal sinus sampling was consistent with Cushing's disease resulting from the left side of the pituitary gland. The patient refused trans-sphenoidal surgery.
Conclusion
ENV is a very rare disease. This case demonstrates that ENV may also developes secondary to Cushing's disease. Therefore, patients with ENV should also be searched for Cushing's disease.

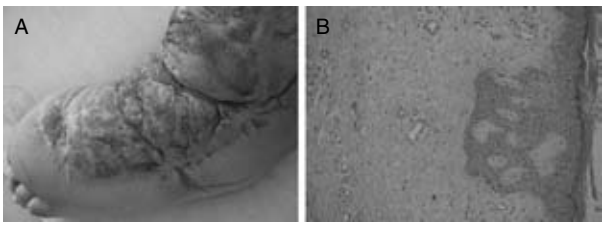


**Figure 1** (A) ENV on the left lower extremity of the patient. (B) H&E stained tissue section demonstrates dense dermal fibrosis, edema of the papillary dermis and extensive pseudo-epitheliomatous changes consistent with ENV.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P388

**Syndrome of inappropriate secretion of antidiuretic hormone in a elderly women affected with herpes zoster infection: a case report**

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Introduction
SIADH is a disorder of sodium and water balance characterized by hypotonic hyponatremia and impaired urinary dilution in the absence of renal disease or any identifiable non-osmotic stimulus known to relase ADH. SIADH is usually observed in hospitalized patients and its prevalence may be as high as 35%.
Case Report
A 70 year old women was admitted to the Medicine and Rehabilitation Clinical Institute of Aosta on October 2011. She had a change of mental status. Physical examination was normal. There was a vesicular erythematous rash at the chest level, which was consistent with a diagnosis of HZI. The patient was disoriented and confused. Laboratory results showed blood count in the normal range, serum concentration of sodium was 119 mEq/l, potassium 4 mEq/l, creatinine 0.8 mg/ml. Urine sodium was 45 mEq/l and potassium 50 mEq/l. Urine was highly concentrated with an osmolality of 620 mOsm/kg $H_2O$, while plasma osmolality was 243 mOsm/kg $H_2O$. Thyroid and adrenal function was normal. Plasma antidiuretic hormone (ADH) was 3.8 pg/ml (normal range 0.3–3.5 pg/ml). Given these results, a diagnosis of SIADH was made. Under water restriction, infusion of 3% saline, treatment with loop diuretics and with complete acyclovir therapy, mental function returned to normal within 2 day. Seven day later, serum sodium concentration was increased to 136 mEq/l with plasma osmolality of 275 mOsm/kg.
Conclusion
We postulate that although the association between HZI and SIADH has been rarely reported in the literature, this is likely to be an under representation of its true incidence.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P389

**Hyponatremia and cytolysis as first signs of decompensated pituitary insufficiency: case report**

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Introduction
Pituitary insufficiency of the adult is a rare pathology (~30–40/1 000 000 per year). Among the acquired causes, Sheehan syndrome (SS) is often characterized by an insidious evolution, which allows it to pass unnoticed for a long time.
Case report
R Maria, 58 years-old, known with SS for 10 years, was hospitalized in the ER with severe asthenia, obnubilation, symptomatic arterial hypotension. Laboratory studies revealed severe hyponatremia (115 mmol/l) and important cytolysis (CK = 21 520 U/l, CKMB = 326 U/l, LDH = 731 U/l) with normal ECG. A myocardial or skeletal muscle etiology was excluded. After successful intensive care she was transferred to Endocrinology. Anamnesis revealed cessation of corticotherapy and intermittent thyroid substitution. Physical examination showed bradylalia, generalized myalgia, pale, infiltrated, depigmented, depilated skin. Biology confirmed pituitary insufficiency: low $FT_4$ = 3.72 pmol/l, paradoxically normal TSH = 2.15 mUI/ml, low ACTH = 15.5 pg/ml, low LH = 0.4 mUI/ml despite the menopausal status. Under substitution therapy (glucocorticoides and L-thyroxin) and hydroelectrolytic equilibration, patient's general status improved progressively, as well as the biological parameters (Na = 137 mmol/l, $FT_4$ = 8.17 pmol/l, CK = 129 U/l, CKMB = 10 U/l, LDH = 370 U/l).
Conclusions
Due to the rarity of both the SS and its therapeutic non-compliance, few studies have evaluated its evolution. High enzymes are found in 1/3 of the cases of hypothyroidism, but the values are rarely significant. Due to normal TSH (as in our case), secondary hypothyroidism can be ignored in the differential diagnosis. Our case is particular because of the severe hypothyroidism manifestations, despite the coexistence of the pituitary corticotrope insufficiency.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P390

**Acromegaly and pregnancy: two acromegalic patients treated with somatostatin analogues**

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Pregnancy in acromegalic patients is an infrequent event, but with earlier diagnosis and advanced surgical/medical management, more affected women get pregnant.
The use of Somatostatin analogues (SSA) during pregnancy seems safe, but there are few reported pregnancies under this treatment.
We report two acromegalic patients treated with SSA that became pregnant.
Case 1
Thirty-three year old woman diagnosed with acromegaly at the age of 31 (March 2008: IGF1: 901 ng/ml; Pituitary MR: intrasellar macroadenoma). She underwent two selective adenomectomies by transphenoidal route (April 2008; March 2009), without cure. Lanreotide, 120 mg – 6/6 weeks was prescribed since June 2008. At appointment (7/10/09) she referred a 12 weeks pregnancy and last Lanreotide injection at 19/8/09. SSA was discontinued. Pregnancy was uneventful and a full-term boy was born, with a normal postnatal development.
Case 2
The patient developed secondary amenorrhea and progressive enlargement of her hands/feet since age 24, 2 years after a normal pregnancy. In 2007, (33-year-old), acromegaly was diagnosed (invasive macroadenoma) that persisted after transphenoidal and transfrontal adenomectomy, (IGF1:912 ng/ml). In June 2008 after stereotaxic radiotherapy she began octreotide 30 mg 4/4 weeks. After 14 months (IGF1: 512 ng/ml) on evaluation for leucorrhea, a pelvic ultrasound revealed a 6 weeks pregnancy. She chooses to abort.
Conclusions
Case 1: although the embryo had been exposed to Lanreotide during the first trimester, pregnancy, fetal and postnatal development were uneventful. Despite reassuring reports in literature, it seems safest to discontinue SSA before or as soon as pregnancy is detected.
Case 2: pregnancy occurred despite prolonged amenorrhea. Women in fertile age should receive adequate contraception if treated with SSA, as it may improve fertility.
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## P391

**Particular course of a case of acromegaly associated with Waldenström's macroglobulinaemia**

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Introduction
Acromegaly and Waldenstrom's macroglobulinaemia are two rare diseases (about 3–4 and 10 cases/million per year respectively), the association between them is even more infrequent, one case being reported in the available literature.
Case report
We present the case of a 74-years-old female (I.I.) suffering with both diseases. In 1997 acromegaly diagnosed by dysmorphic syndrome, than in 2004 hormonal/imagistical investigations (GH at baseline: 2.5 ng/ml, suppressed GH with OGTT: 2.3–2.9–11.6–7.5 ng/ml; 12/15 mm pituitary adenoma on MRI) confirmed the diagnosis. She refused any therapeutical approaches. Cardiovascular diseases (hypertension, left ventricular hypertrophy, major left bundle branch block, left ventricle failure NYHA III) and osteoarticular involvement (osteoporosis with right hip fracture and multiple vertebral fractures) have slowly progressed in time. In 2008 Waldenström's disease was diagnosed and chemotherapy was administered (cyclophosphamide, vincristine, corticosteroids), the outcome being favourable. Affirmatively the dysmorphic syndrome has not aggravated during the last 7 years, but in May 2011 a sudden severe aggravation of the general status, weight loss, tongue-mandibular hypertrophy, malocclusion, swallowing and respiratory disturbances developed within 6 weeks. Surprisingly, hormonal results (GH at baseline: 3.3 ng/ml, GH after OGTT: 1.58–1.58–1.76–1.24 ng/ml; PRL: 72.3 ng/ml), IGF1: 114 ng/ml (101–267) and pituitary CT (11 mm adenoma) did not showed the reactivation of acromegaly. In August–September 2011 a severe gastroparesis, malabsorption syndrome and cachexia developed.
Conclusion
We present a particular course of acromegaly: a very slow progression without any specific treatment, than a rapid aggravation of facial dysmorphic signs. The favourable laboratory/imagistic evolution of acromegaly, confirmed by the involution of adenoma on CT, the slightly elevated GH and normal IGF1, may be attributed to a partial apoplexy in the adenoma or eventually to the medications used for the haematological disease. The discordance between evolutive clinical signs and non-evolutive laboratory/imagistic results needs supplementary investigations (directed towards a local viscerocranial/buccal process).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P392

**A case of acromegaly with nephrogenic adenoma of the bladder: a rare association?**

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A 60-year-old woman with a previous history of twice transsphenoidal pituitary surgery due to acromegaly,had a surgery with a presumed diagnosis of bladder stone. She was reevaluated after the surgery when her hematuria persisted. Percutaneous nephrolitotomy was planned. when multiple stones were identified in the left ureter, but the operation had to be postponed till the achievement of glycemic control. At the meantime endocrinological evaluation revealed a 30×15 mm macroadenoma in the pituitary invading both cavernose sinuses.

Her random GH level was >40 ng/ml and IGF1:1225 ng/ml (age and sex adjusted reference range 71–263 ng/ml).
Octreotid LAR 30 mg/4 weeks was started and conventional radiotherapy was performed. When glycemic contol was maintained she underwent cystoscopy. A mass lesion was identified and resected. The pathology of the specimen revealed nephrogenic adenoma of the urinary bladder. Immunohistochemically lesion was cytoceratin 7 positive, CD 10, *P*63 and TTF-1 negative.
Nephrogenic adenoma, also referred to as nephrogenic metaplasia, is an uncommon benign lesion of the urothelial tract.It is a benign metaplastic response of the urothelium to injury or insult. It is most commonly seen in bladder (80%) however urethra (12%) or ureter (8%) can be involved. Nephrogenic adenoma can be a significant diagnostic pitfall as certain histologic features, such as the presence of enlarged nuclei with prominent nucleoli, degenerative nuclear atypia, tiny tubules with blue mucin simulating signet ring cells, and focal invasion into superficial muscle, when taken out of context, can mimic malignancy.
Up to date there is no reported case of acromegaly with nephrogenic adenoma of the bladder. Considering the increased neoplasia risk in acromegaly, more data are needed to explain if there is a causal relationship between acromegaly and nephrogenic adenoma, or if this is a coincidental finding.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P393

**Recovery of prolactin function following spontaneous pregnancy in a patient with Sheehan's syndrome**

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Introduction
Sheehan's syndrome presents with hypopituitarism of variable severity after parturition usually preceded by *post partum* hemorrhage. Recovery of lactotroph function after initial insult has not been reported in the literature.
Case report
A 41-year woman delivered her 1st child in 1994 at the age of 25 years, complicated by profuse vaginal bleeding and received two blood transfusions at a city hospital. She had lactation failure and did not resume menstrual cycles after. She was investigated in 1996, revealing central hypothyroidism, GH, prolactin and cortisol deficiency on insulin tolerance test. She was put on prednisolone 7.5 mg/day, thyroxine 0.75 mg/day and cyclic estrogen/progesterone. She came to the hospital with pregnancy of 12 weeks duration and delivered in Dec 2003. After the present delivery, she lactated normally. She resumed infrequent cycles after the lactation amenorrhea. In 2007 incidentally she was again found to have pregnancy of 20 weeks on a routine ultrasound, she delivered after a cesarean section and again lactated normally. Metochlopramide test revealed normal stimulable prolactin. MRI pituitary revealed empty sella.
Conclusion
Recovery of lactotroph function after initial failure has not been reported in patients with Sheehan's syndrome. We presume that recovery of lactotroph function after the second pregnancy in this patient is possibly because of stimulatory effect of estrogen and progesterone on residual lactotroph cells.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

**Table 1** Hormonal parameters before and after lactotroph recovery

| Before 2nd pregnancy | | | After 2nd pregnancy | | |
|---|---|---|---|---|---|
| Hormone | Basal | Peak | Basal | Peak | Normal value |
| $T_4$ (μg/dl) | 3.43 | – | 6.5[a] | – | 5.5–13.5 |
| TSH (U/l) | 0.83 | | 0.85 | – | 0.5–6.5 |
| LH (U/l) | 5.56 | 12.21[b] | 4.04 | – | >10 |
| FSH (U/l) | 9.56 | 12.89[b] | 14.09 | – | |
| PRL (ng/dl) | 12.63 | 13.56[c] | 6.13 | 29.99[c] | 5–16.2 |
| GH (U/l) | <1.2 | <1.2[d] | – | – | >3 |
| Cortisol (μg/dl) | <1 | <1[d] | 1.58 | – | >20 |

$T_4$, thyroxine; PRL, prolactin. a=on levothyroxine; B=post GnRH; c=post metoclopramide; d=post ITT.

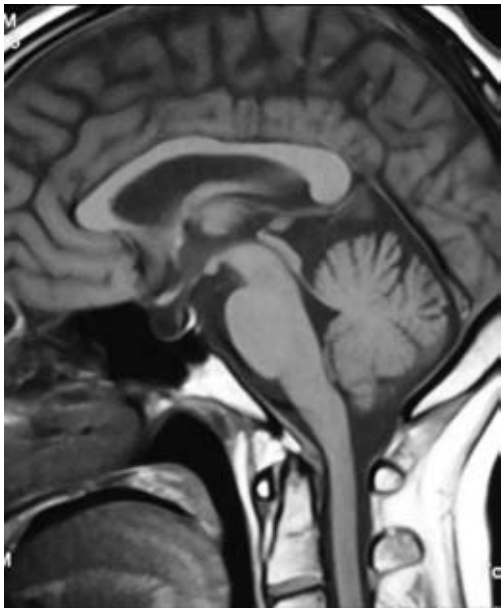

**Figure 1** MRI pituitary sagital view showing pituitary fossa filled with cerebrospinal fluid and stalk touching the base of pituitary floor; features suggestive of empty sella.

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## P394

**Giant macroprolactinoma: recidive after 12 years (case report)**

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Introduction
Prolactinomas are rare in childhood and adolescence, representing a half of pituitary adenomas (1% of intracranial tumors); macroadenomas are more frequent than microadenomas. The signs and symptoms depend on age, sex, tumor size and prolactin level. Due to a higher frequency of macroadenomas in boys, they present more often neuro-ophthalmologic findings (impaired vision, headache)
Case report
We present a case of 25-year-old pacient diagnosed at age 13 with giant prolactinoma. He came to our observation after neurosurgery was performed for symptoms of tumor expansion. No pituitary insufficiency was observed after surgery. Dopamine-agonist therapy was required postoperatively due to persistent hyperprolactinemia (PRL>250 ng/ml *n* 0.7–17 ng/ml). The pacient developed normal puberty, reaching a final high=172 cm. After 7 years of prolactin level control and no IRM signs of recidive under medical therapy, we lost follow-up. At current evaluation (nov. 2011) PRL level was very high=3575 μIU/ml (*n*86–324 μIU/ml); clinical and biological signs of pituitary insufficiency were absent. Visual field was normal. Pituitary X-ray revealed a raised intrasellar volume, undefined sellar walls and a deeply declined sellar floor in the sphenoidal sinus. IRM described a 2/1.3/2.5 cm tumor residue with extension in the right cavernous sinus. Cabergoline therapy was reinitiated.
Discussions
Surgery is reserved for pacients resistant to medical therapy and those with severe neurological symptoms at diagnosis. First-line treatment is represented by dopamine-agonists.
Conclusions
The pacient had severe symptoms at diagnosis. Neurosurgery was performed before endocrinological evaluation and was not curative. Dopamine-agonists were needed postoperatively. After 7 years of good evolution we lost follow-up. The absence of symptoms at current evaluation raised the suspicion of macroprolactinemia. Dopamine-agonist therapy was reinitiated and might be necessary life-long.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P395

**An uncommon cause of hypoglycaemia: a case report**
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Introduction
Hypoglycaemia in a non-diabetic patient is not a common condition and is often a diagnostic challenge.
Case report
A 78-year-old gentleman was admitted to hospital by paramedics when his neighbours found him unresponsive. He had a background history of primary hypothyroidism. Capillary blood glucose reading done on site was 1.0 mmol/l. He received intravenous glucose which resulted in prompt recovery. All other vital signs were normal.
Clinical examination was unremarkable. He had normal blood count, inflammatory markers, renal and liver function tests. He had an inadequate response to synacthen with basal serum cortisol of 137 nmol/l and peak serum cortisol of 381 nmol/l at 60 min. Serum ACTH level is still pending but we expect it to be suppressed, consistent with secondary hypocortisolism. There was evidence of secondary hypogonadism with low testosterone level at 3.2 nmol/l (NR 7–26 nmol/l) and inappropriately normal gonadotrophins (FSH 9.3 U/l (NR 2–17 U/l), LH 9.2 U/l (NR 1–7)). Rest of the basal anterior pituitary profile included a low IGF1 of $<$3.3 nmol/l (NR 7.7 to 24.6 nmol/l), TSH 0.33mu/l (NR 0.25–5mu/l), free $T_4$ 19.1 pmol/l (NR 12–25 pmol/l) and prolactin 144 mu/l. An MRI scan of the pituitary gland was consistent with an empty sella. Patient was treated with intravenous hydrocortisone and is now maintained on oral hydrocortisone. We shall review him in the clinic to discuss testosterone supplementation and assess his GH axis given his low IGF1 levels.
Conclusion
Empty sella is usually an incidental finding on an MRI scan. Approximately one third of these patients present with endocrinopathies and neurological symptoms. Our patient presented with a significant hypoglycaemic episode which is a recognized though a rare presentation of empty sella syndrome and this case serves as a reminder of this rare cause of hypoglycaemia in a non-diabetic patient.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P396

**Central diabetes insipidus: about two clinical cases**
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Introduction
Central diabetes insipidus (CDI) is produced by the destruction of the magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei which results in decreased arginine vasopressin (AVP) synthesis and secretion.
Case report 1
Forty-five year old female, previously healthy, was observed in April 2011 complaining of polydipsia, polyuria, nocturia and weight loss since January. Diabetes mellitus (DM) was excluded and she was admitted for study of possible diabetes insipidus. Water deprivation test was suggestive of CDI. Magnetic resonance imaging (MRI) showed infundibular hypophysitis and no hyperintense signal in the neurohypophysis. Autoimmune diseases, infections and infiltrative diseases were excluded. Imaging (chest x-ray, abdominal ultrasound, mammography, breast ultrasound and thoracoabdominal CT) was normal. No other pituitary deficits were shown. She started therapy with oral desmopressin with clinical improvement.
Case report 2
Fourty-three year old man, previously healthy, was seen in August 2011 complaining of polydipsia, polyuria and nocturia during the previous 3 months. DM was excluded. Water deprivation test was positive for CDI. Pituitary MRI was normal, with normal signal of high intensity in the neutohypophysis. He had no other hormonal deficits. Autoimmune and infectous diseases were excluded. After initiation of oral desmopressin the symptoms disappeared.
Discussion
In both cases it was not determined the etiology of CDI, as it may occur in 20–50% of CDI cases. In our institution is not possible to determine antibodies towards vasopressin secretory cells, which does not allow the diagnosis of this autoimmune form of CDI. The infundibular hypophysitis, observed in the first case, can occur in about 50% of idiopathic cases and more frequently in women. The lymphocytic hypophysitis can be diagnosed by pituitary biopsy, but it's a very aggressive procedure and almost never performed. These cases highlight the difficulty of the etiologic diagnosis of CDI. However, proper treatment allows the symptoms control.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P397

**Macroprolactinomas: therapy compliance (case report)**
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Prolactinomas represent 60% of primary pituitary tumors, galactorrrhoea and menstrual disturbances beeing the most frequent clinical features in women. The mass effect of macroadenomas is expressed by headache, visual field defects and hypopituitarism. The aims of therapy are reduction of tumour size, prevention of tumour expansion and restoration of gonadal function. Dopamine agonists normalize prolactin secretion and reduce tumour size in 40% of patients with macroadenoma.
We are presenting a case of an 49 year old female, who came to us first in 1995, presenting headache, galactorrrhoea and secondary amenorrhea. Her prolactine level was very high with the rest of the pituitary hormones in normal range. We performed an MRI, which showed the presence of a macroadenoma. After the patient refuses surgery, we started dopamine agonists therapy, with reevaluation every 6 months, until 2004, when the patient discontinues therapy (at that moment the prolactin levels were still high, and the adenoma offered signs of protrusion in the sphenoidal sinus). The patient came to us in november 2011 with a prolactine level of 7742 μUI/ml (102–496) and a MRI image of empty sella and a tumoral mass which evolves in the sphenoidal sinus. Again, the patient refuses surgery, so we started once more dopamine agonists therapy.
Discussions
In routine practice, hyperprolactinaemia recurs early in most macroprolactinomas (93%) and microprolactinomas (64%) following dopamine agonists discontinuation. For most macroprolactinomas, cesssation of dopamine agonists cannot be recommended even after 7 years of therapy.
Conclusions
The patient refused surgery, although it was an early diagnosis and all the treatment options were discussed. Dopamine agonists therapy was initiated and after 9 years of intermitent therapy she stopped the treatment and we had no contact afterwards. For this type of patients in particulary, the 1 year followup is very important.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P398

**Endocrine hypertension due to primary aldosteronism**

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Introduction
Primary aldosteronism (PA) is currently believed to be the most frequent form of secondary endocrine hypertension, accounting for 5–10% of all hypertensive patients. After confirming the diagnosis, adrenal venous sampling (AVS) is considered the most accurate means of distinguishing between unilateral and bilateral adrenal disease.
Case report
Female patient, 36 years-old, referred to an Endocrinology appointment in May 2009 due to left adrenal nodule and hypertension refractory to treatment. Hypertension diagnosed at age 24 with progressive drug resistance since February 2009. Left adrenal nodule known since 2004 showing an increase from 15 to 20 mm in 5 years. Family history of hypertension. On physical examination was noteworthy a blood pressure value of 210/110 mmHg. Laboratory assays showed hypokalemia (2.3 mEq/l; *n*: 3.5–5.1), increased aldosterone (ng/dl)/direct renin (μU/ml) ratio (12.6; *n*: 2.4–4.9) and negative screening for other secondary causes of hypertension. Oral potassium supplements were prescribed although with potassium levels difficult to control. Diagnosis of PA was confirmed by saline infusion test (post-infusion plasma aldosterone 62.9 ng/dl) and captopril challenge test (plasma aldosterone remained elevated and direct renin suppressed). AVS was performed but results were inconclusive. Abdominal MRI performed in July 2010 showed a 31 mm nodule in the left adrenal. Spironolactone 100 mg/day was initiated in July 2011 due to symptomatic hypokalemia. A left adrenalectomy was performed in September 2011 and spironolactone and potassium supplements were suspended in the postoperative period. Histological examination showed an adrenal adenoma. In October 2011 she presented blood pressure values of 136/83 mmHg (only medicated with verapamil) and normokalemia (4.3 mEq/l).
Conclusions
Early recognition and treatment of PA is important to overcome the high cardiovascular morbidity and mortality rates associated with this condition. PA case detection is recommended in patient groups with relatively high prevalence of the disease, such as in the clinical case described.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P399

**Unusual association between pheochromocytoma, adrenocortical nodular hyperplasia and empty sella: case report**

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Introduction
Pheochromocytoma occasionally associates with pathological lesions of the adrenal cortex. We report a case of non-functional adrenocortical nodular hyperplasia with a concomitant pheochromocytoma in the controlateral adrenal and empty sella.
Case report
We describe the case of a 52 year old women with a history of essential hypertension and type 2 diabetes mellitus. She complained of: nausea, abdominal pain, vomiting and constipation. The computed tomography revealed a 5.3 cm left adrenal highly vascular tumor with multiple necrosis areas and a 3.5 cm well-encapsulated right adrenal mass. ACTH and plasma cortisol (after 1 mg dexametasone overnight test) levels were normal but the levels of urinary metanefrines were high. The patient was put on preoperative α-adrenoreceptor blockade and after 4 days due to important abdominal pain and lack of transit the surgery was performed and both adrenal glands were removed. Histopathological examination disclosed typical pheochromocytoma on the left side and nodular hyperplasia of the fasciculata on the right side. One month after surgery a full evaluation was made in order to exclude a multiple endocrine neoplasia: PTH and calcitonine levels were normal and the pituitary MRI revealed empty sella.
Conclusions
The present report is a rare case of pheochromocytoma with nodular hyperplasia of the fasciculata in the controlateral adrenal and empty sella. Also the diagnosis circumstances were unusual due to the clinical picture of acute abdomen and lack of endocrine manifestation of both adrenal masses.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Clinical case reports - Thyroid/Others

## P400

**New diabetic emergency; acute rhabdomyolysis complicating hyperglycemic hyperosmolar coma successful management and insight into pathogenesis**

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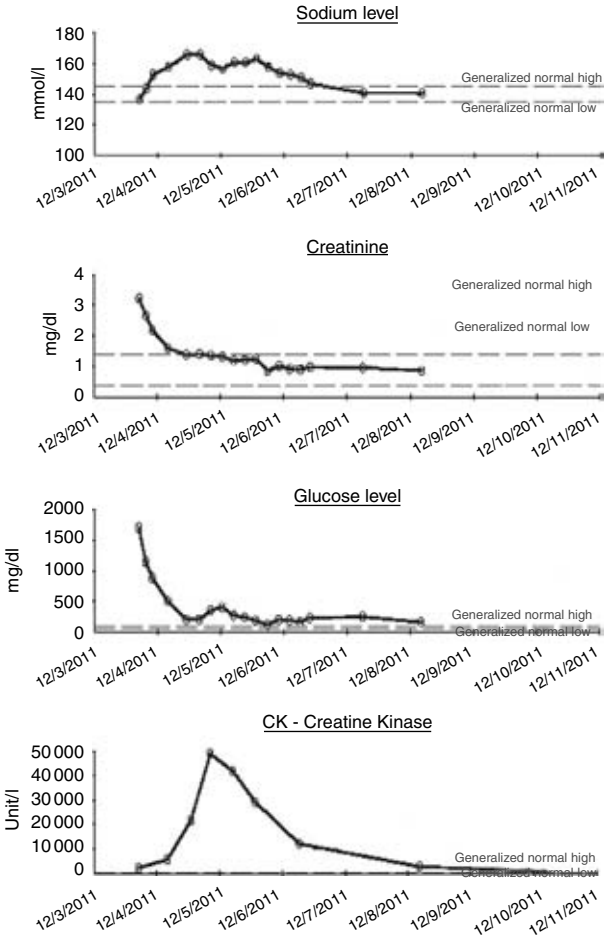
We describe a case of severe rhabdomyolysis(peak CK 48897 μ/ml and massive myoglobinuria) complicating hyperglycemic hyperosmolar coma. A 57y/o M with DM2 on metformin presented with polyuria, polydipsia, weakness and confusion. On admission he had GCS E2M3V1 no signs of trauma or infection. Na 137 mEq/l K 4.8 mEq/l $HCO_3$ 22 mEq/l Cl 93 mEq/l BUN 116 mg/dl Cr 3.2 mg/dl glucose 1710 mg/dl pH 7.22 ketones +small, Mg 5.2 mg/dl P 3.3 mg/dl Ca 9.6 mg/dl TSH 1.47 μU/ml Ethanol <5 mg/dl, toxicology screen negative, osmolality 364 mOsm/kg. Initial Troponin 0.03 ng/ml, peak 0.12 ng/ml 28 h later. Initial CK 2379 μ/l, 21767 μ/l at 24 h, peak 48 897 μ/l 28 h later. CK isoenzyme 100%MM. Urine myoglobin >10 000 ng/ml (nl<1). As he was



on flexeril, serum and urine cyclobenzaprine level were checked and were negative. He received aggressive i.v. hydration with NS and Bicarb drip along with insulin drip. Blood glucose control was achieved over 48 h. Cr normalized in 28 h. Na peaked to 166 mg/dl and corrected over 3 days. P and K levels remained nl. CK trended down to 881 μ/l on day 7. Mental status was at baseline by day 3. Hyperglycemic hyperosmolar state precipitates rhabdomyolysis which aggravates acute renal failure. Pathogenesis of this nontraumatic rhabdomyolysis is multifactorial including, inhibition of the Na pump by hyperosmolar state, acidosis, hypernatremia, K deficiency, decrease in intramuscular energy supply from insulin deficiency[1]. Resultant fall in transmembrane potential and elevated intracellular Ca, activates proteases with leakage of muscle enzymes resulting in rhabdomyolysis[2]. We also hypothesize that the prothrombotic state induced by severe hyperglycemia causes muscle tissue infarction and elevated CK, confirmed in our case as 100% MM. Prompt recognition and treatment with bicarb drip as in our case can avert acute renal failure precipitated by this unusual cause of rhabdomyolysis. CK is not routinely measured in hyperosmolar hyperglycemic states. We recommend routine monitoring of CK in these cases with particular attention to cases with very high Na as without early recognition and treatment of rhabdomyolysis, patients could have potentially fatal outcomes. We suggest CK be remeasured a day after peak glucose and serum osmolality given the temporal association demonstrated by our case.

1. *Am J Nephrol* **11** 447–450.
2. *Nephron* **47** 202–204.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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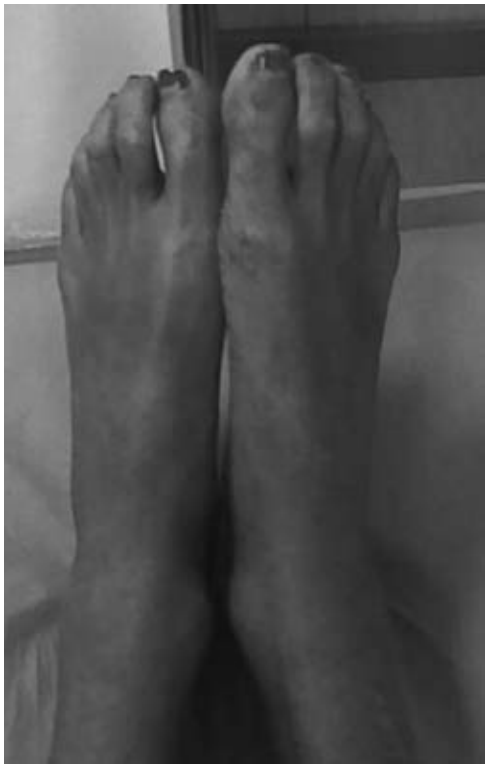

## P401

**Laminopathy and auto-immune sclerodermia: chance or necessity?**

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Some laminopathies associate both progeria and sclerodermic components (*Am J Med Genet A* 2006 Hennekam, *J Cell Sci* 2008 Sagelius), but most sclerodermic syndromes are not associated to LMNA mutations despite the presence of nuclear envelope antibodies, mainly directed to lamin (*PNAS* 1983 McKeon, *Eur Respir J* 2011 Dib).

A 15-th year old girl was referred for severely imbalanced diabetes and a voracious appetite interpreted as binge eating disorders. This girl had been diagnosed at 8 years old with auto-immune sclerodermic and insulin resistance syndromes. Hypertension, polycystic ovaries and insulin requiring hypertriglyceridemic diabetes occurred at 13 years old. Clinical features associated: weight 43 kg, height 160 cm, normal blood pressure with treatment, mandibular hypoplasia, face-sparing generalized lipoatrophy, muscular–skeleton disorders, distal sclerodermia with tendinous retractions and telangiectasies confirmed by capillaroscopy. Fasting blood glucose and C-peptide levels were respectively 2.5 g/l ($n<1$) and 13 ng/ml ($n<2$). HbA1c was 9.2% ($n<6$), ALAT 126, ASAT 307 ($n<40$ UI/l), leptin 2 ng/ml ($n$ 3.5–11), total fat mass 11% ($n$:30–40 DEXA), intra/ total abdominal fat mass 52/84cc with severe liver steatosis 26% (MRI) and fibrosis F2 (biopsy). Cardiac investigations showed mild anomalies. Anti-nuclear autoantibodies have been found constantly positive since the age of 8 (1/640ème)), without other auto-antibodies. A *de novo* D47Y LMNA mutation was found.

This sclerodermic syndrome associated to this laminopathy could be linked i) to the severity of the glucose imbalance, generating a disorder of glycosylation ii) to the N-terminal type of LMNA mutation, rather encountered in progeria with sclerodermic disorders iii) to the cell or nucleus stress induced by the laminopathy leading to a release of nuclear envelope antigens, generating a non-specific occurrence of anti-nuclear auto-antibodies. Altogether, this case report offers new insights on the mechanisms linking inherited and auto-immune forms of lipodystrophies and sclerodermia, in line with those reported in PSMB8 mutations.

Sclerodermia with telangiectasies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P402

**Pendred's syndrome: genetics and phenotypic variability**

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Background

Pendred syndrome (PS) is an autosomal recessive disorder characterized by defective organification of iodine, goiter and deafness. It is caused by mutations in pendrin gene (SLC26A4), a transporter of chloride/iodide that mediates the efflux of iodine from thyroid follicular cells to the follicular lumen.

Clinical case

Case-index: MJFS, female, refered to consultation at 35 years for enlarged neck. Personal history: congenital deafness, thyroidectomy at age 19, under levothyroxine therapy. Family history: deafness and goiter in several family members. Physical examination: goiter. Evaluation: normal thyroid function; thyroid hypertrophy with bilateral homogeneous hyperfixation in cintigraphy. Clinical evolution: at 55 years presented a nodule in the right lobe with cytology suspicion of papillary carcinoma; underwent right lobectomy with isthmectomy. The histology revealed a microfolicular adenoma, nodular hyperplasia and papillary hyperplasia. At 63 years presented left lobe enlargement, with suspicious nodule. The cytology was benign (colloid). The molecular study of SLC26A4 gene was performed and revealed mutations c.367C>T (p.Pro123Ser) and c.412G>T (p.Val138Phe), in heterozygosity in exon 4, confirming the diagnosis of PS.

The daughter of case-index: MSF, female, consultation at 3 years for enlarged neck. Personal history: congenital deafness. Evaluation: diffuse goiter without thyroid dysfunction, autoimmunity negative. Evolution: at 11 old years presented thyroid enlargement; levothyroxine therapy was initiated. At 18 years had a multinodular goiter. At 22 years had complains of pain and cervical compression; the cytology was colloid. A total thyroidectomy was performed. Histology: diffuse hyperplasia without malignancy. The molecular study of SLC26A4 gene revealed mutation c.367C> T (p.Pro123Ser), in homozygosity in exon 4, confirming the diagnosis of PS.

The husband of index case, without genetic relashionship, but also with goiter and neurosensorial deafness, as well as other family members, waits for genetic study.
Conclusions
The association of familial goiter and congenital deafness should cause suspicion of this entity. Although thyroid function isn't usually affected, the structural change is often significant, putting difficulties in differential diagnosis. The diagnosis is confirmed by molecular studies, which must be performed in all family members.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P403

**A rare case of vanishing fetal goiter and role of colour doppler ultrasound in the diagnosis**

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Introduction
Fetal goiter is a rare condition. The incidence of goitrous hypothyroidism is 1/30 000–50 000 live births. We describe a case of antenatal fetal goiter that vanished upon delivery and was not detected in the newborn. The case report is followed by a discussion wherein we review the literature on thyroid problems in pregnancy and management of fetal goiter.
Case report
A 27-year-old known hypothyroid woman with bichorial biamniotic pregnancy had antenatal ultrasound (USG) at 24 weeks of gestation and found to have a butterfly shaped, uniformly echogenic and highly vascular solid mass on the anterior aspect of fetal neck of one twin. Polyhydramnios and other complications were absent. Thyroid swelling was confirmed on fetal MRI. The other twin was normal. Color Doppler indicated hypofunction of thyroid which was confirmed with cordocentesis. Mother was hypothyroid with very high anti TPO antibody titre. The case was managed by titrating maternal thyroxin and serial monitoring of fetal thyroid size and function. Patient delivered preterm at 30 weeks of gestation. There was no thyroid swelling in any of the twins which was confirmed on USG. Both babies were euthyroid at birth had normal development on follow up so far.
Conclusion
Fetal goiter results in compression of the esophagus and the trachea and leading to polyhydramnios, hyperextension of the neck and dystocia during labor and mental and motor retardation later in life. Therefore, diagnosis of fetal hypothyroidism should be established at an early stage and appropriate hormone replacement treatment should be started. Fetal thyroid color Doppler is a new modality that helps in determining fetal thyroid function and can help to avoid invasive procedures like cordocentesis in appropriate cases. We emphasize on importance of noninvasive USG and maternal biochemical monitoring and correction as required. Hence, a strategic approach to fetal goiter is required to achieve optimal benefits at minimal fetal and maternal risk.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P404

**Gastrostomy solves severe hypoglycemias after bariatric surgery**

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Introduction
Patients with bariatric surgery require close monitoring because of possible metabolic complications. It has been published some cases of severe and persistent hypoglycemias resistant to diet and pharmacologic treatment being necessary a pancreatectomy. We present a case report of nesidioblastosis after gastric bypass that required subtotal pancreatectomy but despite this, hypoglycemias persisted. Before performing total pancreatectomy we placed a tube feeding in remnant stomach and hypoglycemias disappeared.
Case report
45 years woman with morbid obesity (BMI $=48$) underwent gastrojejunal bypass. One year later (BMI$=25.9$) she began with postprandial hypoglycemia that did not improve after diet and acarbose. Basal analysis and fasting test were normal. OGGT revealed hyperinsulinism and hypoglycemia. Imaging test were normal. After oral load of three enteral nutrition products (1, rich in fast absortion carbohidrates; 2, slow absorption carbohydrate with insoluble fiber and 3, monounsaturated fatty acids rich) were observed descending degrees, respectively, of hyperinsulinism and hypoglycemia. Mesenteric arteriography with intra-arterial calcium stimulation and measurement of insulin and C peptide in hepatic vein revealed a right hypersecretion in splenic and superior mesenteric area (pancreatic body and tail) Given the persistence of hypog. partial pancreatic resection was decided. Pathologic examination revealed diffuse hyperplasia of islets supporting diagnosis of nesidioblastosis. Despite initial improvement, hypoglycemias returned so it was decided to perform a gastrostomy for enteral nutrition, while orally she only ate proteins and fats. After this, hypoglycemias disappeared.
Conclusion
The clinical significance of this case resides in the few cases reported to date with this evolution to our knowledge (only one previously published case) and the possible therapeutic implications:published literature advises complete pancreatectomy if hypoglycemia persists after partial pancreatectomy, however the performance of gastrostomy may be a much less aggressive therapeutic approach. Likewise, the persistence of hypoglycemia after subtotal pancreatectomy with subsequent resolution after gastrostomy in remnant stomach supports a major role of rapid release of food to the ileum and production of gastrointestinal peptides more than the cell hyperplasia of islets in itself as main cause of hypoglycemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P405

**Pubertal induction with testosterone of a boy with bilateral anorchia guided by the development of his monozygotic twin brother**

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Introduction
We describe a monozygotic twin pair, of which one boy was diagnosed with anorchia. Both were followed-up till age 17.
Case report
At birth, in one twin 46 XY boy (A), testes were not palpable while his brother (B) was unaffected. Stimulation with human chorionic gonadotrophin (hCG) and orchidopexia were unsuccessful at age 3. A second hCG-stimulation test was performed at age 8, where serum testosterone response failed to increase. No testicular tissue was detected by abdominal laparoscopy. At the age of 10.5, when the bone age was 11.6 years both in A and B, low dose testosterone substitution therapy (25 mg/2 weeks) was started. Before puberty induction, A and B had similar weight and height. During puberty, a slightly faster increase in weight (A–B 11–19%) and height (A–B 3–7%) was observed in A. A and B ended up with a similar and normal final height, weight, arm span and sitting height. Secondary sexual characteristics developed normally in both brothers. At the age of 17, bone mineral density, body composition (dual X-ray absorptiometry, DXA-scan), volumetric bone parameters at forearm and calf (peripheral quantitative CT-scan) were evaluated. We observed similar bone mineral density at the lumbar spine, total hip, distal radius and whole body (DXA, A–B $<5\%$). Fat percentage was 14% in A vs 11% in B. Trabecular (distal radius) and cortical volumetric bone parameters (mid and proximal tibia) were comparable (A–B $<5\%$). However, at one cortical site (proximal radius), A had a smaller cortical bone size with a thicker cortex (all A–B 10–20%).
Conclusion
Low dose testosterone substitution in bilateral anorchia, guided by the pubertal evolution in the healthy twin, led to a comparable pubertal development, final height and bone mineral density. Moreover, testosterone did not seem to be necessary for normal increase in length before puberty.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P406

**Pseudohypoparathyroidism type Ib: a case of occult familial disease**

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Introduction
Pseudohypoparathyroidism (PHP) constitutes a heterogenous group of disorders characterized by end-organ resistance to parathyroid hormone (PTH), due to a defect in the stimulatory G protein-activated cAMP formation. PHP type Ib is characterized by renal resistance to PTH, without the phenotypic features of Albright hereditary osteodystrophy (AHO).
Case report
A 31-year old woman was referred to our clinic for investigation of severe hypocalcemia, detected on routine laboratory examination. Apart from mild hand numbness, no further symptoms of hypocalcemia or signs of AHO were evident. There was no relevant family history and both proband's parents were normocalcemic and unrelated. Her serum calcium levels were 6.6 mg/dl (normal range 8.8–10.6), serum phosphate: 4.9 mg/dl (normal range: 2.5–4.5), PTH: 1629 pg/ml (normal range: 10–52), 25-hydroxyvitamin-D: 15.7 ng/ml (normal $>30$ ng/ml), 1,25-dihydrohyvitamin-D3: 31 ng/ml (normal range: 19.6–54.3), 24 h-urinary calcium: 45 mg (normal range 0–250) and 24 h-urinary phosphate: 681 mg (normal range: 400–1300). Thyrotropin levels were 5.67 mIU/ml (normal range 0.4–4), and anti-thyroid autoantibodies were negative.
GNAS methylation analysis showed a methylation defect in the maternally derived exon A/B, with a normal methylation pattern of the paternal NESP55-DM allele, presumably caused by microdeletions disrupting the upstream *STX16* gene. Treatment with alfacalcidol and calcium was initiated, which restored calcium and PTH levels.
One year after diagnosis, the patient successfully carried a twin pregnancy to term, following *in vitro* fertilization for idiopathic infertility. During pregnancy, she remained metabolically stable. GNAS methylation analysis in the offspring, revealed the same methylation defect in the male infant, establishing an autosomal dominant mode of inheritance pattern.
Conclusions
PHP Ib is a rare disease that can remain undetected to mid life. Establishing the diagnosis is important not only for appropriate case management but also for genetic counseling.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P407

**Considering familial benign hypocalciuric hypercalcemia on differential diagnosis of primary hyperparathyroidism**

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Introduction
Primary hyperparathyroidism is the most common cause for hypercalcemia. Familial benign hypocalciuric hypercalcemia (FBHH) is an unusual autosomal dominant disease. The mutation in the calcium sensing receptor (CaSR) determines a shift to the right in the calcemia set-point that inhibits PTH secretion. Generally asymptomatic, these patients present with mild hypercalcemia and hypophosphatemia, normal or slightly increased PTH levels and hypocalciuria. Daily calciuria lower than 50 mg and calcium to creatinine clearance ratio below 0.01 favour FBHH hypothesis instead of typical primary hyperparathyroidism.
Family case report
We describe a family whose propositus was a 37-years-old male, referred to our Department due to diabetes secondary to alcoholic pancreatitis. His routine analysis revealed hypercalcemia (11.2 mg/dl; RR: 8.4–10.4). He didn't exhibit any cardiac, gastrointestinal or psychiatric symptom that could support a primary hyperparathyroidism hypothesis. Absence of ancestor family history suggesting hypercalcemia. The following investigation confirmed hypercalcemia (10.9 mg/dl), hypophosphatemia (1.7 mg/dl; RR: 2.7–4.5), high PTH (75 pg/ml; RR: 9–72) and regular magnesium, albumin and creatinine levels. He showed hypocalciuria (85.6 mg/24 h; RR: 100–300) and a calcium to creatinine clearance ratio of 0.004. Cervical ultrasonography and 99 mTc-sestamibi scintigraphy did not display any abnormality. Bone mineral density was preserved at dual energy X-ray absorptiometry. The propositus has three children, two of them presenting hypocalciuric hypercalcemia. Genetic study has identified R648X mutation in the CASR gene (heterozigoty).
Conclusion
Family history and analytical abnormalities led to FBHH diagnosis, which was genetically confirmed.
We emphasize the need of ruling out FBHH through an investigation triggered by hypercalcemia. Propositus and affected relatives should be warned against undergoing parathyroidectomy, as long as it would be unnecessary (considerable renal reabsorption of calcium and subsequent hypocalciuria still remain after total parathyroidectomy) and hazardous.
Due to neonatal severe hypercalcemia risk, consanguineous relationships are strongly discouraged.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P408

**The diagnostic challenge of a parathyroid adenoma undetectable by Tc-99m Sestamibi scintigraphy or computed tomography in a patient with newly diagnosed sarcoidosis and hypercalcaemia**

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Hypercalcaemia is a common finding in numerous diseases processes but is non-specific to its cause. We report a case of a 55-year-old lady with known sarcoidosis who developed hypercalcaemia and elevated parathyroid hormone due to a parathyroid adenoma. Both these disease processes are independently associated with hypercalcamia, but it is unusual to have both in combination and difficult to establish which is the primary cause. In our case the diagnosis was complicated by a negative Technetium Sestamibi and computed tomography (CT) scan specifically looking for a parathyroid adenoma. This led to an incorrect diagnosis of sarcoid hypercalcaemia with tertiary hyperparathyroidism secondary to osteomalaciea. This diagnosis was reconsidered when a trial of steroids showed an unexpectedly poor response. A lesion noted in the patients CT scan behind the patients left clavicle was investigated further by surgical exploration. Although this lesion was identified as a sarcoid granuloma, the opportunity was taken to explore the patients thyroid. A solitary parathyroid adenoma was detected and following parathyroidectomy her hypercalcaemia resolved. This rare combination highlights the importance good clinical judgment and a healthy scepticism of advanced investigations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P409

**Severe gestational hypothyroidism due to anti-TSH receptor blocking antibodies**

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Autoimmune hypothyroidism due to thyroid stimulation blocking antibodies (TSB-Ab) is uncommon. When occurring in pregnancy, this condition may be responsible for feto-neonatal complications as a result of both maternal hypothyroidism and trans-placental TSB-Ab transfer to the fetus.
Clinical case
September 2010: a 27 year-old woman was diagnosed with autoimmune severe hypothyroidism (TSH 325 mIU/l (n.v 0.4–4.0), $FT_4$ 1.54 pmol/l (n.v. 11.5–22.6), $FT_3$ 0.67 pmol/l (n.v. 3.7–8.3); TPOAb and TgAb positive). Levo-thyroxine (L-$T_4$) treatment was started (100 μg/day) and euthyroidism achieved in November 2010 (TSH 0.34 mIU/l, $FT_4$ 18.6 pmol/l, $FT_3$ 7.0 pmol/l). In December 2010 pregnancy was ascertained (5 weeks) and thyroid function tests revealed

l–$T_4$ dose inadequacy (TSH 13.9 mIU/l (trimester-specific n.v.: 0.03–2.3); $FT_4$ 10.3 pmol/l (trimester-specific n.v.: 11.9-21)). L-$T_4$ dose was therefore increased (1300 μg/week) with prompt restoration of euthyroidism (January 2011, 8 weeks: TSH 0.16 mIU/l). Thyroid receptor antibodies (TRAb) at 10 weeks of gestation were found to be positive with high titer (>40 UI/l). TSH receptor bioassay revealed these antibodies to be only partially active (30%) in blocking TSH receptors. In the further follow-up L-$T_4$ dose was progressively adjusted to maintain TSH and $FT_4$ within the normal range for pregnancy. Both TRAb titer and Ig-activity proved consistently unchanged (at 20 and 30 weeks).
Fetal/neonatal data. Morphological US, umbilical flowmetry and fetal echo-cardiography were normal for gestational age at 20 and 30 weeks. Fetal thyroid volume and vascularization, bone maturation, and fetal mobility were normal as well, at 32 weeks of gestation. August 2011 (38 weeks): cesarean section delivery. Neonatal data: TSH 29.4, 25.9 e 4.4 μU/ml and $FT_4$ 22.0, 31.4 e 16.0 pm/l, at 12 and 36 h and at 3 weeks of post-natal life, respectively. TRAb >40 UI/l at 36 h. Thyroid US was normal.
Severe hypothyroidism in child-bearing age women should advice routinely TRAb bioassay.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P410

**A case of refractory hypoglycemia**

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A 74-year-old diabetic lady presented to emergency-department following a collapse due to hypoglycaemia (blood glucose 1.7 mmol/l) and responded dramatically to intravenous–glucose. She had recurrent hypoglycaemic episodes in the last-fortnight, despite discontinuation of her oral hypoglycaemics and no other obvious precipitating factors/toxins like alcohol. She was on prednisolone, 10 mg along with azathioprine, 150 mg, atenolol, 25 mg, and candesartan, 8 mg. She has rheumatoid-arthritis, skin-psoriasis and essential-hypertension. She was diagnosed with a large right sided intra-thoracic fibrosarcoma (Fig. 1) for which she underwent surgery in 1995 and was confirmed histologically. Subsequently, she had further recurrence which deemed inoperable. These hypoglycaemic-episodes were investigated by ruling-out any obvious cardiac, renal, hepatic, or adrenal-dysfunction or sepsis. Perhaps coexistent fibrosarcoma and recurrent hypoglycaemia pointed towards possibility of non-islet cell tumour-hypoglycaemia. She was managed initially with i.v./oral-glucose and high-dose prednisolone along with carbohydrate rich diet; without controlling her hypoglycaemic attacks. Diazoxide, which inhibits pancreatic insulin-release, was introduced at a low-dose and up titrated to 150 mg TDS. Hypoglycaemia are usually fully-reversible after surgical removal of tumours; however, in our case with an inoperable-tumour, alleviating hypoglycaemia was becoming a therapeutic challenge. With limited options available, the role of GH was considered; as it is well established that GH stimulates hepatic gluconeogenesis and glycogenolysis, probably suggesting a role in managing non-islet cell tumour hypoglycaemia. She was thereby treated with GH which reduced frequency of her hypoglycaemic episodes and rendered her near-euglycemic.
Conclusion
Hypoglycaemia being a common reversible medical-emergency is frequently associated with oral hypoglycaemic-agents/insulin-therapy; however, it can rarely be a paraneoplastic manifestation of underlying tumour. Our case report illustrates therapeutic challenges encountered in a patient with inoperable fibrosarcoma, presenting with resistant hypoglycaemia and perhaps suggesting role of GH in similar cases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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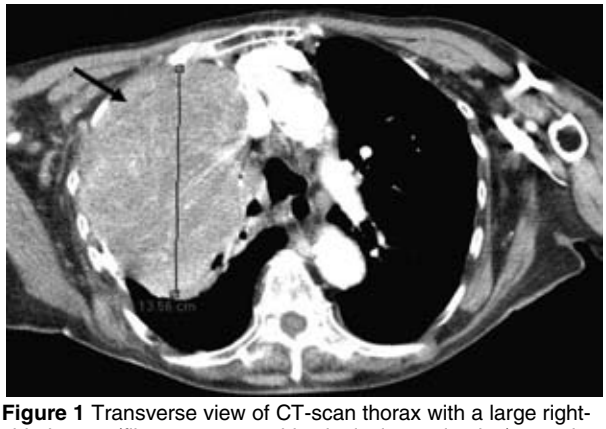

**Figure 1** Transverse view of CT-scan thorax with a large right-sided mass (fibrosarcoma on histological-examination) seen in the upper-zone of the lung.

## P411

**Double jeopardy in pregnancy: a case of autoimmune polyglandular syndrome type 2 in a pregnant patient**

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This is a case of an adult female who presented with 1 year history of palpitations, heat intolerance, easy fatigability, hyperdefecation and proptosis. She was admitted to this institution due to 3 month history of nausea, vomiting, abdominal pain, flank pain and generalized weakness. During admission, she was diagnosed with hyperthyroidism supported by laboratory findings of suppressed thyroid stimulating hormone and elevated free thyroxine levels and was then treated with methimazole. However, patient's condition did not improve. She remained hypotensive and tachycardic for 3 days which was initially attributed to septic shock secondary to urinary tract infection. Further work-up which includes serum cortisol level determination and co-syntropin test revealed adrenal insufficiency. She was given glucocorticoids, and eventually she became hemodynamically stable. Unfortunately, patient had abortion necessitating dilatation and curettage. With the presence of hyperthyroidism and adrenal insufficiency, she was then diagnosed with autoimmune polyglandular syndrome (APS) in Pregnancy. At present, there are very few published case reports of APS in pregnancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P412

**Pseudo malabsorption of levothyroxine**

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Background
Therapy with levothyroxine (L-$T_4$) is essential in hypothyroidism treatment. The marked elevation of thyrotropin (TSH) in patients treated with appropriate doses of L-$T_4$ is rare and can result from malabsorption, drug interaction or poor adherence. The non-adherence, omitted by the patient, is called pseudo malabsorption.
Clinical report
ACCS, female, 30 years old, hospitalized for persistent hypothyroidism despite L-$T_4$ therapy. Personal history: submitted to total thyroidectomy in March 2010 because cytological suspicion of follicular tumor in a simple multinodular goiter. The histology revealed a papillary microcarcinoma (T1N0M0), without other changes. Started replacement therapy with L-$T_4$. After increasing doses of L-$T_4$ (125 to 400 μg/day) there was no normalization of TSH or thyroid hormones

(TSH 74.3→93→50 μUI/ml (RR: 0.4–4.0), $FT_4$ 0.2→0.4→0.5 ng/dl (RR: 0.8–1.9)), with reappearance of thyroid tissue in surgical loca. Admitted to hospital for surveillance of taking L-$T_4$ and study of possible L-$T_4$ malabsorption.No drug habits. Physical examination: BMI 29 kg/$m^2$, without major clinical signs of thyroid dysfunction. Laboratory: TSH 74 μUI/ml, $FT_4$ 0.4 ng/dl. Blood count, biochemistry, folic acid, vitamin B12, iron metabolism, stool examination, research and degree of fat digestion of feces, autoimmunity for celiac disease or pernicious anemia: all normal. Esophagus and gastric endoscopy was macroscopically normal; a gastric biopsy was performed and showed chronic antral gastritis, non-atrophic, with mild activity and colonization by *H. pylori*. The test of oral overload with 1 mg of L-$T_4$ revealed: TSH 60 μUI/ml at baseline→33 μUI/ml after 4 h, $FT_4$ 0.7 ng/dl at baseline → 1.0 ng/dl after 4 h. This result confirmed the diagnosis of pseudo malabsorption of L-$T_4$. The therapeutic regimens included confronting the patient with the results and the eradication of H. pylori.

Conclusions

The poor adherence to therapy is the most common cause of persistent hypothyroidism in patients receiving adequate doses of levothyroxine. Clinical suspicion should be investigated excluding malabsorption syndromes or drug interaction. In this patient, the high dose overload of levothyroxine allowed the confirmation of the diagnosis. Psychological counseling may be necessary.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P413

### Vitamin D deficiency and elevation of para-thyroid in Thalassemia minor: a case study

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βThalassemia Minor cases unlike Thal-Major don't require immediate medical attention, due to effective compensatory haematopoiesis and remain transfusion independent and patients may remain metabolically deficient lowering quality of life. To impose an artificial genetic bottleneck and suppress the dispersion of Thal-minor and Thal-major mutationsas in a population early detection is the only way out. Hypocholestreloemia, Vitamin D deficiency and marginally elevated Parathyroid hormone may be found in Thal-minors as reported in the case study. Absence of significant anaemia may make the condition cryptic or latent, delaying diagnosis of the condition and the situation may be complicate in longterm. Parathyroid level may be evaluated for secondary hyperparathyroidism due to deficiency of vitamin D. If several findings of Ca crystals (++/+) are present in urine RE, Bisphosphonate therapy may provide relief. This case study reports occurrence of pancreatic insufficiency, resultant steatorrhoea, Vitamin D (25-OH) deficiency (13.86 ng/ml) with Hypocholesterolemia (85 mg/dl). Para thyroid hormone was in upper limit (62 pg/ml) and Ca+2 was 9.5 mg/ml in a 30 years old male Thal-minor patient (fetal hemoglobin 2.10%, HbA2 4.60% and Hb Adult 84.80%).

**Table 1** Some diagnostic parameters of the patient case

| Hemato-logical profile | Endocrineand related par-ameters profile | Serum enzymes | Serum anti bodies | Metabolic par-ameters and vitamin pro-file | Minerals and electrolytes |
|---|---|---|---|---|---|
| Hemoglobin 13.8 mg/dl | Para thyroid hor-mone 62 pg/ml | SGPT 333 IU/ml | Anti CCP antibody negative | F sugar 85 mg/dl | Serum calcium was moderately low at 8.6 mg/dl |
| Reticulocyte count 1.08% | Serum aldoster-one 304.99 pg/ml | Alk phos 400 IU/ml | ANA negative | PP sugar 100 mg/dl | Serum bi-carbon-ate mod-eraly elevated at 63 mmol |
| Fetal hemo-globin 2.10% | Erythropoietin 22.80 mIU/ml | | CRP negative | Total choles-terol 80 mg/dl | Serum chloride WNR |
| HbA2 4.60% | Homocysteine 11.97 μmol/l | | | HbA1c was 5.60% | Serum sodium WNR |
| Hb adult 84.80% | TSH WNR | | | Total bilirubin 3 mg/dl | Serum pot-assium was 5 mg/dl |
| TIBC was normal | $FT_3$ WNR | | | Total protein 6 mg/dl | |
| First hour ERS 08 mm (Wes-tergren) | $FT^4$ WNR | | | Vitamin B 12 level 466 pg/ml | |
| TC of WBC 6900/ cm | Urine osmolarity 295 mmol/kg | | | Folic acid level 14.15 ng/-ml | |
| DLC WNR | Urine volume 3560 ml/24 h | | | Vitamin D (25 OH) 13.86 ng/-ml | |
| | Creatinie clear-ance was 117 ml/min | | | 72 h stool fat was esti-mated to be 25.6 g | |
| | | | | 132 mg/dl protein excreted in 24 h | |

WNR - with in normal range for Indian males.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P414

### Study of hormone and thyroid antibodies of auto-immune thyroid disorders

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Auto-immune thyroid diseases (AITD) such as Graves' disease (GD) and Hashimoto's thyroiditis (HT) which are archetypical organ specific auto-immune diseases are increasing in recent years and about 2–4% of women and up to 1% of men are affected worldwide, and the prevalence rate increases with advancing age. The AITD are characterized by the presence of raised serum antibodies directed against thyroid antigens. The development of antibodies to antithyroid microsomal antibody, antithyroglobulin antibody and antithyrotropin receptor antibody (TR-Ab) is the main hallmark of AITD. HT is diagnosed by TPO-Ab, and/or Tg-Ab positively, a negative TR-Ab level and, the presence of diffuse goiter. Thyroid antibody testing is not routinely available in developing countries, and few studies have measured thyroid antibodies in Mongolia.

Thyroid hormones in serum, Tg-Ab, TPO-Ab using an ELISA technique in 209 patients with various thyroid pathologies attending an endocrine department in Shastin Central Hospital. Patients were clinically grouped into Graves' disease ($n=160$) and Hashimoto's thyroiditis ($n=39$). Blood donors without thyroid disease ($n=30$) acted as controls. During the study we performed blood test for thyroid hormones in nine patients with enlarged thyroid gland in euthyroidism, 30-hypothyroidism with unknown etiology.

The levels of thyroid hormones were analyzed in patients with Graves' disease. To determine thyroid hormones in patients with GD, thyroid hormones were $3.97\pm0.3$ nm/l, determine thyroid hormones in the euthyroidism were $1.72\pm0.13$ nm/l, in patients with hypothyroidism $0.29\pm0.03$ ng/ml. Thyroid TPO–Ab was 100% positive in all euthyroidism and hypothyroidism. In subjects with euthyroidism TG–Ab was $189.27\pm94$ IU/ml, and during hypothyroidism TG–Ab was $888.8\pm216.7$ IU/ml.

Study results showed the increase in $T_3$ and $T_4$ levels decreased TSH with Graves' disease. In the euthyroidism of thyroid hormones were revealed normal, in patients with hypothyroidism $T_3$, $T_4$ were low, TSH was high with Hashimoto's thyroiditis.Thyroid autoimmunity appears more common in patients with GD and HT, and Tg-Ab, TPO-Ab were significantly associated with auto-immune thyroid disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P415

**New insulin receptor mutation in a patient with primary amenorrhea**

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Primary amenorrhea is a rare disease that affects <1% of adolescent girls. The most common causes of primary amenorrhea include chromosomal abnormalities, hypothalamic and pituitary disorders, lack or structural abnormality of reproductive organs.
18 years old woman was referred to us by her pediatric physician for evaluation of primary amenorrhea and hirsutism. She had low birth weight, 1.9 kg, and hypostaturism. At the age of 6 years underwent to clonidine provocation test that showed normal values of growth hormone secretion. At the age of 17 the patient noticed some signs of masculinization (fine hairs on her cheeks) in the absence of menarche or evidence of sex development.
She is currently eighteen years old, her height is 132 cm (20 cm <3rd percentile), her weight 33 kg (BMI=18.9). Clinical evaluation of hirsutism according to Ferriman and Gallway has given a score of 23 (severe), no signs of acanthosis nigricans on the neck.
Endocrinological evaluation showed high levels of testosterone (3.4 ng/ml), with normal gonadotropins, estradiol, TSH and thyroid hormones, PRL, GH, IGF1, PTH, ACTH, 17-OH-P, DHEA-S. Pelvis ultrasonography showed microplolycystic ovaries and a small uterus. Metabolic assessment revealed fasting hyperinsulinemia (88 μU/ml) with normal fasting plasma glucose (89 mg/dl), but diabetes at 2 h of oral glucose tolerance test (289 mg/dl) with very high insulin levels (2123 μU/ml). Given the basal and glucose tolerance test insulin levels, considering the possibility of insulin receptor gene alterations, DNA direct sequencing of the exons 17–21 of the insulin receptor gene, encoding the tyrosine kinase domain, was maden.
An heterozygous mutation in exon 19 with arginine substituting glycine in position 1146 was found. The rest of the family is currently under investigation. Patient started therapy with flutamide 125 mg once a day *per os*, and metformin 850 mg three time/daily. After 6 month from the beginning of therapy menarche occurs.
Conclusions
To the best of our knowledge, this is the third Italian patient with severe insulin resistance due to a mutation of IR gene and the first patient presenting with primary amenorrhea.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P416

**Plasmapheresis therapy in a case with recurrent pancraetitis attacks as a consequence of hypertryglyceridemia**

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Hypertygiceridemia is responsible from the 1–4% of acute pancreatitis cases. When serum triglyceride level rises above 1000–2000 mg/dl, risk of having acute pancreatitis also increases. Medical therapy is usually inadequate for treatment of hypertryglyceridemia cases which cause severe and recurrent pancreatitis attacks. In our case we have reported a patient having recurrent attacks under medical therapy and underwent plasmapheresis.
Case
45 years old female patient has admitted to the hospital with epigastric pain and had the diagnosis of billiary pancreatitis and underwent cholecystectomy. After that operation she was hospitalized in every 2 or 3 months with severe abdominal pain, detected to have pancreatitis and further investigation was made to explain the reason of recurrent attacks. Severe hypertyglyceridemia was detected in laboratory tests. Although she was receiving fibrate therapy with the maximum tolerable and allowed dosage, her triglyceride levels did not fall below 2000 mg/dl. She was referred to our department in 2011. At the time of hospitalization her TG level was 9000 mg/dl.She underwent plasmapheresis with albumin for once. Her TG level was 600 mg/dl on the first day and 400 mg/dl at the third day after plasmapheresis. It has been 4 months from the plasmapheresis and she did not have any pancreatitis attack. Her triglyceride levels are below 400 mg/dl under fibrate therapy.
Result
Acute pancreatitis occurring secondary to hyperlipidemia is principally treated with hydration and pain palliation. Severe and recurrent attacks can be counted as plasmapheresis indication.Plasmapheresis reduces tryglyceride levels, removes active enzymes and preinflammatory molecules from the circulation. Although there is information about benefits of plasmapheresis for prevention of recurrent pancreatitis in hypertrygiceridemic patients there is no consensus about the ideal frequency and duration of therapy. As we see from our case sometimes only one plasmapheresis might be cost-effective and adequate for secondary prevention.
Declaration of interest
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## P417

**Association of thyroid hormone resistance and hypogonadotropic hypogonadism**

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Introduction
Patients with resistance to thyroid hormone (RTH) have variable tissue hyporesponsiveness to thyroid hormone (TH) due to a defect in the TH receptor (TR) β gene. So far, 124 different mutations have been identified among 343 unrelated families. Despite the TH resistance, some patients have symptoms and signs of hyperthyroidism and may have goiter. RTH is characterised by high TH and normal or high TSH concentrations. Differential diagnosis includes a TSH-producing pituitary adenoma, TH cell membrane transport and metabolism defects and euthyroid hyperthyroxinemia. RTH is associated with autoimmune thyroid disease but not with other endocrinopathies.
Case report
A 47-year-old male with antecedents of atrial flutter, ischemic ictus and hypercholesterolemia was sent to rule out hyperthyroidism. He complains of flushing, sexual dysfunction and infertility. On examination there was no goiter, heart rate: 72 beats/min, weight: 78 kg, height: 173 cm, BMI: 29 kg/m$^2$, normal genitalia and secondary sexual characters. His IQ was slightly low. He had high $T_4$ and $RT_3$, normal $T_3$ and high-normal TSH levels: $TT_4$:17.5 ng/ml (5.0–12.0), $FT_4$: 2.96–4.32 ng/ml (0.70–1.85), $FT_3$: 2.8–5.1 pg/ml (2.0–6.8), RT3: 0.38 ng/ml (0.10–0.35) and TSH: 2.11– 4.03 μU/l (0.40–4.00). A TSH producing pituitary adenoma was excluded (normal pituitary MRI, SHBG: 22.6 nmol/l (10–50), α-subunit, TSH and α-subunit responses to IV TRH (0, 15, 30, 60 min: TSH: 1.70, 11.10, 14.80, 8.33 μU/l;α-subunit (0.02–0.8): 0.23, 0.49;0.42, 0.29 UI/l). Ultrasound scan showed mixed nodules with citology of nodular hyperplasia. Antithyroid antibodies were negative. Thyroglobulin: 48 ng/ml (1.5–56) and binding proteins were normal: TBG 18 mcg/ml (10–40), albumin 4.6 g/dl (3.7–5.4), prealbumin 30 mg/dl (10–40). Gonadal function tests showed total testosterone 2.41–2.60 ng/ml (2.70–10.69), FSH 7.09–11.10 mUl/ml (1.5–15), LH 0, 89–1.68 mUl/ml (1.4–7.7), PRL basal 4.79–6.56 ng/ml (2.50–17.00) and ferritin 316 ng/ml (20–300). The other pituitary hormones were normal as well as TH in his relatives (parents and a daughter). A TR β gene mutation was detected: switch G>T in heterozygosis in the c.959 position that produces a switch of the aminoacids p.Arg320Leu (R320L). This mutation have been described before.
Conclusions
We present a case of RTH clinically euthyroid due to a de novo mutation in the TR combined with idiopathic hypogonadotropic hypogonadism. This association have not been reported previously.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P418

**5-Alpha reductase type 2 deficiency: a case report**

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5-Alpha reductase deficiency is a rare 46, XY disorder of sex differentation caused by mutations in the 5-alpha reductase type 2 gene (SRD5A2) located on chromosome 2p23. Affected patients have a deficiency of the 5-alpha reductase type 2 enzyme, which becomes partially or totally unable to convert testosterone into dihydrotestosterone, the latter being responsible for the development of the external genitalia, prostate, and urethra in the male fetus. Most affected individuals are characterized at birth by predominantly female external genitalia and are often raised as girls. Patients raised as girls exhibit spontaneous virilization at puberty. Some patients are sufficiently masculinized at birth and are raised as boys.

In this article we describe a 22-year-old patient who had female external genitalia at birth and was raised as a girl. When puberty was reached, she had amenorrhea, virilization and lack of secondary sex characteristics. For these reasons, she was brought to our clinic and 5-alpha reductase deficiency was diagnosed. The woman exhibited a masculine build and muscle development, male hair characteristics, micropenis, and perineoscrotal hypospadias. Testes were palpable in the right and left inguinal canals. Chromosome analysis revealed a 46,XY karyotype. On pelvic MRI, ovaries and uterus were absent. A 41×11 mm vagina-like structure was seen, as were testes within labia-like scrota bilaterally, and seminal vesicles with corpus cavernosa. Hormonal studies included serum total testosterone level 1.54–2.67 ng/mL (normal: 0.1–0.75), free testosterone level 13.79 pg/ml (normal: 0.29–3.18), free androgen index 40%, 17-OHP level 2.34 ng/ml (normal: 0.5–2.4), FSH 28.23 IU/ml (normal: 1.7–19.26), and LH 7.93 mIU/ml (normal: 1.24–8.22). While the serum testosterone was 9.80 nmol/l, serum DHT was 0.52 nmol/l and T/DHT ratio was calculated to be 18.8 (normal is $<12$). Upon further analysis of the 5-alpha reductase type 2 gene, a previously unreported heterozygous R178S mutation was found.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P419

**Subacute or hashimoto thyroiditis? a diagnostic dilemma: case report**

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Introduction

Subacute thyroiditis (SAT) is characterized by cervical pain. Rarely, other thyroid diseases, like Hashimoto's thyroiditis (HT), may be associated with cervical pain, leading to confusions with SAT. We report a particular case of painful goitre.

Case report

D Maria, 50 years old, presented with cervical pain, low grade fever, inflammatory syndrome (ESR = 132 mm/1 h), and was diagnosed with SAT. Thyroid function was normal. Ultrasound (US) aspect of goiter (30 ml) was in homogeneous, hypoechoic, pseudo-nodule in the left lobe. Treatment with prednisone (30 mg/day) induced a rapid improvement of pain and inflammatory syndrome (6 weeks after ESR = 26 mm/1 h). After 3 months, with 10 mg Prednisone/day, she had a relapse (thyroid pain, ESR = 95 mm/1 h) with new remission after increasing dose followed, 2 months later, by a new episode of severe cervical pain and high ESR = 87 mm/1 h. Euthyroidism, positive antiTPO antibodies. Thyroid US revealed a hypoechoic irregular left nodule, with suspect cytology. This aspect and the intolerance to anti-inflammatory drugs have led to surgery – quasitotale thyroidectomy. Histology described an association of lymphocytic thyroiditis with giant cells. Postoperative hypothyroidism is compensated until present with 100 mg $LT_4$/day.

Discussion

The overlap of symptoms may determine the confusion between SAT and HT. Painful HT is rare; in most literature cases the response to anti-inflammatory treatment was transitory and surgery was imposed by the evolution of the disease. The histological exam of our case suggests a rare association between the two forms of thyroiditis. We may speculate that inflammation of SAT has determined an immune response inducing the appearance of TH.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P420

**True hermaphroditism with a rare 46,XX/47,XXY Klinefelter's mosaicism: a new unique case and review of previous reports**

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Introduction

Only few cases of true hermaphroditism with male phenotype and 46,XX/47,XXY Klinefelter's mosaic karyotype with clinical features different among cases have been previously described.

Case report

A 12-year-old patient was referred to our clinic for mixed gonadal dysgenesis. Since birth he has ambigous genitalia: unilateral criptorchidism on the right and perineal hypospadia; left gonad was palpable in the scrotum since birth. At the age of 1.5 months the child underwent surgery for the strangulated right inguinal hernia: fallopian tube and a necroticised right gonad were removed. Histopathological examination revealed ovarian tissue. Cytogenetic analysis of peripheric blood at the age of 1.5 years detected 46,XX (80%)/47,XXY (20%) karyotype. He had hypospadia repairing in several steps. Further, the Mullerian remnants were removed laparoscopically also on the left. At presentation, his stature was 145.5 cm (−0.2 SDS), weight: 34 kg (−1 SDS), bone age: 13 years, pubertal stage: Px3Ax1; left testis 1 ml, penis 4.5×2.5 cm; bilateral ginecomastia. Both serum LH and FSH levels were evevated, basal testosterone and estradiol were low. At ultrasound, a structure typical for testicular tissue with the epididimal cyst on the left and the rudimentary uterus were present. As the patient is raised as a male, testosterone replacement therapy for primary hypogonadism has been prescribed; surgical removal of any Mullerian structures left was recommended. The left gonade, presumably testis, requires biopsy and further monitoring for malignancy.

Conclusion

This case of disorder of sexual differentiation with male phenotype and 46,XX/47,XXY Klinefelter syndrome's mosaic karyotype is intriguing for finding of Mullerian remnants on both sites. A comparative review of previoulsy reported ten cases will be done to empaphazyse doctors's awareness and the necessity of multidisciplinary approach to cover differnt aspects of follow-up for every such a patient.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P421

**Genetics of HLA-identical monozygous twins with different manifestations of polyglandular autoimmune syndrome**

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Summary

The autoimmune polyglandular syndromes (APS) comprise a wide spectrum of autoimmune disorders and are divided into a very rare juvenile (APS 1) and a

relatively common adult type with (APS 2) or without adrenal failure (APS 3). APS 1 is caused by mutations in the autoimmune regulatory (AIRE) gene on chromosome 21 and is inherited in an autosomal recessive manner. Mutations of the AIRE gene result in defective proteins which cause autoimmune destruction of target organs by disturbing the immunological tolerance of the patients. Genetic testing may identify patients with APS 1, but not those with APS 2 and 3. For APS 2/3, susceptibility genes are known which increase the risk for developing autoimmune disorders, but without being causative. These are certain HLA genes, the cytotoxic T lymphocyte antigen (CTLA-4)gene, and the protein tyrosine phosphatase nonreceptor type 22 (PTPN22) gene on chromosomes 6, 2, and 1, respectively. Actual diagnosis of APS involves serological measurement of organ-specific autoantibodies and subsequent functional testing.

Our patients

We present the history of a 30-year-old monozygotic female twin pair. One of them (KDN) had Hashimoto's thyroiditis as first manifestation of APS and, 4 years later, Addison's disease occured and gestational diabetes was found during her first pregnancy. The first clinical sign of the second patient (NDN) was type 1 diabetes with severe hyperglycemia and, at the same time, hypothyroidism was also found due to Hashimoto's thyroiditis, which was followed with Addison's disease 5 years later. These monozygotic twins presenting polyglandular autoimmunity had different manifestations of diabetes mellitus although they were HLA-identical. Genetic testing was performed for trying to find the difference in their genome and subseptibility genes.

Results

The DNA chip data showed in both patients insertion in the 12. chromosome (q21.1) and gain of ANTXRL gene the GDM (KDN) positive patient (pseudogene) and different LCHSs (loss of heterozygosity) in both patients. The RNA chip and real time PCR data revealed in the patient with T1DM (NDN) overexpression of the IFNinduced transmembrane protein, IFN$\gamma$R 2, HLA DOB, PTPN2

Key words

Polyglandular autoimmune syndrome, HLA identical twins, variability of clinical manifestation, genetics.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P422

### Papillary thyroid-type carcinoma arising from struma ovarii: a case report

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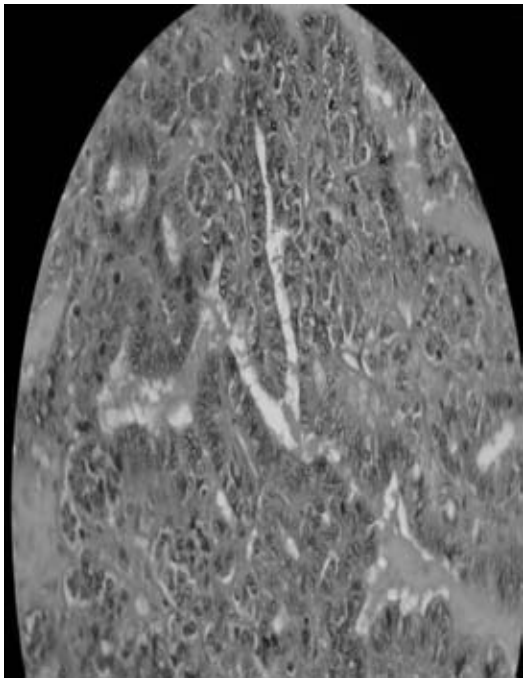
Struma Ovarii and its malignant transformation to thyroid-type carcinoma are rare conditions. In recent reviews of reported literatures, only 53 cases of papillary thyroid-type carcinoma arising from struma ovarii were documented from 1924 to 2008. Due to the rarity of the disease, lack of uniform histological criteria for malignancy and protracted clinical course, its management is not also universally accepted by physicians. The aim of this paper is to present a very rare case of papillary thyroid-type carcinoma arising from struma ovarii, and review literatures on the suggested consensus for diagnosis and management of case.

A 41-year-old Filipino woman presented to our section after she underwent total abdominal hysterectomy with bilateral salphingectomy for suspected primary ovarian carcinoma. She has been complaining of vague abdominal pain and distention for almost a year. Her pre-operative CT scan of the abdomen revealed large cystic mass with calcification suggestive of ovarian teratoma. Intra-operatively, the left ovary was converted to an $11\times8\times3.5$ cm multiloculated, multiseptated mass densely adherent to the bowels and uterus. On pathologic examination, the large ovarian mass proved to be a papillary thyroid-type carcinoma arising from a struma ovarii. She underwent platinum-based chemotherapy used for ovarian teratoma.

On review of available case reports and case series done on papillary thyroid-type carcinoma, surgical resection of the tumor has been shown as effective treatment modality. Total thyroidectomy to facilitate whole body scan, radioactive iodine ablation, and thyroxine suppression therapy have shown to significantly decrease tumor recurrence and mortality. Chemotherapy and radiotherapy did not show any benefits on clinical outcomes.



Our patient underwent total thyroidectomy, radioactive ablation therapy and levothyroxine suppression therapy after tumor resection.

Papillary thyroid-type carcinoma arising from stuma ovarii as presented in our case should, therefore, be diagnosed and managed as primary papillary carcinoma of thyroid gland.

Low power view showing papillary architecture with fibrovascular core and overlapping nuclei.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P423

### Hypothyroidism with extensive pericardial effusion: different therapeutical approaches

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Introduction

Disturbances of the thyroid gland are the most common diseases of the endocrine system. Their prevalence is increasing with age and can be easily under diagnosed. Advanced forms of thyroid disease can mimic cardiovascular, psychiatric disorders, malignancy or other disorders and can result in life threatening conditions.

Case reports

In the current study we present two case reports concerning patients with cardiovascular symptoms. In both patients an extensive pericardial effusion was found as the first sign of undiagnosed hypothyroidism. The first patient (50-year-old woman), a pericardiocentesis was performed because of clinical and

echocardiographic signs of cardiac tamponade. The pericardiocentesis resulted in a removal of effusion of 2560 ml. The second patient (34-year-old woman), the pericardial effusion was concentrated predominantly in a hardly accessible place. Therefore a conservative approach was decided also because of absence of clinical symptoms of tamponade and despite of a presence of a right atrial collapse. Both patients received levothyroxine replacement therapy in order to treat the hypothyroidism. No refill of pericardial effusion has occurred in the patient treated by pericardiocentesis. The volume of the pericardial effusion was reduced substantially in the patient treated conservatively.

Conclusions

In both patients insidious non-treated hypothyroidism can result in major cardiologic impairment. Correct diagnosis of hypothyroidism was of essential importance concerning successful treatment of pericardial effusion. A matter concerns the rate of replacement therapy: long lasting hypothyroidism could induce a recurrence of a pericardial effusion after pericardiocentesis or its progression in patient treated conservatively. In the contrary a rapid increasing of replacement dose could induce a heart failure or acute forms of coronary heart disease in patient with underlying coronary artery disease.

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Declaration of interest

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## P424

Abstract withdrawn.

## P425

**A case of persistent hypoglycemia in the setting of connective tissue disease**

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Introduction

Autoimmune forms of hypoglycemia are uncommon. However, they should be considered in any patient with hyperinsulinemic hypoglycemia. Here we present a case of persistent hyperinsulinemic hypoglycemia caused by insulin receptor antibodies.

Case report

A 65-year-old chinese woman with mixed connective tissue disease (MCTD) and hypothyroidism presents with a 2-week history of persistent hypoglycemia since discontinuation of her immunosuppresants. A 72 h fast was consistent with hyperinsulinism; serum glucose was 1.1 mmol/l, insulin was 823 pmol/l (normal fasting: $<$118 pmol/l), c-peptide was 615 pmol/l (normal fastng: 264–1026 pmol/l). Adiponectin was 56 μg/ml (normal: 8.3–13.9 μg/ml). There was no history of insulin use. Screening for plasma sulfonylureas, biguanides, insulin analogs and insulin antibodies were negative. Imaging of the abdomen and pancreas was normal. Given the hyperinsulinism, screening for insulin receptor antibody was performed which confirmed the presence of anti-insulin receptor antibodies. She was diagnosed with Type B insulin resistance and started on immunosuppressants resulting in improvement of her hypoglycemia.

Conclusion

In patients with hyperinsulinemic hypoglycemia, especially in the setting of connective tissue disease, the diagnosis of Type B insulin resistance needs to be considered. In this syndrome, insulin receptor antibodies are present and are either antagonistic or agonistic to the insulin receptor. Typically, patients present with severe hyperglycemia. Initial presentation of persistent hypoglycemia, as in our patient, is rare. The insulin:c-peptide ratio is usually 0.2–0.5. In our case, the insulin:c-peptide ratio was 1.3. Possible explanation includes altered metabolism and degradation of insulin. A paradoxical elevation of adiponectin is seen and may be caused by altered insulin action on adipocytes. The presence of elevated adiponectin can aid in the diagnosis of this rare syndrome. Despite the rarity of this condition, awareness of type B insulin resistance is essential to allow for proper management since treatment usually includes multi- modal immunosuppresion to target pathogenic antibodies, and to prevent unnecessary pancreatic surgery.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P426

**Successful pregnancy in a patient with biologically inactive LH or partial LHCGR resistance**

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Introduction

LH resistance is very rare and there are only a few case reports in the literature. We describe a patient who presented to us with secondary amenorrhea and very high isolated LH levels who subsequently had a successful pregnancy with IVF.

Case report

A 16 year old girl was referred to us to investigate the cause of her oligomenhorrea. She had attained menarche at the age of 13 and had only 5 periods in 3 years. She had no symptoms/signs of any endocrine abnormality.

Her blood results showed elevated LH levels more than 250 U/l. Her FSH, prolactin and the rest were normal. There was no evidence of interference from heterophile antibodies. MRI scan of her pituitary was normal. We suspected she had LHCGR mutation causing LH resistance, however genetic analysis did not identify any such mutation in exon 11.

She was started on the OCP. There was some suppression of LH from the baseline although incomplete. She was having regular bleeding on the OCP. In 2008 she wanted to start a family. Since she did not have LHCGR gene mutation, we wondered if the receptor function was normal and hence decided to give a trial of IVF cycle. She received Zoladex to induce pituitary down regulation followed by recombinant FSH to induce follicular growth and hCG. She had a successful pregnancy. Currently her LH is not fully suppressed in spite of being on the OCP confirming that the hypothalamic pituitary dysfunction persists.

Conclusion

LH resistance in women is a very rare phenomenon. Our patient is likely to have either partial LHCGR resistance or biologically inactive LH. Our patient had a positive pregnancy with IVF making it a success story however the molecular diagnosis for her LH resistance is still unclear.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P427

**Quadruple metachronous malignancy in a single patient with multiple sclerosis: case report**

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Introduction

Quadruple primary malignancies occur with an incidence of $<$0.1% and $<$100 cases have been published to our best knowledge. We are presenting a patient with multiple sclerosis and triple thyroid carcinoma, double melanoma and a breast cancer.

Case report
Over the course of 4 years we treated a single patient due to stage III (T3, N1a, M0) medullary thyroid carcinoma size 45 mm in the right lobe and two micropapillary carcinomas in the left lobe, 1.24 mm thick scapular melanoma (Clark II, Breslow II) and 0.85 mm thick lumbar melanoma (Clark II, Breslow II) with negative sentinel node, lobular invasive 4 mm breast carcinoma with clear resectional margins and negative nodes followed by 20 mg tamoxifen daily. FDG–PET scan peformed 1 month ago was negative, CA 15–3 level was within normal values and calcitonin level was 83 ng/l. There are no signs of disease reccurence. From 1980 to 2011 only 89 cases of primary quadruple malignancy have been reported. The number of multiple malignancies report is slowly but gradually increasing.
Conclusion
Quadruple malignancy is a rare phenomenon in medicine and most cases of multiple malignancies affect one organ in a female patient. Our patient had family history of malignancy on mother's and father's side (father, grandmother and uncle) . She has not received chemotherapy with alkylating agents which are well known cause of secondary cancers. The patient received mitoxanthrone, drug extensively used as a disease-modifying therapy for multiple sclerosis. However, this treatment could be linked to non-malanocytic skin tumors and increased susceptibility to develop acute promyelocytic leukemia.
Further studies and closer clinical attention is needed to clarify the relation between secondary malignancies, applied treatment and endogenous and exogenous carcinogens.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P428

**Hungry bone syndrome in a patient with McCune–Albright syndrome after total thyroidectomy: case report**

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McCune–Albright syndrome (MAS) is a rare disease. It is characterized by a combination of polyostotic fibrous dysplasia, autonomous overproduction of hormones and skin hyperpigmentation.
Thirty-year-old patient with significant bone deformities and the history of multiple fractures of long bones despite treatment presented with multinodular goiter with long standing hyperthyroidism. During thyroidectomy in 2009 two enlarged nodules suspected for being inferior parathyroid adenomas were removed. In the postoperative period the patient developed severe symptoms of hypoparathyroidism: overt hypocalcaemia with tetany and the need for parenteral and then oral substitution of calcium and vitamin D. The doses required to achieve normal calcaemia are shown in Fig. 1. High bone turnover in fibrous bone dysplasia and removal of the functioning parathyroid glands were probably the causes of hungry bone syndrome described. We also assume hyperthyroidism as the aggravating factor. Primary hyperparathyroidism could also be one of the manifestations of autonomous endocrine overproduction in MAS but it was not confirmed in our case (biochemical parameters prior to operation, no parathyroid hyperplasia on histology). Two years postoperatively parathyroid hormone levels are within the normal range, but the doses of vitamin D and calcium supplementation remain unusually high, Fig. 1. Currently the patient has no symptoms and is surprisingly free of fractures since the operation with marked improvement in *T*-score.
In our report we describe a case of hungry bone syndrome with severe hypocalcaemia after accidental removal of two parathyroid glands during total thyroidectomy and subsequent need of unusually high doses of calcium and vitamin D substitution in a patient with McCune–Albright syndrome.

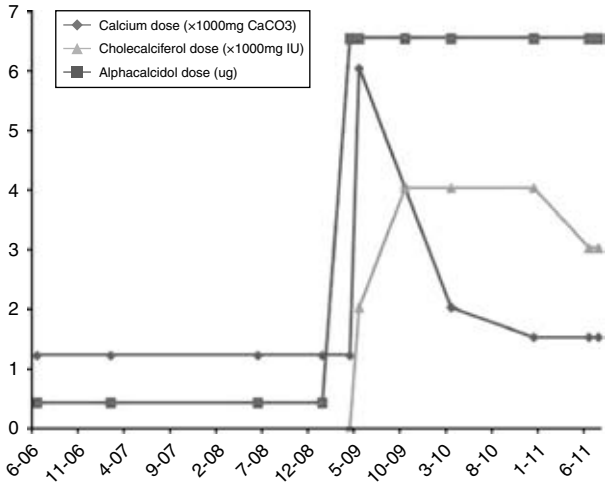


**Figure 1** Doses of vitamin D and calcium.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P429

**A case with thyroid metastasis from small cell lung carcinoma**

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Background
Among the lung carcinomas metastasizing to the thyroid, adenocarcinomas are the commonest followed by squamous, small cell, large cell and bronchioloalveolar carcinomas.
Case presentation
A 55-year-old man who presented with superior vena cava syndrome was found to have an 11 cm mediastinal mass encasing superior vena cava and compressing trachea and esophagus. The mass reached right pulmonary hilus. A bronchoscopic biopsy showed small cell carcinoma of the lung. The thyroid appeared normal on computed tomography (CT) of the chest. The patient was given chemotherapy with cisplatin and etoposide and external radiotherapy. Six months after the presentation, multiple brain metastases were detected on magnetic resonance imaging. Chemotherapy was changed to topotecan and cranial irradiation was performed. Ten months after the presentation a right thyroid nodule was detected on CT scan of the chest. On physical exam, a right thyroid nodule was palpable. $FT_4$, $FT_3$, TSH levels were within normal limits at that point, as well as antithyroglobulin and antithyroid peroxidase antibodies which were performed at a later date. Thyroid ultrasonography (USG) showed a $26.2 \times 16.8 \times 15.7$ mm hypoechoic solid nodule with irregular borders in the right thyroid lobe. USG-guided fine needle aspiration showed metastasis from small cell lung carcinoma. His cranial metastases worsened. He developed right cervical lymph node, pancreatic and meningeal metastases and passed away 15 months after the initial presentation.
Discussion
We report a rare case of thyroid metastasis from small cell lung carcinoma. After reviewing the literature and this case, we conclude the following:
1) Metastasis to the thyroid gland indicate poor prognosis, especially metastasis to the thyroid from lung carcinomas represent preterminal events.
2) In any patient with a known history of malignant disease, the appearance of a new thyroid mass should trigger a thyroid fine needle aspiration biopsy to search for metastatic disease.
Declaration of interest
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## P430

**Central hypothyroidism following peripheral hyperthyroidism: a brief case report**

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Objective
Measuring thyroid stimulating hormone levels alone may be insufficient to appropriately evaluate thyroid function. Reduced thyroid stimulating hormone

levels associated to normal/reduced $FT_4$ levels should prompt investigation of pituitary function.
Case
A 31-year-old man underwent biochemistry and thyroid function assessment for asthenia. He developed thyrotoxicosis due to painless thyroiditis with. positive.antithyroid antibodies. He had TSH level $<0.010$ mU/l (*n*: 0.35 to 4.9 mU/l), $FT_4$ level was 2.5 ng/dl (*n*: 0.7 to 1.5 ng/dl) and $FT_3$ level increased to 4.5 pg/ml (*n*: 1.7 to 3.7 pg/ml).
Subsequently 5 months later, we showed without any treatment reduced thyroid stimulating hormone levels associated to slightly decreased $FT_4$ levels and low-normal $FT_3$ levels. Endocrine assessment revealed deficiency in ACTH-cortisol, GH and IGF1 with hyperprolactinemia. Dynamic endocrine tests confirmed panhypopituitarism.
In fact, central hypothyroidism followed peripheral hyperthyroidism.
Magnetic resonance imaging showed a large pituitary tumor with cystic component with extension intra-and suprasellar suggestive of a craniopharyngioma.
The patient has a neurosurgical endoscopic transphenoidal allowing removal of the tumor and the histological analysis confirmed the diagnosis.
Conclusion
This association of peripheral hyperthyroidism with central hypothyroidism must be a rare occurrence, as a literature search has found only one case almost similar. Especially as the craniopharyngioma is a rare brain tumor and accounts for ~1.2–4.6% of all intracranial tumors. Our case illustrates coexistence of craniopharyngioma with hypopituitarism and clinically significant autoimmune thyroid disease. The presence of hypopituitarism does not preclude the development of autoimmune thyrotoxicosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P431

**Impact of glibenclamide therapy in a patient with neonatal diabetes and intermediate DEND syndrome with the V59M mutation in the KCNJ11 gene**

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[2]Hospital Maria Pia, Centro Hospitalar do Porto, Oporto, Portugal.

Introduction
Neonatal diabetes is a rare condition diagnosed within the first months of life. Activating mutation of KCNJ11, the gene encoding the Ki6.2 subunit of the ATP-sensitive potassium channel, is the most common cause of permanent neonatal diabetes, and ~20% of patients have neurological features.
Patients with the severe neurological phenotype exhibit developmental delay, motor weakness, and epilepsy in addition to diabetes (DEND syndrome). Intermediate DEND syndrome is a less severe clinical picture.
Sulfonylurea therapy is associated with metabolic and neurologic improvement in these patients.
Case report
We report the case of a boy diagnosed diabetes at the age of 6 weeks, which proved to be permanent, and who was treated with insulin from diagnosis. He immigrated and was lost in follow-up since the age of 18 months until he was 19 years. When we reviewed him, there was a past of behavioural and psychomotor disabilities since the age of 4, so DEND syndrome was suspected, and he was tested for K ATP channel defects. A heterozygous mutation *p*.VAL59Met (c.175G>A) was found in exon 1 of the KCNJ11gene, confirming the diagnosis of intermediate DEND syndrome.
Glibenclamide treatment was started at age 19 and increased up the dosage of 0.6 mg/kg per day, and insulin doses were gradually reduced, leading not only to improved glycaemic control (HbA(1c) fell from 11.6 to 8.9%), but also an impressive improvement in several aspects of cognitive function.
Conclusion
Although neonatal diabetes is a rare form of diabetes mellitus, genetic studies are critical in the diagnosis and treatment of these patients.
In this case neurological and glycaemic control improvement was seen after starting glibenclamide, but did not result in freedom from exogenous insulin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P432

**Parkinson-like syndrom as a unusual manifestation of primary hypoparathyroidism: case report**

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We describe case of 50-year patient with poliomyelitis in childhood, no other concomitant ilnesses, admitted in 2010 to neurologic departement of our hospital for grave parkinson-like syndrom with loss of motor function, symetric quadruparesis with spasticity, epileptic seizures and mental deterioration. These problems started about 12 months before and progressively worsened, patient was treated by levodopa and primidone. Patient was after bilateral intraocular lens implantation for cataracta of unknown origin in 40 years.
Brain CT scan reveals basal ganglia and cerebellum calcifications, blood calcium level was extremely low (1.04 mmol/l) as well as parathormone level (<5 pg/ml). Neck sonography, renal sonography, electroencephalography was without pathologic changes.
After intensive calcium i.v. supplementation (at beginning 20 mmol/day) and calcitriol supplementation patient status rapidly ameliorated. Symetric quadruparesis almost disappeared after 14-days treatment and patient was dismissed to out-patient care on peroral calcium and calcitriol medication. Next was antiparkinsonic and antiepileptic therapy diminished and 6 months after event was discontinued with no side effect.
Now, 1 year after diagnosis, patient is completely asymptomatic, with normal EEG findings, with no motor problems, fully self-sufficient, with normal ionisated and total calcium level with only substitution of calcitriol (1.5 µg daily). Screening for concomitant autoimmunities (thyroid, adrenal, pancreatic antibodies) was performed with no abnormality.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P433

**Van Wyk e Grumbach syndrome: case report**

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Introduction
In 1960, Van Wyk and Grumbach described the association of hypothyroidism and precocious puberty. Hypothyroidism leads to delayed bone age and a reduction in growth rate by reducing the amplitude of GH pulses. When precocious puberty is associated with thyroid hypofunction, estrogen action on the epiphyseal plates reduce this delay. We report the case of a child who developed incomplete puberty and reduced growth rate, caused by primary hypothyroidism.
Case report
G. 7th, female, complaining to grow 1 cm in 1 year and weight gain. Father 172 cm tall and the mother 168 cm. Child with 115 cm, 29 kg, BMI: 22 and short stature. FC: 52, TSH> 100, IO 4–5 years. Treatment with levothyroxine, TSH normalize and child thinned, antibodies. After 6 months of starting treatment developed central precocious puberty: 0.66 LH, FSH 2 TO 9.4 and Prolactin 6:36 ng/ml - normal skull CT. M2 P1, TSH 0.09, T4 1.33, IO 5 years.
Discussion
Hypothyroidism is a rare cause of precocious puberty. Van Wyk and Grumbach proposed that chronic hypothyroidism cause a negative feedback to the absence of TSH, gonadotropins and prolactin and their removal would be responsible for increased levels of TRH. The high HRT cause the increase of FSH which in turn would trigger puberty. In this case the patient did not have hyperprolactinemia.
Conclusion
This syndrome, when untreated and recently diagnosed as well as physical damage loss in stature, can provide psychosocial impairment. Therefore, immediate action is necessary to lessen or prevent the damage height and provide psychological support to children and parents in order to avoid major behavioral disorders.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P434

**Primary hyperparathyroidism associated with autoimmune polyglandular syndrome type III and isolated IgA deficiency**

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Introduction
Isolated IgA deficiency (SIgAD) is the most common form of primary immunodeficiency. Patients with SIgAD have an increased risk of both systemic and organ-specific autoimmune diseases. Autoimmune thyroid diseases (AITD) and type 1 diabetes mellitus (T1DM) are the most common autoimmune endocrine disorders. They occur frequently together, and this combination is nominated as autoimmune polyglandular syndrome type 3 (APS 3). Primary hyperparathyroidism (PHPT) is the most common cause of hypercalcemia, most cases occur in women, mainly as a sporadic disease, most often caused by a single adenoma.
Case report
A 55-year-old woman with a past history of diabetes mellitus was admitted to the hospital for hypercalcemia found on routine laboratory testing. Her family history is negative for hypercalcemia or endocrine tumors. In addition to the presence of vitiligo, the patient was found to have AITD (anti-thyroid peroxidase antibody-positive subclinical hypothyroidism) and anti-glutamic acid decarboxylase antibody-positive diabetes mellitus (T1DM). Subsequent workup confirmed the presence of PHPT (elevated total 3.21 mmol/l and ionized serum calcium 1.70 mmol/l; serum creatinine level was normal; urinary calcium excretion was 7.7 mmol/24 h; serum intact PTH 393 pg/ml inappropriately elevated; bone densitometry showed *T* scores at the lumbar spine of $-2.5$, at the total hip of $-1.7$, and at the distal radius of $-3.0$; (99 m)Tc-sestamibi scintigraphy localized abnormal lower right parathyroid gland). Immunological investigation revealed SIgAD with serum IgA $<$ 0.24 g/l in the presence of normal levels of other immunoglobulin isotypes and no history of recurrent infections. The patient was cured after parathyroidectomy, histopathological diagnosis was parathyroid adenoma.
Conclusion
There are only rare cases of immune-mediated hyperparathyroidism, associated with anti-calcium- sensing receptor autoantibodies, frequently in context with other immune diseases. The case presented here describes association of PHPT caused by adenoma with APS 3 and SIgAD. Genetic basis for this antibody deficiency and associated autoimmunity remains to be defined.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P435

**Reversible Hashimoto's thyroiditis: clinical, hormonal, autoimmune and ultrasonographic evidence**

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Introduction
Hashimoto's thyroiditis (HT) and Graves' disease (GD) constitute a spectrum of autoimmune thyroid diseases (AITD). An abnormal thyroid echographic pattern characterized by a diffuse low echogenicity has been described in both AITD. This hypoechogenicity is due to three components: increase of intrathyroidal flow, functional changes in thyroid follicles with increased cellularity and decrease of the colloid content, resulting in the reduction of the cell/colloid interface, variable degree of lymphocytic infiltration. The first two components may be reversible during medical treatment and seem to be characteristic for GD, whereas lymphocytic infiltration may rather represent mostly HT.
Case report
A 19-year-old boy manifested initially, at the age of 15 years, the typical clinical signs of hyperthyroidism (excessive sweating, heat intolerance, sleep disturbances firm goiter (II degree), tachycardia, palpitations, nervousness, excessive sweating and tremor). Laboratory tests evidenced mild T3-dependent hyperthyroidism with decreased TSH and a positive titer of anti-TPO and anti-TG antibodies whereas TRAb were negative. Thyroid ultrasound (TUS) was heterogenous, predominantly hypoechogenic. Hashitoxicosis was diagnosed. 2.5 years later, after more than 1.5 years of no control, he was readmitted to the ward with clinical an hormonal features of hypothyroidism of autoimmune origin. Ultrasound examination showed hypoechogenicity of the gland and enhanced vascular flow based on Power Doppler analysis. L-thyroxine (L-T4) was added. 6 months later, the primary care physician withdraw L-T4 since TSH was normal as well as 3 months later. 6 months after L-T4 withdrawal the patient was still euthyroid with no goiter and full recovery of normal blood flow and normal echogenicity of the thyroid was documented (Fig. 1) thus confirming the ultrasonographic remission and reversibility of the disease however anti-TPO were still mildly elevated. Conclusion: This is the first evidence that HT, with its classic manifestation, might be reversible on clinical, hormonal and ultrasonographic grounds.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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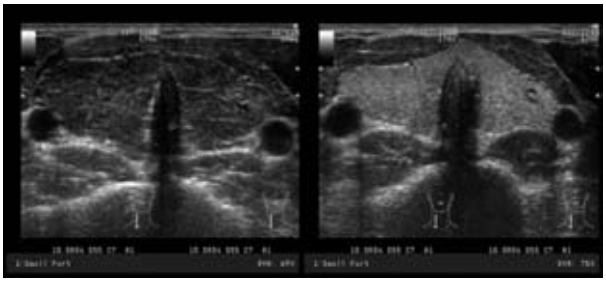

**Figure 1.**

## P436

**Hypoparathyroidism - a rare cause of heart failure**

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Introduction
Calcium plays a key role in myocardial contractions and relaxation. Heart failure induced by hypocalcemia is rare but potentially reversible case.
Case report
We present the case of 50 years old woman with muscle cramps, cataract, chest pain and dyspnea that lasts 2 years. In regional health center she was treated as psychiatric patient and COPD. Last few months she became extremely malaise with crural edemas and walking distance of 100 m. She was referred to Clinic for Cardiology and rtg of chest show cardiomegaly and bilateral pleural effusion. ECG report show negative T waves between V1-6. CK levels were extremely high, while CKMB and troponin levels were normal. Echocardiography suggested systolic and diastolic dysfunction of both left and right ventricules with mitral and tricuspid regurgitation, while EF was 37%. Standardized therapy for heart failure was started but there was no clinical effect. Coronarography was normal. Endocrinologist was consulted due to high CK levels, clinical signs of hypocalcemia were observed and calcium (1.3 mmol/l) and PTH (6 ng/ml) levels suggested hypoparathyroidism. Substitution therapy with calcium and vitamin D was started. After 1 year of therapy patient was asymptomatic. Control echocardiography was performed and both systolic and diastolic function of ventricules were normal, EF was 51%.

Conclusion
In patients with heart failure calcium levels should be tested, especially if heart failure is refractory to therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P437

**Hypogonadism with subsequent multi-organ involvement: a mystery solved**

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A 53-year-old gentleman was seen following a recent diagnosis of type 2 diabetes in May 2009. He had suffered a subarachnoid haemorrhage in 1993 and remained under the local tertiary centre after developing secondary hypogonadism treated with testosterone replacement. The cause had never been established.
The patient had previously been diagnosed with seronegative HLA B27 arthropathy and in December 2008 was admitted with acute cardiac failure and atrial fibrillation. An echocardiogram demonstrated a moderately dilated right and left heart, global left ventricular hypokinesia, an ejection fraction of 20% consistent with dilated cardiomyopathy. He responded well to medical therapy.
He had mildly abnormal liver function tests in the past but his AST level was twice the upper limit of normal in May 2009. Subsequent iron studies revealed a transferrin saturation of 90% and ferritin of 8989 μ/l. Testosterone replacement was therefore discontinued.
Hepatitis screen and liver autoantibodies proved negative. AFP level was $< 2$. Abdominal ultrasound confirmed 23 cm hepatomegaly but no splenomegaly. The patient was confirmed to have primary haemochromatosis (codon 63-HH, codon 282-YY).
Baseline tests revealed: $FT_4$ 12.5 pmol/l, TSH of 0.6 mU/l; 0900 h cortisol 454 nmol/l; prolactin 101 mIU/l; testosterone 0.2 nmol/l, LH $<0.1$ IU/l, FSH $<$ 0.2 IU/l; IGF-1 7.1 nmol/l and GH 1.26 μ/l. A TRH test was normal but a glucagon test confirmed GH deficiency with normal cortisol response.
An MRI did not demonstrate pituitary enhancement but cardiac MRI has shown a grossly dilated LV with globally impaired function and severe myocardial iron loading. The latest ferritin is now back to normal with regular venesection and IGF1 is also normal. A recent ECHO has demonstrated return of good systolic function.
Primary haemochromatosis is an autosomal recessive disorder characterised by excess iron deposition especially in the liver, heart, pancreas, and pituitary. This case is an excellent example of why this diagnosis should be excluded in patients with unexplained cardiac failure or hypogonadism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P438

**Thyrotoxicosis-associated acute myocardial infarction and ventricular fibrillation in a patient with normal epicardial coronary arteries**

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Introduction
Hyperthyroidism-associated myocardial infarction with normal epicardial coronary arteries on angiogram is seldom reported.
Case report
A 61-year-old Caucasian man, resident in an iodine sufficient area, presented with chest pain in the emergency unit. ECG revealed inferolateral myocardial infarction with ST-segment elevation. Soon after admission, the patients developed collapse due to primary ventricular fibrillation, requiring defibrillation. Coronary angiogram revealed normal epicardial vessels. These findings suggest that the acute myocardial ischemia was secondary to coronary vasospasm. Biochemical data showed hypokalemia ($K+=3$ mmol/l) and elevated $TT_4$ (18.26 μg/dl). After cardiac stabilization and kallium repletion, the patient was referred for endocrine assessment. Severe hyperthyroidism was confirmed (TSH $<0.03$ mUI/l, $FT_4=47.5$ pmol/l, TT3$=352$ ng/dl). TPO antibodies were slightly increased (48 IU/ml). Thyroid ultrasound revealed small diffuse goiter. Antithyroid therapy was started (Methimazole $45\rightarrow30\rightarrow20\rightarrow15$ mg/day). Despite iodine overload via coronary angiography, normalization of $FT_4$ levels (11.2 pmol/l) was noticed seven weeks later. The patient did not repeat angina, arrhythmic events or hypokalemia after normalization of thyroid function.
Conclusion
This case highlights the importance of considering hyperthyroidism in cases of myocardial infarction with normal epicardial coronary arteries. We suggest thyroid function screening in patients with coronary spasm.
Declaration of interest
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## P439

**A case of papillary thyroid carcinoma with esophageal invasion treated by targeting PEI and transesophageal argon plasma coagulation**

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There are several choices for treating esophageal cancer. But only operation is the standard therapy of papillary thyroid carcinoma with esophageal invasion. Esophageal stent and gastric fistula are chose when the patient can't swallow because of esophageal stenosis by tumor invasion. But those put a lot of strain on the patient, and lower their quality of life. To reduce the burden on patient, it is useful to adopt targeting percutaneous ethanol injection (targeting PEI) and transesophageal argon plasma coagulation (APC). Targeting PEI can reduce the volume of main tumor with cutting off feeding artery. APC is used treating benign esophageal disease like a varix generally. Recently, it began to be used for treating early esophageal cancer, and some reports say it is effective treatment for tracheal invasion of head and neck tumor. We present a patient of Papillary Thyroid Carcinoma (PTC) with esophageal invasion who kept prognosis well with treatment of targeting PEI and transesophageal APC. The patient was 83 years old woman who complained dysphagia. There was a tumor at right lobe of the thyroid gland, its size was $30\times29\times24$ mm. Lung metastasis and invasion to esophagus and trachea were found by CT. Endoscopic examination detected globular tumor 20 cm away from front tooth, and it occupied 80% of esophageal lumen. We treated main tumor with Targeting PEI twice. After treatment, the size was reduced about $22\times17\times16$ mm. Next, we treated esophageal invasion with APC and PEI. After first time treatment, its size reduced to 50% of lumen. Total 5 times we did, and its size reduced to 20% finally. It has been about 40 months from last treatment, no any problems appeared.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P440

**Association between diabetes mellitus and sarcoidosis: a case report**

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Introduction
Sarcoidosis is a multisystemic inflammatory disease of unknown etiology, characterized by noncaseating granulomas, predominantly in the lymph nodes, lungs, eyes and skin, although any organ may be involved.
Sarcoidosis may also be associated with endocrine autoimmune diseases such as type 1 diabetes, autoimmune thyroiditis.
Case report
We present a case of a 24-year-old young man who was diagnosed in August 2010 with type 1 diabetes mellitus with a fasting plasma glucose value over 300 mg/dl. We initiated insulinotherapy with biphasic insulin aspart 30/70 22 units per day for 6 month and then insuline Glargine 26 units per day because the patient refused intensive insulin treatement. After 2 month the patient complained of chest pain and dispnoea. The chest radiography showed bilateral hilar limphadenopathy and the chest computed tomography (CT) revealed many mediastinal adenopathy (1.7–2.5 cm) and some hilar adenopathy (3 cm). A tuberculin skin test was nonreactive. Serum ACE level measured at 36.7 μ/ml (normal: 15–28 μ/ml). The performed tests infirmed tuberculosis diagnosis. Bronchoscopy: bilateral diffuse bronchitis aspect without active lesions or mucosal proliferative elements.
Bronchial aspirate revealed isolated and grouped cylindrical epithelium, squamous epithelium, posters of squamous metaplastic epithelium, relatively frequent macrophages and lymphocytes, rare neutrophils. After the diagnosis of sarcoidosis grade I–II the patient was treated with NSAIDs because corticotherapy would be negatively affected glycemic status.
Conclusion
The association between type 1 diabetes and sarcoidosis is rare and the question is if sarcoidosis was caused by the therapy with insuline Glargine. Patient should be followed up for other autoimmune diseases as sarcoidosis may be associated with autoimmune hemolytic anemia, autoimmune idiopathic thrombocythaemia, Sjogren's syndrome, autoimmune thyroid disease.
Declaration of interest
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## P441

**Noninsulinoma pancreatogenous hypoglycemia syndrome as a rare cause of hyperinsulinemic hypoglycemia: case report**

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Introduction
Adult-onset noninsulinoma pancreatogenous hypoglycemia syndrome (NIPHS) associated with hyperinsulinemic hypoglycemia is a very rare entity. Clinical features of NIPHS are similar to those of insulinoma. Hypoglycemia with neuroglycopenic symptoms presents the main diagnostic condition. NIPHS is often poorly responsive or unresponsive to medical management necessitating surgical intervention with resection of pancreatic tissue.
Case report
We report case of a 50 years old man that was addmited to our hospital 6 years ago with symptoms of postprandial hypoglycemia with low level of glucose and high level of insulin. Fasting 72 h test was performed and it was positive. CT scan showed small pancreatic lesion and patient underwent surgical resection of pancreas in pursuit of insulinoma. Microscopically and immunohistochemicaly, the pancreas exhibited nodular islet cell hyperplasia and high positivity to chromogranin A and synaptophysin. Six months after surgery patient had symptomatic hypoglycemia again, and fasting 72 h test was positive. Abdominal CT and MRI showed small multifocal pancreatic lesions and suspected hepatic metastasis, whole body scintigraphy with Tc99 m-Tektroyd and CT confirmed pancreatic lesions without hepatic metastasis. Medical management with lanreotide (120 mg subcutaneous injections once monthly) was introduced. For 2 years during treatment with lanreotide patient was euglycemic. Because we excluded metastatic disease we changed medical management from lanreotide to diazoxide. Introduction of diazoxide was associated with improvement. Unfortunately patient stopped treatment with diazoxide and after 2 years he came back with hypoglycemic symptoms and extreme obesity. We again confirmed hyperinsulinemic hypoglycemia with unchanged radiological finding on pancreas and without metastasis. Diazoxide therapy was introduced again.
Conclusion
A rare cause of hyperinsulinemic hypoglycemia in adults can be diffuse pancreatic islet cell hyperplasia. Differentiating between NIPHS and insulinoma is tricky and can present difficulty for treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P442

**A case of pneumomediastinum and tracheomalasia in thyroid storm with longstanding goiter**

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Tracheomalasia is extremely rare condition in Graves' disease and may result in pneumomediastium with fistular formation from longstanding compression by a large goiter. There is no report that Graves' disease combined with tracheomalasia and pneumomediastinum. A 24-year-old woman presented with abdominal pain, diarrhea and fever. On physical examination, a large goiter was detected on her anterior neck. Computed tomography of chest showed large amount of air in anterior mediastinum. Twelve years ago, she was diagnosed Graves' disease and had not taken antithyroid drug regularly. The laboratory findings were markedly decreased TSH compared to increased thyroid hormone level and positive auto-antibody for thyroid (TBII, TSH binding inhibitory immunoglobulin). Thyroid storm was diagnosed and appropriate management was performed by using antithyroid drug, lugol solution, dexamethasone and propranolol. Dyspnea was developed because of aspiration pneumonia, mechanical ventilation was needed. After being normal range of free $T_4$, total thyroidectomy was performed. four soft and floppy tracheal rings with fistular formation were found on operation field. Extubation of endotracheal tube was difficult due to tracheomalasia, so tracheostomy was done. Weight of thyroid gland was 119.5 g. Airway obstruction is also possible due to flaccid tracheal ring after removing hard goiter encasing trachea.Therefore, in cases of total thyroidectomy with large longstanding goiter, extreme caution should be used.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P443

**A case of pancytopenia with hyperthyroidism**

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Atypical manifestations of hyperthyroidism include hematological, cardiovascular, dermatological manifestation. Especially single lineage abnormalities such as anemia (34%), leukopenia (5.8%), thrombocytopenia (3.3%) are reported, but pancytopenia is a rare presentation of hyperthyroidism. The suspected etiologic mechanisms include ineffective hematopoiesis, reduction in blood cell life span, autoimmune process, toxicity of thyroid hormone.
We report a case of pancytopenia that was precipitated by hyperthyroidism. A 69-year-old woman presented to emergency department with more than 2 months history of general weakness, easy fatigability, weight loss of 12 kg. She took no regular medications and had no significant past medical history or family history. Laboratory data on admission were as follows: WBC 3.12 K/ul, Hb 8.4 g/dl, PLT 17 K/ul, thyroid function tests showed abnormally high concentrations of free $T_4$ ($FT_4$) and total $T_3$ ($TT_3$)($FT_4$: 51.64 pmol/l, $TT_3$: 4.58 nmol/l) with a TSH concentration below the detection limit. TRAb 264.2 U/l, anti-Tgn 773.1 u/ml, anti-TPO 3000 u/ml. Thyroid ultrasound and thyroid scan (Tc-99 m) suggested diffuse thyroiditis. Abdominal ultrasonogram showed no abnormal findings

including hepatosplenomegaly. Normocellular marrow was noted in Bone marrow aspiration and biopsy. From these results, the patient was diagnosed with pancytopenia with primary hyperthyroidism. She was started on propylthiouracil 100 mg qd, propanolol 40 mg bid and parenteral dexamethasone 1 mg bid. $FT_4$ and $TT_3$ levels decreased gradually and pancytopenia improved after 2 weeks (TSH $<0.16$ mu/l, $FT_4$ 24.42 pmol/l, $TT_3$ 2.34 nmol/l).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P444

**Struma ovarii and the thyroid surgeon**

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Introduction
Struma ovarii is a rare ovarian monodermal teratoma. The most common presentation is an abdominal pelvic mass with pain, traditionally managed by gynaecologists. The malignant form is extremely rare and consists of differentiated thyroid cancer. It is rare for a struma ovarii to present with features of hyperthyroidism. We present a case series of struma ovarii and discuss the role of the thyroid surgeon in their management.
Methods/Results
Case 1: A 40 year old lady presented with a 2 month history of swelling in the lower abdomen. Investigations revealed a mass arising from the left ovary. Surgical histology revealed a follicular carcinoma arising in struma ovarii. She needed a total thyroidectomy prior to radio iodine therapy.
Case 2: A 60 year old lady underwent thyroidectomy for thyrotoxicosis.Three months post operatively she remained thyrotoxic despite stopping thyroxine. A whole body radio iodine scan revealed a high uptake in the left ovary. Surgical histology of the ovary showed a benign struma ovarii.
Case 3: A 69 year old lady presented with a post menopausal vaginal discharge. Investigations showed a left ovarian mass. Surgical histology revealed malignant struma ovarii and she required total thyroidectomy and radio iodine treatment.
Conclusion
This case series highlight the diagnostic and therapeutic role of thyroid surgeon in the management of benign and malignant form of struma ovarii.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P445

**Multiple myeloma associated with primary hyperparathyroidism**

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Introduction
The association of multiple myeloma (MM) with primary hyperparathyroidism (pHPT) is very infrequent. In addition pHPT may be overlooked or later diagnosed, because on the one hand certain clinical and laboratory characteristics of MM, such as hypercalcaemia, asthenia, osteoporosis and impaired renal function overlap with the elements of pHPT, on the other hand modern pHPT include non-classical (e.g. normocalcemic or mild clinical) forms. We present the case of a 75-years-old female (P.E.) known with operated recurrent kidney stones (1976, 1997, 2001), multiple fractures (both forearms 2001, clavicle 2004), and hypertension, chronic ischemic heart disease, heart failure NYHA III. In 2006 MM was diagnosed and specific haematological treatment (among others corticosteroids) was applied. In 2008 osteoporosis was discovered and clodronate (800 mg/day) therapy was started. Intermittent hypercalcaemia associated with lumbar pain, asthenia and dizziness were considered related to the hematological disease. In August 2011 arose the suspicion of primary hyperparathyroidism: iPTH: 540.4 pg/ml (normal:15–68.3), se-phosphate (se-P): 0.72 mmol/l ($n$:0.87–1.45), se-Ca: 2.28 mmol/l ($n$:2.15–2.57), Tc-99 m sestamibi scintigraphy suggested diffuse hyperplasia of both superior parathyroid glands. DXA osteodensitometry showed persistent osteoporosis ($-2.49$SD lumbar and $-3.2$SD femoral neck $T$-score). In October 2011 high total se-Ca-level was measured (12.45 mg/dl, $n$:9–11) with iPTH:283 pg/ml ($n$:8.7–79.6), creatinine-clearance: 66.1 ml/min, 25-OH-vitaminD:11 ng/ml ($n>30$), associated with aggravated asthenia. We increased the dose of clodronate to 1.200 g/day, and 40 mg po furosemide was given three times weekly. Within 2 weeks total se-Ca decreased to 11.18 mg/dl, albumine corrected se-Ca:10.74 mg/dl, but iPTH:407.6 pg/ml, so furosemide was reduced to 1 tablet/week. Low-dose calcitriol (0.50 μg/day) was carrefully introduced, taking into account vitamin D deficiency, osteoporosis and reduced kidney function in an aging person. Medical therapy was recommended instead of surgery: cinacalcet in association with bisphosphonate and calcitriol, with monthly control of calcaemia.
Conclusion
A very rare association between two infrequent diseases but with certain clinical/laboratory similarities, as well as the outcome under medical treatment is presented.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P446

**Graves' disease with a transient lung mass**

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Introduction
Involvement of different tissues and organs may be seen during the course of Graves' disease (GD). We here aimed to present a case with GD and lung mass whose lung lesion disappeared after recieving antithyroid treatment.
Case report
A 60 year old man admitted to our hospital with back pain. His thorax computed tomography (CT) revealed a $1.5\times3$ cm mass in lower right lobe. He was consultated by our clinic preoperatively before thoracotomy for his thyroid function test (TFT) revealing overt hypertyroidism. On laboratory evaluation; TSH:0.004 μIU/ml (0.4–4.0), $fT_3$:9.1 pg/ml (1.57–4.71), $fT_4$:3.23 ng/dl (0.8–1.48), anti-TPO(+) anti-Tg(+) CRP:1.2 mg/l ESR:10 mm/h were recorded. Thyroid ultrasonography revealed hypervascular enlarged thyroid lobes with heterogeneous apperance. Radioactive iodine uptake performed 2 months after iodine exposure from the radiocontrast substance revealed increased uptake. He was started methimazole tretment and his lung surgery was postponed. Two months later his TFT was euthyroid and referred for thoracotomy. He had another thorax CT which showed that the mass in the right lobe of the lung almost totally resolved after which the surgery was cancelled (Fig. 1). He didn't show any sign or symptom of a systemic inflamatory disease that could explain the transient mass lesion in the lung.
Conclusion
Thionamides used in the treatment of hyperthyroidism has also been shown to exhibit immunmodulatory effects, direct anti-inflammatory and immunosupressive properties. This drug group has been associated with protection from chronic inflammation and autoimmune disorders. During the course of antithyroid treatment, remission of some immune-related diseases have been reported. However, there is not enough evidence to associate the disapperance of the lung mass with the antithyroid treatment in our case. This is expected to be coincidental rather than causally related.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

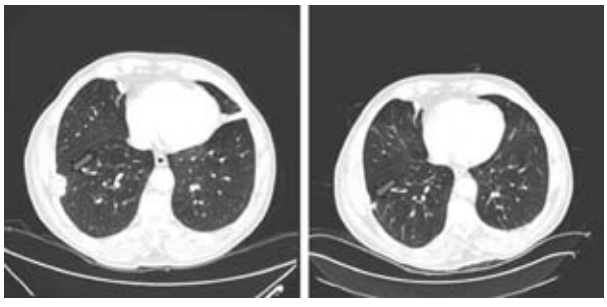

**Figure 1.** Chest CT before and after methimazole treatment

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## P447

### Combination of recombinant TSH (rhTSH) and lithium overcomes amiodarone-induced low radioiodine uptake in a thyrotoxic female

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Background
Recombinant human TSH (rhTSH) promotes $^{131}$I uptake in selected populations. Lithium increases RAI retention by reducing intra-thyroidal release. In this report, combination of rhTSH and lithium overcame low RAIU in Graves' disease.
Objective
We aim to present the case of a Graves' disease given combination of rhTSH and lithium to overcome amiodarone-induced low $^{131}$I uptake.
Methods and results
A 39-year old female with Graves' disease acquired thionamide-induced agranulocytosis and went into cardiorespiratory arrest. She had ventricular tachyarrhythmias on resuscitation and cardioverted with amiodarone. She was subsequently placed on hydrocortisone (150 mg/day), amiodarone (200 mg/day) and propranolol (60 mg/day). She was referred to our institution for definitive Graves' disease management. On admission, she was normotensive, tachycardic, and afebrile, with fine tremors, hyperreflexia, and diffuse thyromegaly. She had normal complete blood count, hypokalemia, elevated ALT and low TSH (0.03 μIU/l, NV 0.27–3.75). Thyroid ultrasound showed diffuse thyromegaly with uniform echopattern and normal color flow doppler. RAIU was low at 4 and 24 h (6 and 7%, respectively). In preparation for RAI therapy, she was given lithium 900 mg/day and two doses of 0.9 mg i.m. rhTSH. Repeat RAIU after the 2nd dose of rhTSH showed a more than 5-fold increase in 4 h uptake (32 vs baseline 6%). She was given 131-I 25 mCi after the 2nd dose of rhSTH. Clinical and biochemical course continuously improved thereafter.
Conclusion
This is the first case to demonstrate the efficacy of combining rhTSH and lithium to overcome amiodarone-induced low 131-Iodine uptake in Graves' disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P448

### Primary hypoparathyroidism presenting as recurrent TIAs with intracranial calcification

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Intracranial calcification seen on CT may be an incidental finding or it can be a direct cause of neurological symptoms depending on which areas of the brain are affected. The differential cause for the formation of intracranial calcium deposits include hypoparathyroidism, congenital infections i.e. toxoplasmosis and Fahr's syndrome.
A 50–year–old woman was referred to the transient ischaemic attack (TIA) clinic in December 2011, with recurrent episodes of dysphasia and right-sided jaw tightening and weakness, each episode lasting 10–20 min. She developed similar symptoms with no other focal neurology during the consultation in the TIA clinic and it was noted that her baseline adjusted calcium done the same morning was very low at 1.13 mmol/l (2.15–2.60). She was immediately admitted to hospital and was treated with 10 ml of i.v. 10% calcium gluconate, followed by infusions of 40 ml of 10% calcium gluconate in 1l of 5% dextrose daily over 3 days. Alphacalcidol 1 microgram twice daily and Adcal D3 two tablets twice daily were started.
The CT findings and blood results (Table 1) were consistent with intracranial calcification caused by primary hypoparathyroidism. Since discharge from hospital she has remained asymptomatic on oral calcium and vitamin D. Addison's, pernicious anaemia and hypothyroidism have been excluded to rule out type 1 polyglandular autoimmune syndrome.
Our case highlights the importance of always checking the calcium when patients present with 'neurological' symptoms as symptomatic hypocalcaemia can mimic various conditions. Secondly, it also brings to attention that hypoparathyroidism, with or without significant hypocalcaemia, must be considered as a cause of intracranial calfication found on imaging of the brain.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Baseline investigations and results obtained

| Investigation | Result | Reference range |
|---|---|---|
| Adjusted calcium | 1.13 mmol/l | 2.15–2.60 |
| Parathyroid hormone | $<$0.5 pmol/l $<$0.5 pmol/l(2nd sample) | 1.1–6.8 |
| Phosphate | 2.47 mmol/l | 0.80–1.40 |
| Magnesium | 0.79 mmol/l | 0.70–1.00 |
| 25–OH Vitamin D | $<$10 nmol/l | 70–150 |
| TSH | 0.38 mu/l | 0.30–4.20 |
| ECG | Prolonged QT interval | |
| CT Head | Extensive symmetrical intracerebral calcifications are seen in the basal ganglia, centrum semi ovale, subcortical regions in both occipitallobes and cerebellum. There is some effacement of the cerebral sulci and temporal horns are mildly dilated | |
| Creatinine | 85 umol/l | 60–125 |

## P449

### Ampullary NET presenting as Virchow's node

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Troisier's sign names a visible left cervical adenopathy (Virchow's node) as the first sign of a pancreatic tumor, mainly adenocarcinomas, by dissemination through the thoracic duct. Neuroendocrine tumors (NET) of the ampulla of Vater are extremely rare, no more than 2% of ampullary malignancies, and account for $<$0.3% of all gastrointestinal NETs.
A 59–year old woman presented with an enlarging painless left cervical node. Ultrasonographic guided biopsy of a round-shaped and hypervascular lymph node showed a small round cell proliferation with solid pattern positive for chromogranin A (CgA) but negative for calcitonin. Thyroid was normal in sonography. Laboratory results showed normal levels of tumour markers (CgA, gastrin, PP, somatostatin, glucagon and calcitonin), normal bilirrubin and elevated transaminases, mainly γGT(1167 U/l) and alkaline phosphatase. Abdominal CT revealed a dilated bile-duct (15 mm) with prominent papilla. A hypoechoic mass in the ampulla and periportal adenopathies were evident in endoscopic ultrasonography (EUS), both positive for NET when biopsied. She underwent the Whipple resection with extended lymph node dissection and pathological study showed an ampullar NET of 1.7$\times$1.4 cm invading the duodenal mucosa and pancreas with lymphatic permeation in tumour and preaortic and mesenteric nodes, Ki-67$<$2%. One month later, a functional cervical left dissection was performed, showing 9/41 lymphatic nodes affected by the NET, the biggest reaching 4.5 cm (Ki-67$<$2%). At present the patient remains asymptomatic, without evidence of disease neither by CT nor Octreoscan.
Ampullary NETs are very rare tumors, frequently associated with von Recklinghausen's disease. They usually present as obstructive jaundice without carcinoid syndrome. Our case is a new one of this uncommon tumour with a singular first complaint as a cervical node (Troisier's sign), not previously described in ampullary NETs. We remark the vast asymptomatic lymphatic extension in spite of the low Ki-67 and size $<$2 cm of primary tumour.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P450

**Unusual cause of hypokalemic paralysis**

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Introduction
Thyrotoxic hypokalemic paralysis is a disorder most commonly seen in Asian men. The condition primarily affects the lower extremities. The main characteristic features of this condition are elevated thyroid hormones, hypokalemia and proximal muscle weakness or paralysis.
Cases
Case 1: A 28 year old patient presented to emergency department with 3 day history of progressive weakness of both lower limbs which made him unable to walk. Later the weakness progressed to upper limbs.
Case 2: A 24 year old male presented with a history of recurrent cramps and spasms of the lower limb muscles for a month. He also had a history of acute onset weakness of both lower limbs for 2 days.
Case 3: A 35 year old male presented with repeated episodes of weakness of lower limbs. He was hospitalized thrice for the same complaints and hypokalemia was documented in each episodes.
The laboratory results were notable for a very low potassium level. After excluding other possible causes, thyroid function was done in these cases. $T_3$ and $T_4$ were high and TSH was suppressed in all the three. Technituim scan showed diffuse increased uptake in cases 1 and 2 suggesting Graves' disease. Third case had patchy uptake suggesting MNG. They were started on Carbimazole 30 mg/day and propranolol 60 mg/day. The muscle weakness improved gradually and were discharged on anti-thyroid medications.
Discussion
Thyrotoxic paralysis has a predilection for males of Asian descent. The pathogenesis is not clear but enhanced activity of Na+, K+-ATPase might be the possible etiology. Definitive treatment is correction of the thyrotoxic state along with cautious potassium replacement during the attack.
Conclusion
Hypokalemic paralysis must be included in the differential diagnosis of acute muscle weakness and thyrotoxicosis should be kept in the differential diagnosis especially in Asian men.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P451

**Paraneoplastic severe hyponatremia in a patient with GIST: case report**

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Hyponatremia may manifest with nausea, disorientation, seizures, coma, cerebral edema and even death. The etiology of hyponatremia as paraneoplastic syndrome has been attributed most often to high levels of vasopressin. Correction of hyponatremia is usually successful at moderately low sodium levels, although it must be done slowly to prevent osmotic demyelination. Gastrointestinal stromal tumors (GISTs) rarely present paraneoplastic reactions, a few cases have been reported.
An 81 year-old man presented with anemia (HGB = 8.5 g/dl), mild hyponatremia (Na = 128 mEq/l) and low Plasma Osmolality (273 mmOsm/l). A CT scan showed a large mass originating from the gastric wall, without gastrointestinal obstruction or distant metastases. A tumor, 12 cm in diameter, was completely removed by proximal gastric resection. Histological examination diagnosed a GIST.
During post-operative period, the patient developed arrhythmia, pneumonia, vomit and confusion, requiring recovery in intensive care unit. Blood sample evaluation showed severe hypotonic hyponatremia (Na = 114 mEq/l, Plasma Osmolality = 239 mOsm/l) and worsening anemia. Red blood cell transfusion, antibiotics and supportive care were administered. Treatments to correct hyponatremia (water restriction and hypertonic saline) weren't effective, with persistent and symptomatic hyponatremia. Treatment with tolvaptan 15 mg started 30 days after surgery once daily for five consecutive days. Natremia have gradually reached 130 mEq/l, and plasma osmolality 282 mOsm/l; the treatment was continued weekly for 4 months, and then monthly for 2 months. Tolvaptan treatment was necessary to maintain natremia within normal value. Drug withdrawal occurred six months from the start, with stable normal natremia. PETscan performed 2 months after surgery showed abnormal uptake in the stomach, of uncertain significance. Because of patient's poor performance status a watchful waiting approach was preferred continuing tolvaptan treatment. PETscan performed 4 months after surgery showed a decreased uptake. After 15 months, the patient is well and free from disease.
Vaptans may be useful and safety to treat hypotonic hyponatremia associated with paraneoplatic syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P452

**Papillary thyroid carcinoma in two patients with primary hyperparathyroidism**

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Introduction
Co-existence of medullary thyroid carcinoma and primary hyperparathyroidism (PHPT) is well described, however, the association of non-medullary cancer and PHPT is less recognised. Herein, we report two cases of patients with PHPT and papillary thyroid carcinoma (PTC).
Case 1
A 55-year-old woman received a diagnosis of PHPT during investigation of her anemia. (Ca: 11.4 mEq/l, PTH: 256 pg/ml, urinary calcium: 960 mg/day). Thyroid function tests were totally normal. Neck ultrasonography demonstrated multinodular goitre. There were six nodules; one had 20×12 mm diameter and microcalcification. There was a 14×9 mm, hypoechoic, solid lesion compatible with a parathyroid adenoma at the left side. A fine needle aspiration biopsy (FNAB) was performed for the microcalcific nodule. The FNAB was positive for PTC. The patient gone under total thyroidectomy, central lymph node dissection and parathyroidectomy. A parathyroid adenoma and two foci of PTC (2.5 cm and 1.5 cm) with two metastatic lymph nodes were determined.
Case 2
A 60-year-old man presented with back and lombar pain referred for evaluation of PHPT. His laboratory findings confirmed the diagnosis of PHPT: Ca: 15.2 mg/dl, PTH: 1900 pg/ml, urinary calcium: 720 mg/day, serum creatinine: 2.1 mg/dl, alcaline phosphatase: 2142 U/l. Thyroid function tests were normal. Neck ultrasonography showed multinodular goitre. One of 5 nodules was 23×19 mm, hypoechoic, had a satellite nodule and calcification. There was a hypoechoic, solid, 18×17 mm lesion compatible with a parathyroid adenoma at the right side. The FNAB of the nodule was suspicious for PTC. Total thyroidectomy, central lymph node dissection and parathyroidectomy were performed. The pathology report confirmed parathyroid adenoma and a 2 cm of PTC.
Conclusion
These cases represent the probability of concomitant PHPT and PTC. Preoperative evaluation with ultrasonography may be suggested to patients with a diagnosis of PHPT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P453

**Marked improvement of gastrointestinal pseudo-obstruction after debulking surgery of malignant pheochromocytoma by Intravenous administration of α-blocker phentolamine**

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We present a case of severe malignant pheochromocytoma complicated with intestinal pseudo-obstruction, which was refractory to conventional therapies but reacted to intravenous administration of an α-blocker, phentolamine.

Malignant pheochromocytoma typically metastasizes to bones, liver, lungs, and lymph nodes, and average 5-year survival rate in the patient with metastases is approximately 50%. Treatment options for malignant pheochromocytoma include surgical debulking, external irradiation and systemic antineoplastic therapy. The survival rate worsens in proportion to tumor size, so the essential therapeutic goal is tumor reduction by debulking surgery even if the surgery is not curative, and control of symptoms of excessive catecholamine secretion.

We present a case of 65-year-old man who suffered from transient ischemic colitis and persistent paralytic ileus complicated with megacolon due to pseudo-obstruction developing after the surgical manipulation of malignant pheochromocytoma. Even though other symptoms including hypertension were well controlled, the abdominal symptom was fatal. The pseudo-obstruction showed poor response to conventional treatment including oral administration of α- and β-blockers, yet was relieved promptly with chronic intravenous administration of an α-blocker, phentolamine.

In summary, surgical treatment can trigger this kind of ileus in a patient with pheochromocytoma producing higher levels of catecholamines, even though other symptoms are well controlled. We suggest that intravenous administration of an α-blocker, phentolamine, should be initially considered in such gastrointestinal pseudo-obstruction due to overproduction of catecholamines, especially for the patient who is unable to take meals or drugs efficiently, prior to embarking on surgical decompression of severe megacolon.

Declaration of interest

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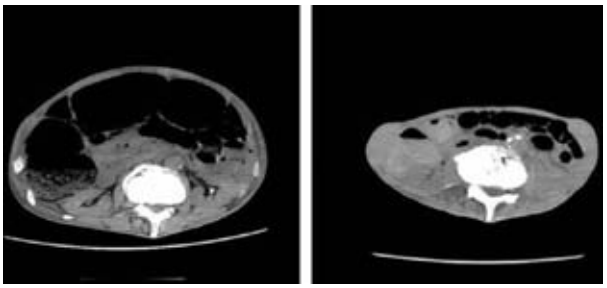

**Figure 1** Abdominal CT findings of the gastrointestinal pseudo-obstruction after surgery (left) The abdominal CT findings of the megacolon on postoperative day 2. (right) The abdominal CT findings after treatment with intravenous administration of phentolamine.

## P454

### Primary and secondary hyperparathyroidism coexisting in patient with liver cirrhosis and coeliac disease: efficiency of preoperative treatment with vitamin D

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Introduction

Primary and secondary hyperparathyroidism may coexist unrelated to chronic nephropathy. It can occur more often than expected, especially in elderly population with malabsorption syndrome or /and liver diseases.

Case report

A women 60 years of age was admitted to hospital due to long standing ostealgia. The primary hyperparathyroidism was suspected. The medical history presented recently diagnosed coeliac disease and cryptogenic hepatic cirrhosis.

Lab tests during hospitalization: PTH level was surprisingly high 2200 pg/ml (*n*: 15–65 pg/ml), calcium serum level and calcium urine excretion in 24 h collection in the normal range, phosphates 1.8 mg/dl (*n*: 2.5–4.8 mg/dl), ALP 660 U/l (*n*: 36–123 U/l), 25(OH)D3 extremely below the normal range: 6.7 ng/ml (*n*: 30–80 ng/ml). The level of creatinine was normal. Osteopenia was confirmed. Scintigraphy, USG and CT revealed of single parathyroid adenoma of diameter 3.0 cm.

Gluten-free diet and oral vitamin D (gtt. calcifediol 75 μg each day) was recommended.

Elimination of ostealgia, PTH reduction (540 pg/ml), slight elevation of calcium level: (11.8–12.2 mg/dl), normalization of 25(OH)D3 level (47 ng/ml) were obtained. Surgical removal of parathyroid adenoma was successfully performed five months later. Hungry bone syndrome was not observed post operatively. Vitamin D supplementation was maintained. PTH, calcium, phosphates, ALP, 25(OH)D remains in the normal range.

Conclusions

1. Gastrointestinal diseases especially liver diseases can trigger secondary hyperparathyroidism and enhance primary hyperparathyroidism.
2. High levels of PTH/Ca ratios can be caused by profound deficit of Vitamin D3.
3. Early recognised deficit of D3 followed by preoperative D3 supplementation with strict control of calcium serum levels eliminate post operative severe 'hungry bones syndrome'.
4. Vitamin D3 treatment should lasts at least 3 months considering PTH, D3, Ca receptors regulation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P455

### Primary hyperparathyroidism and metastatic breast cancer: a simultaneous presentation

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Introduction

Hypercalcaemia is a frequent complication of breast cancer with bony metastasis. There is also an increase incidence of primary hyperparathyroidism among patients with breast cancer. We report a patient with breast cancer presenting with hypercalcaemia secondary to both parathyroid hormone- related peptide (PTHrP) from liver metastasis and possibly co-existing primary hyperparathyroidism.

Case report

A 53 year old woman, with a history of right sided breast cancer presented as an emergency due to general deterioration and was found to have hypercalcaemia. She had undergone mastectomy, completed six cycles of chemotherapy and 25 fraction of radiotherapy 10 months ago. Blood test revealed an adjusted calcium of 5.54 nmol/l (NR 2.10–2.55 nmol/l), PTHrP of > 60 pmol/l (NR 0.0–1.8 pmol/l), 25-OH Vitamin D of 12 nmol/l (NR 50–200 nmol/l), acute renal failure and deranged liver function tests. Further investigation included a bone scan which excluded any bony metastasis and an abdominal ultrasound revealing liver metastasis.

Despite appropriate treatment with intravenous fluid, bisphosphonate and steroid therapy, patient continued to deteriorate and died.

Conclusion

Hypercalcaemia in malignancy mostly results from bony metastasis and PTHrP. Recent data suggests a strong correlation between breast cancer and primary hyperparathyroidism. Although the exact pathogenesis is unclear, it has been attributed to be the possibility of common etiological pathways shared by the two conditions.

Our patient presented with extreme levels of hypercalcaemia, secondary to PTHrP associated with metastatic breast cancer, without bony involvement. Despite this high level of calcium level, PTH was still measurable, indicating probable primary hyperparathyroidism as well although it could also possibly be because of severe vitamin D deficiency and renal disease.

Hypercalcaemia secondary to PTHrP carries a poor prognosis and co-existing primary hyperparathyrodism in such setting can be an interesting observation but it does not dictate the course of the patients' treatment or outcome.

Declaration of interest

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## P456

**Insulinoma presenting as post-prandial hypoglycaemia**

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A 52 year old lady with hypothyroidism and hypertension was referred by her GP for unexplained low blood glucose on several occasions. There was history of vacant spells with confusion and disorientation over the previous year. When referred for neurological assessment, no abnormality was identified. Her medications included amlodipine 5 mg, ramipril 10 mg, thyroxine 50 mcg, loratidine 10 mg. Her BMI was 44 kg/m$^2$.

Additional investigations arranged by GP included, cortisol (0920 h) 184 nmol/l, short synacthen test (0 min) 331 nmol/l and (30 min) 602 nmol/l, eGFR 62 ml/min.

She reported significant weight gain over the last few years, compared with her twin sister who had a normal BMI. She drinks Lucozade when feeling 'under the weather'.

A prolonged OGTT was performed as an outpatient. She did not complain of any symptoms of hypoglycaemia, no insulin/C-peptide samples were taken.

Analysis of the plasma glucose samples showed hypoglycaemia occurring at 3 h (1.6 mmol/l), rising slowly to 2.3 mmol/l at 4½ h.

An admission was arranged for further assessment. Initially her FBG was 3.6 mmol/l, insulin 197 pmol/l, C-peptide 443 pmol/l. After breakfast she developed postprandial hypoglycaemia (plasma glucose 2.0 mmol/l with a further increase in insulin and C-peptide levels: 682 and 1537 pmol/l respectively. A sulphonylurea screen was negative.

CT scan showed 14 mm hypervascular tumour within tail of pancreas consistent with insulinoma. Cytology from an FNA showed features in keeping with a well differentiated neuroendocrine tumour.

She underwent distal pancreatectomy and splenectomy in June 2011. She was reviewed subsequently in clinic, asymptomatic and successfully losing weight.

Insulinoma presenting as post-prandial hypoglycaemia only is an uncommon presentation of a rare tumour. These patients often have hypoglycaemia unawareness, so close clinical evaluation and monitoring of capillary glucose is vital to avoid unnecessary delays in diagnosis.

Declaration of interest
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## P457

**Malignant, ectopic ACTH secreting occult neuroendocrine tumor: a case report**

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Introduction
Occult neuroendocrine tumors are still a difficult diagnostic/therapeutic challenge.
Case report
In November 2007, a 29-years-old Caucasian woman was admitted to our in-patients clinic with a 2-month history of rapidly progressing signs and symptoms of chronic hypercortisolism, including a bipolar disorder in psychosis. On admission the patient presented with hypokalemia (3.2 mEq/l), high levels of plasma (3164 nmol/l) and urinary free (26 945 nmol/24 h) cortisol and high ACTH levels (354 ng/l), consistent with ACTH-dependent Cushing's syndrome (CS). High-dose-dexamethasone failed to suppress ACTH and cortisol. Magnetic resonance imaging (MRI) of the pituitary was normal and bilateral inferior petrosal venous sampling did not show any central/peripheral or right-to-left gradient. Computed tomography (CT) of the chest and abdomen resulted negative as well as In-111 pentetreotide scintigraphy. No decrease was observed in plasma ACTH after 100 μg s.c. octreotide. Total body bone and MIBI scintigraphy, FDG-PET-CT, Ga-DOTANOC-PET, upper and lower bowel endoscopy, breast and thyroid ultrasound were uninformative. Neuroendocrine oncologic markers such as Cromogranine A (CgA), Ca19.9 and Tpa were only slightly increased. In February 2008 a pelvis MRI showed sacral and iliac osteoblastic lesions; bone biopsy revealed athypical epithelial cells AE1/AE3 citokeratin positive. In June 2008, because of unsuccessful medical therapy, bilateral adrenalectomy was performed, with complete remission of CS. In July 2008, a FDG-PET-CT showed an increased uptake in one left para-aortic and two left supraclavear lymphnodes, which were removed: at histology, an infiltrating neuroendocrine carcinoma AE1/AE3+, CD56+, CgA+, synaptofisine+, ACTH+, was diagnosed. After 14 months of stable disease, a tumor progression was found with bone, chest lymphnodes and hepatic metastases, associated to a hypophosphoremic and hyponatriemic paraneoplastic syndrome. Chemoterapy was started in December 2008, but uneffective. In March 2010, peritoneal tumor dissemination with ascites developed. In June 2010 the patient died, primitive tumor still occult.
Conclusions
Neuroendocrine tumors can manifest with different signs/symptoms/paraneoplastic syndromes. Their management may need change over time.

Declaration of interest
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## P458

**Noonan Syndrome in adulthood: two cases reports**

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Introduction
Noonan syndrome (NS) is an autosomal dominant disorder or may occur on a sporadic basis, characterized by short stature, typical face dysmorphology and congenital heart defects. NS is a clinical diagnosis. Establishing the diagnosis can be very difficult, especially in adulthood. There is a great variability in expression and the phenotype become less pronounced with increasing age. The scoring system of Van der Burgt *et al.* has been devised to help the diagnostic process. In 50% of the patients with NS, a missense mutation is found in the PTPN11 gene on chromosome 12. The incidence is reported to be between 1/1000–1/2500 live births.
Subjects
Case 1: A 54-year-old male was studied in the endocrine practice because of subclinical hypothyroidism. He had medical history of mental retardation, bilateral cryptorchidism, hearing loss, cataract and myeloproliferative disorder. Face dysmorphology, pectus excavatum and other characteristic features confirm clinical diagnosis of NS. Karyiotype and molecular study of fragile X premutation were normal. Mutational screening was carried out by direct sequencing of all coding exons of the genes PTPN11, SOS1 and RAF1 and we did not detected heterozygous point mutations or polymorphisms.
Case 2: Fourty-one-year-old woman was refereed for clinical hypothyroidism monitoring. Pulmonary stenosis intervention was performed at the age of eight. Physical examination showed pectus carinatum and face dysmorphology suggesting NS. Clinical diagnosis was confirmed by the finding of pArg552Gly heterozygous mutation in SOS1 gene.
Conclusions
Although NS diagnosis is usually performed in the first childhood, diagnosis in the adulthood should be also suspected because of the implication in genetic advice. NS is genetically heterogeneous and only 50–60% of patients show mutations in the involved genes.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P459

**Unusual course of Amiodarone-induced hypothyroidism: case report**

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Introduction
Amiodarone a potent antiarrhythmic medication induces thyroid dysfunction in about 20% of patients. Amiodarone-induced hypothyroidism (AIH) usually

occurs early in the course of treatment, more often in iodine-sufficient areas, in women, and in the presence of thyroid autoantibodies. Long-term treatment with amiodarone is associated with a reduction in prevalence of AIH, which may reflect adaptation of the thyroid autoregulatory mechanisms to iodine excess.
Case report
A 68-year-old man referred to our outpatient clinic with a history of myocardial infarction, CABG, hypertension, and ventricular extrasystoles (Lown class IVb). He had been treated with amiodarone for the past 8 years. His thyroid hormone status had been checked in regular 6-month intervals showing normal TSH, FT4, FT3 concentrations. However, the last results were as follows: TSH- 33.7 mIU/l, FT4- 13.9 pmol/l (12.4–22.0), FT3- 4.7 pmol/l (2.6–5.7), thyroid autoantibodies assays were negative. He was then started on 50 μg of levothyroxine and amiodarone was discontinued shortly after, as cardiologists considered it safe. For the next one year patient still required substitution with the same dose of levothyoxine and his TSH and FT4 concentrations returned to the middle of reference range. After one year amiodarone was restarted due to deterioration of the arrhythmia, this time permanently. In the time of next 5 years his TSH levels were gradually increasing up to 32 mIU/l, in spite of increasing levothyroxin dose up to 125 μg. FT4 level after initial rise to 27.07 pmol/l was decreasing to high normal values near 22 pmol/l. FT3 concentrations remained close to the lower limit, however recently FT3 concentration fell below normal range to 2.29 pmol/l.
Conclusions
Late onset of AIH in our patient (after 8 years of amiodarone therapy) indicates on a necessity of a life-long monitoring of thyroid function in all patients treated with amiodarone. TSH and FT3 rather than FT4 concentrations should be used to determine levothyroxine dose in AIH patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P460

**Anaplastic thyroid carcinoma: case report**
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Introduction
Anaplastic thyroid carcinoma (ATC) is characterized by an aggressive clinical course and refractoriness to currently available local and systemic modalities of treatment. It is considered the most aggressive solid tumor, with few patients alive more than 1 year following diagnosis.
Bible et al have reported a combined therapy of radiation and radiosensitizing adjuvant chemotherapy, with some positive results.
Case report
The authors report a case of ATC in a 37-year-old woman, with irrelevant past medical history. She referred a left anterior cervical mass, which was detected one year ago. Because of recent fast enlargement, although no compressive symptoms, she was submitted to ultrasound guided biopsy of the dominant node, which revealed ATC.
Total thyroidectomy with central node dissection was performed.
ATC was histologically confirmed. Staged pT4N0MxR1.
Post-operatory US showed no remaining thyroid tissue on the surgical bed, or suspicious nodes.
No pulmonary metastases were found on the CT.
She was submitted to intensity-modulated radiation therapy and radiosensitizing plus adjuvant chemotherapy, intending four cycles of docetaxel plus doxorubicin, with good therapeutic tolerance.
A CT scan performed six months later showed no recurrence of the disease.
Conclusion
This case of an ATC, was atypical because it occurred in a young patient, treated with surgery a combined radiochemotherapy regimen, and the patient is alive 6 months after diagnosis, with unsuspected signs of disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P461

**'Collision tumor' of the thyroid: a case report**
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Introduction
Differentiated thyroid carcinoma (papillary and follicular) being the most common type of thyroid malignancy (80%), originates from the thyroglobuline secreting follicular cells. Medullary thyroid cancer derived from parafollicular C-cells producing calcitonin is relatively rare (5–10%). In this report we aimed to present a case with simultaneous papillary and medullary carcinoma in the same thyroid nodule.
Case report
Sixty nine year old woman admitted with progressive swelling on the right side of the neck. Her past medical history was unremarkable except for well controlled hypertension. She had no radiotherapy exposure to neck and family history was negative for thyroid malignancy. Thyroid function tests were euthyroid. Her neck ultrasonography revealed multinodular goiter and right submandibular solid mass. Fine needle aspiration biopsy of the right dominant 14 mm hipoechoic thyroid nodule and right submandibular mass revealed papillary carcinoma and pleomorphic adenoma respectively. She underwent total thyroidectomy and right suprahyoid neck disection. Pathology was reported as pleomorphic adenoma (submandibular gland), medullary thyroid carcinoma and focal papillary thyroid carcinoma (14×12 mm) (right thyroid nodule). She recieved radioactive iodine ablative therapy. She is currently on follow up in remission state.
Conclusion
The coexistance of different malignancies within the same mass is termed as 'collision tümör'. Appearance of concurrent papillary and medullary pathological features in the same thyroid tumor, so-called 'collision tumor' of the thyroid, is relatively rare being less than 1% of the all thyroid malignancies. There are no pathogenetic mechanisms available so far to explain the concomitant neoplastic transformation of the C-cells and follicular cells in the same thyroid nodule. Further analyses are needed to detect the etiopathogenesis of this condition.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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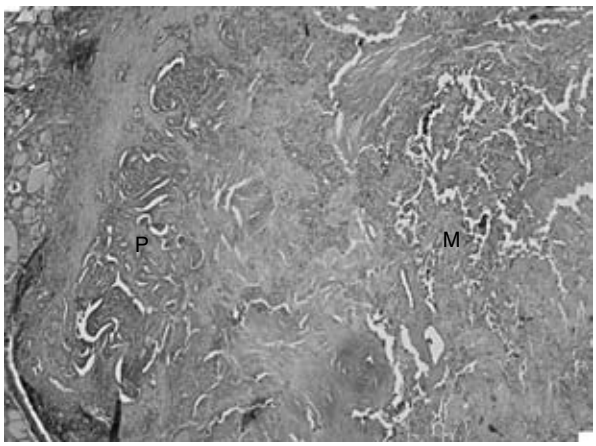


**Figure 1** Microscopic appearance of adjacent papillary (P) and medullary (M) thyroid carcinoma.

## P462

**Unknown reason of continuing hypercalcemia after successful extirpation of parathyroid gland**
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It is to delineate two categories of hypercalcemia: hypercalcemia associated with dysfunction of the parathyroid gland and hypercalcemia that occurs despite appropriate parathyroid suppression.

There is casuistic model of 73 years old woman with several years personal history of hypercalcemia and clinical polyarthritis. After standard examinations there was diagnosed primary hyperparathyroidism. USG screening expected adenoma of parathyroid gland right down, but increased accumulation of radiopharmaceuticals during scitigraphic examination was in area of right submandibular gland. In November 2009 there was executed radionavigated extirpation of parathyroid gland adenoma - adenoma was localized in lower pole of right lobe of thyroid gland.
After operation the level of PTH was normalized, but later the hypercalcemia has continued, temporary with mild increasing of PTH. Based on these laboratory results we assumed the coexistence of another disease, which could participate on hypercalcemia too.
Within differential diagnosis of hypercalcemia there was made complete algorithm of the examinations. At PET/CT expected ectopic hyperplastic parathyroid gland was displayed, but considering of the PTH level we do not assume it is the reason of hypercalcemia.
Considering polyarthritis personal history we executed rheumatologic examination. Within rheumatologic screening the positivity of antibodies ANA, ENA, Ro52, SSA, SSB, SCl 70 was found, but diagnostic criteria of systemic disease were not matched.
Because of polyclonal gammapathy the haematological examination was executed, but the multiple myeloma was not positively proved too. Considering continuing hypercalcemia we add to treatment ex juvantibus the corticosteroids which reduced calcemia under 3 mmol/l.
Hypercalcemia is a common electrolyte abnormality with a wide differential diagnosis. The reason of hypercalcemia is in 90% primary hyperparathyroidism and malignity. In our patient there is no positive reason of hypercalcemia so far. Except above mentioned comes into consideration the autoimmune inflammatory disease, and diagnostic criteria are not matched that time.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P463

**Catastrophic bone deformities associated with primary hyperparathyroidism in a middle-aged man**

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Background
Parathyroid carcinoma is a very rare cause of primary hyperparathyroidism. We aimed to present a case of middle-aged man, who was initially misdiagnosed as parathyroid adenoma, with parathyroid carcinoma and severe bone deformities associated with hyperparathyroidism.
Case
A 36-years old male presented with leg aches and severe hypercalcemia in 2001, when he was diagnosed with primary hyperparathyroidism (corrected serum calcium 18.5 mg/dL (8.4–10.2), parathyroid hormone 400 pg/ml (8–76)). Total spine MRI demonstrated lytic lesions in all thoracal and lumbar vertebra. A parathyroid adenoma (28×20 in diameter, lower right part) was excised. In March 2008, he presented with generalized leg aches and gait disturbances associated with multiple pathological fractures (Figure 1) when his serum calcium was 15.6 mg/dl. Total parathyroidectomy, total thyroidectomy and thymectomy performed. Histopathological examination revealed a parathyroid carcinoma in three different focuses, with an invasion to the adjacent perineural, vascular and lymphatic tissues. He lost his follow-up once again until October 2010, when he presented with malaise, fatigue, gait disturbances and end-stage renal disease. His calcium was 16.5 mg/dl, PTH 1497 pg/ml, serum creatinine 5.5 mg/dL. Radiography of the right femur and hemipelvis demonstrated generalized Brown tumors which are huge and incorporated in distal femur giving it the form of 'woven bone', associated with osteitis fibrosa cystica. Neck ultrasonography showed an irregular shaped parathyroid lesion (17×14 mm), with a surrounding pathological lymph node (14×8 mm). He died of myocardial infarction when he was being prepared for another neck exploration surgery.
Conclusion
In patients presenting with severe hypercalcemia associated with hyperparathroidism, the probability of parathyroid carcinoma must be excluded, in order to avoid a catastrophic bone involvement and mortality associated with hyperparathyroidism severe deformities involving the long bones.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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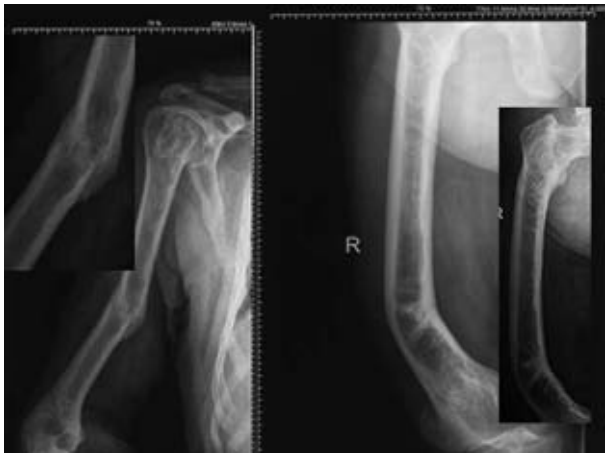


**Figure 1.**

## P464

**Severe fetal and neonatal hyperthyroidism following radioiodine therapy in a woman with Graves' disease**

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Introduction
Radioiodine (RAI)-therapy for Graves' disease (GD) is often followed by worsening autoimmunity, and the increase in TRAb may persist for several years. In pregnant women TRAb pass the placenta and may stimulate the fetal TSH receptor with a risk of fetal and neonatal hyperthyroidism.
Case report
A 21-year old woman with GD was treated with RAI, and subsequently was euthyroid with L-T4-replacement. Before RAI-therapy, TRAb was 8 U/l (reference $\leq$1.0 U/l). She became pregnant 4 months after RAI-therapy, and simultaneously developed opthalmopathy. Fetal tachycardia (176–180 beats/minute) developed at gestational week (GW) 33, and fetal echocardiography revealed dilated right atrium, right ventricle hypertrophy and tricuspid insufficiency with regurgitation (4–6 m/s). At GW 34+5, she delivered a severely thyreotoxic girl with FT4 >100 (12.0 – 22.0) pmol/l, FT3 > 50 (3.9–6.7) pmol/l and TSH 0.02 (0.99–7.77) mU/l. TRAb in serum of the mother and the neonate were >40 U/l. The neonate had growth acceleration without adequate weight gain (birthweight 2270 g, length 51 cm), goiter, advanced skeletal age (6 months), tachycardia (230–250 beats/minute), needed ventilatory support and developed pneumothorax. Treatment included beta-blockers, iodine, carbimazole, and later L-T4. Her condition improved, the echocardiographic findings were normalized, and she has had a normal development. Later, the mother underwent total thyroidectomy, and TRAb level declined. In a subsequent pregnancy, the fetus showed no sign of hyperthyroidism, and the neonate was euthyroid and healthy, despite elevated TRAb in cord blood (10.9 U/l).
Conclusions
This case report illustrates potential risks of RAI treatment in women with GD and documents passage of TRAb across the placenta in two successive pregnancies, with antibody-associated fetal and neonatal hyperthyroidism in the 1st pregnancy. After thyroidectomy in the mother, TRAb declined. In women who plan pregnancy, total thyroidectomy may be a better treatment option than radioiodine.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P465

**Hypercalcaemia in pregnancy**

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A 28 year old female with no significant medical history, 25 weeks into her second pregnancy was referred to endocrinology with adjusted calcium of 2.99 mmol/l. She had a short history of generalised aches and tiredness. Examination did not reveal any stigmata of MEN1 or the HPT-JT syndromes. There was no known family history of endocrine disorders. Her PTH was 7 pmol/l, confirming primary hyperparathyroidism. As parathyroid Tc-scintigraphy would be contraindicated in pregnancy, an MRI of her neck was reviewed by a radiologist with special expertise in parathyroid imaging. The scan showed evidence of a right sided adenoma. An uncomplicated parathyroidectomy was performed under sedation and bilateral cervical block, without the use of opiates. The histology of the mass was compatible with a benign adenoma. Molecular genetic analysis did not show deletions, duplications or mutations in either MEN1 or RET. She has remained normocalcaemic after 1 year of follow up. Definitive treatment during pregnancy is recommended as hypercalcaemia increases the risk of pregnancy associated complications. Maternal complications such as nephrolithiasis, pancreatitis and bone disease are reported in 67% of mothers. Foetal complications are reported in up to 80%, including preterm labour, neonatal tetany, IUGR and foetal death.

After localising the tumour preoperatively without the use of ionising radiation, surgery should be performed during the second trimester where possible, but may be indicated at any stage of gestation. This case highlights the approach to diagnosing and managing a rare presentation of a common condition. Radiological expertise is required for successful pre operative localisation of parathyroid tumours in pregnancy. In addition to timely surgery, patients should also be investigated for underlying genetic causes as in all young patients with hyperparathyroidism. Although not backed by evidence, long term follow up of these patients with periodic calcium measurement is recommended.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P466

**Ectopic Cushing's syndrome and thymic hyperplasia**

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Introduction

Thymic hyperplasia has been described after hypercortisolism resolution. The natural history remains poorly defined: emergence $\geqq$1 month after hypercortisolism resolution, variable duration, usually spontaneous resolution/benign course.

Case Report

♂, 24, referred in 2000 for secondary hypothyroidism: TSH: 0.25 μUI/ml (0.25–5); FT4: 5.55 pmol/l (9–20). Clinical evaluation: insomnia, nocturnal sweating, facial erythema, myalgias, asthenia; acne, abdominal purple striae; BMI: 24.1 kg/m$^2$. Total testosterone-1.8 ng/ml (2.7–11), ACTH-104 pg/ml (9–52), UFC-495 μg/24 h (10–80); Cortisol-17 μg/dl (5–25).

CRH test: ACTH-51, 48, 52 pg/ml (baseline, 15, 30 min respectively); cortisol-110, 188, 211, 176 μg/dl (baseline, 15, 30, 45 min respectively).

Pituitary MRI (07/2000): 'dimensions slightly greater than expected, stalk without deviation; no hypothalamic alterations'. He starts ketoconazole 400 mg/day; dose adjustments (UFC/clinical). Cervico-thoraco-abdominal CT (08/2000):normal. Octreotide scintigraphy (10/2000): 'Focus uptake in the right hemithorax.' Bronchoscopy (01/2001): Normal. In Jan/2001 worsening of hypertension and hypokalemia: KCl + spironolactone were started; at that time UFC 60 μg/24 h, ACTH 159 pg/ml, cortisol 13 μg/dl. Lung surgery (04/2001): '2 lymph nodes without neoplastic aspects removed.' Inferior petrosal sinuses catheterization (05/2001): no gradient. Octreotide scintigraphy (07/2001): 'more evident fixation'. Thoraco-abdominal CT(07/2001): 'Nodular image in the area corresponding to scintigraphy.' Lung surgery(octreotide-labeled probe; 08/2001):'Bronchial carcinoid.' Steroid substitution was done during 9 months, with progressive hypercortisolism stigmata regression. Chest CT (2002): 'anterior mediastinum retrosternal triangular formation; thymus? conglomerate adenopathy?'. Chest CT (2003; 2006): 'mass probably related to thymus…31 × 16 mm'. Chest CT (2011): 'normal appearing mediastinum, centered, no adenopathy'. The patient maintains follow-up.

Conclusions

This case illustrates the frequent difficulty in localizing the source of ACTH production, and the possible association of a thymic hyperplasia occurring after the hypercortisolism resolution. A self-limiting evolution is to consider.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1**

| | **Cortisol** (μg/dl) | |
|---|---|---|
| **Dexamethasone suppression testing** (06/2000) | Baseline | After |
| 1 mg (23 h) | | 15 |
| 0.5 mg every 6 h for 8 doses | | 27 |
| 8 mg (23 h) | 24 | 21 |

## P467

**Synchronous papillary thyroid cancer and astrocytoma: case report**

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Introduction – aim

The incidence of multiple primary cancers is reported to be between 0.3 and 4.3%. The second primary lesion is identified either simultaneously with the primary lesion (synchronous) or after a period of time (metachronous). We report such a rare occurrence of dual malignancy of the thyroid and brain in order to stress the importance of a good preoperative workup to arrive at such a diagnosis preoperatively and also to stress the importance of radical surgery in patients with operable primary tumors.

Case report

A 47-year- old man was admitted to our Medical center on March 18, 2010 with 4-month history of weakness of the right fingers, with a thyroid mass. Magnetic resonance imaging revealed a mass lesion in the right temporal, frontal lobes of brain with diffuse high intensity on T2-weighted and fluid-attenuated inversion images. A few small lesions were enhanced by contrast medium on the T1-weighted images. Ultrasound examination of thyroid: nodules (size 2.6 × 2.1 × 2.0 cm and 5 mm) in the right lobe and 3 nodules (4 mm, 6 mm, 8 mm) in the left lobe. Ultrasonography guided fine-needle aspiration biopsy was performed and the cytological examination revealed cells of papillary thyroid cancer. The absence of familial adenomatous polyposis was determined after a complete colonoscopy. In 24.03.2010 subtotal removal of brain tumor was performed. Histologically the brain neoplasm was astrocytoma grade II. In 5.04.2010 a total thyroidectomy with central and unilateral modified left radical neck dissection was performed. Histology demonstrated a papillary thyroid carcinoma with extrathyroidal extension to parathyroid soft tissue, metastases to the lymph nodes. Postoperatively, he underwent radioactive iodine ablation therapy. In order to explain the molecular basis for such an occurrence, we performed the immunohistochemistry of the operative specimens. The cells thyroid cancer reacted for p53 (reaction +) in 75% sells, Her 2 (+2 in 90% cells), vimentin (+), EMA(+), L-thyroglobulin (+), estrogen receptors – (+), progesterone receptors-(++), pancytokeratin – (++). The astrocytoma was positive only for p53 (+).

Conclusion

In the light of advances in molecular biology we can only conjecture that the mutant p53 which has been found in both papillary thyroid cancers and astrocytomas may be one such putative mutation underlying such an occurrence.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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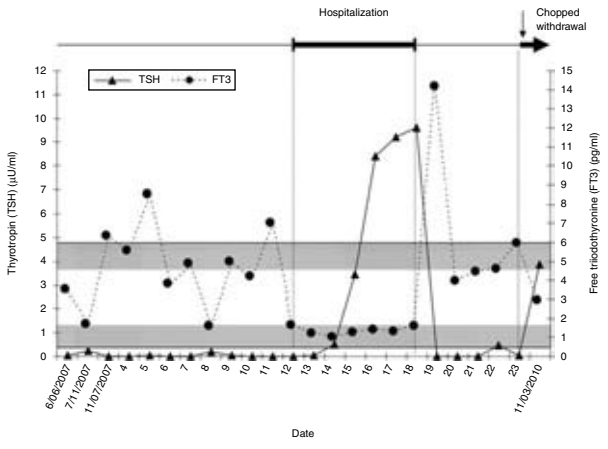
## P468

**Exogenous thyrotoxicosis by consumption of pork sausage**

M. Cano Megias, J. Diez, G. Perez López, M. García Villanueva, P. Iglesias & A. Matei
University Hospital Ramon y Cajal, Madrid, Spain.

Background
Exogenous hyperthyroidism is a rare cause of thyrotoxicosis. It is caused by ingestion of excessive amounts of thyroid hormone, which could be intentional or surreptitious (known as factitious thyrotoxicosis). One of the most exceptional cause is the intake of meat or sausage containing thyroid tissue, inadvertently mixed with traces of muscles and other tissues of the animal's neck (hamburger thyrotoxicosis). The clinical symptoms are indistinguishable from other common causes of hyperthyroidism as Graves disease.
Case report
A 82 years-old woman with recurrent self-limiting hyperthyroidism for three years who was admitted in our Endocrinology department. She had weight loss up to 3 kg and abnormal heart beats sensation. The physical examination was irrelevant. The biochemical test showed not detectable stimulating thyroid, thyroglobulin and peroxidases antibodies. The thyroid scintigraphy with Tc99m pertechnetate displayed a heterogeneous uptake pattern and a small normal thyroid. The thyroglobulin levels were low. A further interview with the patient reported a daily consumption of pork sausage. When she stopped consumption of these sausages, she became asymptomatic and thyroid function was normalized. The pathology analysis of a sausage sample detected tissues from the pork's neck, such as thymus, cartilage and salivary glands, but not traces of thyroid glandule.
Conclusions
Thyrotoxicosis induced by food should be suspected in cases of self-limiting and recurrent episodes of hyperthyroidism with suppressed thyroid stimulating hormone (TSH), high levels of triiodothyronine (FT), low levels of thyroglobulin and not detectable thyroid antibodies. The scintigraphy shows usually a low uptake pattern. The levels of FT3 and thyroxine (FT4) could be useful to differentiate the kind of hormone intake. This case illustrates the importance of a thorough and detailed anamnesis to get a accurate differential diagnosis of a repeat silent thyroiditis.




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P469

**Ovarian hyperstimulation syndrome and autoimmune primary hypothyroidism in two members of a family**

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Guilan University of Medical Sciences, Rasht, Islamic Republic of Iran.

Background
Spontaneous ovarian hyperstimulation syndrome (sOHSS) occurs rarely and has been associated with high level of human chorionic gonadotropin (HCG), mutated FSH receptor (FSHr) gene and hypothyroidism. We report sOHSS in two members of a family with autoimmune hypothyroidism.
Patient Findings
A 15 years old girl presented with abdominal pain and distention and typical features of hypothyroidism.Serum TSH was $>100$ mIU/l and anti-TPO antibody was 290 U/ml. Abdominal ultrasound and CT-scan revealed bilateral multi-lobulated ovarian cysts. FSHr gene sequencing showed no mutation. The second patient, the cousin of the first patient, a 14.5 years old girl was presented with acute abdominal pain and distention after a minor trauma. Hypothyroid feature was remarkable; laboratory test showed TSH: 72.5 mIU/l. Abdominal ultrasound revealed bilateral multilobulated ovarian cysts with one ruptured cyst. Levothyroxine therapy resulted in significant regression of ovarian cysts in both patients.
Conclusion
sOHSS can be simply managed with Levothyroxine in patients with hypothyroidism. However, its association with thyroid autoimmunity and puberty needs more investigation.
Keywords
Ovarian hyperstimulation syndrome, ovarian cyst, Hypothyroidism, Puberty, familial.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P470

**Novel treatment option in the management of SIADH related Hyponatremia: two case reports**

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Introduction
Current treatment for SIADH related hyponatremia is unsatisfactory. We describe two cases of effective Tolvaptan use in SIADH related to disseminated cancer.
Case 1
Fifty-three year old female with metastatic ovarian carcinoma, vomiting from partial intestinal obstruction, was referred with sodium 121 mmol and normal renal function. She was clinically dry and mildly confused. SIADH was confirmed but urine Na was $<10$ suggestive of hypovolemia.
IV fluid was commenced followed by Demeclocycline – latter caused renal failure and was stopped. Cyclizine pump was started and soon she was able to drink more and renal function normalised. Tolvaptan 15 mg was started (sodium 122 mmol) when adequately hydrated and Urine sodium $>20$. Within 48 h sodium was 127 mmol and 135 mmol/l five days after starting Tolvaptan. Family wanted her home as she was now relatively alert and stable. Unfortunately she passed away 3 days later.
Case 2
Fifty year old lady with ovarian cancer and abdominal metastases – was referred with falling Sodium levels – 138 in April, 134 June, 126 in July (admission). Investigations confirmed SIADH. Fluid restriction was poorly followed and Sodium only improved to 128 mmol/l. Started 15 mg of Tolvaptan. Clinically felt better with progressive rise in Sodium over a week – 134, 139, 140, 142 mmol/l. Tolvaptan was stopped and she was discharged to a Hospice. Day 7 post-discharge sodium was 133 and day 14 it was 134 mmol/l.
Discussion
Tolvaptan (new Vasopressin receptor antagonist) has shown promise in treating hyponatremia due to SIADH. Intermittent vomiting in the 1st patient meant that fluid restriction long-term was impractical and ineffective in the 2nd patient. Demeclocycline caused renal toxicity. Tolvaptan raised and maintained sodium levels without compromising safety, enabled removal of fluid restriction and allowed the patient to spend the last few days at home. It is important to correct dehydration before using Tolvaptan.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P471

**Growth hormone: 'beginning of a new life'**

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King George Hospital, Barking, Havering and Redbridge University Hospitals NHS Trust, Greater London, UK.

We report three patients who were referred for possible chronic fatigue syndrome and presented with a long standing history of a multitude of symptoms. Comprehensive investigations revealed a low IGF1 and subsequent insulin tolerance test (ITT) demonstrated growth-hormone (GH) deficiency with a normal MRI pituitary in each case. The first patient presented at the age of 34 years with lethargy, aches and difficulty concentrating. Biochemically the only abnormality in th]e basal pituitary tests was a low IGF1 of 9.7 nmol/l (15–39.9). She underwent an ITT where the peak GH-level was low at 0.2 μg/l and the peak cortisol was suboptimal at 414 nmol/l. Her AGHDA score was 20/25 and her symptoms responded dramatically to GH replacement. Her MRI pituitary was normal and currently she takes hydrocortisone at times of stress.

The second patient presented at the age of 36 years with a multitude of symptoms, including lethargy and generalised aches. Biochemistry revealed a low IGF-1 of 12 nmol/l (15–39.9), but no other hormonal deficiencies. He underwent an ITT where all the GH-levels were $<0.1$ μ/l and the peak cortisol was normal at 597 nmol/l. Her AGHDA score was 25/25 and the MRI pituitary was normal. GH replacement was commenced with an improvement in all the symptoms.

The third patient presented at the age of 33 years with difficulty losing weight and tiredness. He had a low IGF-1 at 9.5 nmol/l (15–39.9). After an ITT all the GH-levels were $<0.1$ μg/l and the peak cortisol was suboptimal at 323 nmol/l. Currently after 2 years on GH replacement he has managed to lose weight and is 'much happier with life'.

Patients with multitude of symptoms and/or chronic fatigue syndrome should have endocrinological investigations, in particular looking for GH deficiency, despite normal MRI pituitary. We recommend that all patients with these symptoms should be referred to an endocrinologist for further assessment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P472

**Severe amiodaron: induced thyrotoxicosis**

A. Dokupilova[1] & J. Payer[2]
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Indroduction

Amiodaron is an potent antiarrhythmic drug used for the treatment of ventricular and supraventricular arrhythmias. Long-term amiodaron therapy can cause a thyroid abnormalities in up to 14–18% patients. There are two main forms of AIT: type 1, a form of iodine-induced hyperthyroidism, and type 2, a drug-induced destructive thyroiditis. Differentiation of these 2 types is essential for determining the best management of the disease.

Case report

We present a case of severe amiodaron-induced thyrotoxicosis. 68 years old man with heart failure and severe systolic dysfunction EF – 25%, after implantation of ICD in secondary prevention of sudden cardiac arrest was admitted to our cardiology department after 3 electrical shocks. Patient has been using amiodaron for at least 2 years. We have established the correct function of ICD. Patient had the following laboratory findings: uTSH 0.005 (0.3–4.2 μIU/l), fT4 $>$ 100 (12–22 pmol/l), fT3 18.2 (3.1–6.8 pmol/l), antiTPO 12 (0–60 IU/l), antiTG 18 (0–60 IU/l), TRAK 0.1 (0–1 IU/l). The clinical presentation has included fatigue, weight loss and profound muscle weakness. Color flow Doppler sonography has shown absent hypervascularity, there were a thyroid nodule 10 mm in diameter. There was no improvement in laboratory finding and clinical asesement after discontinuation of amiodaron therapy for more than 2 months. We started a treatment with thionamides in daily dose 60 mg. No effect of these therapy was recorded, so we have to use a combined treatment with thionamides 60 mg daily dose, corticosteroids in daily dose 60 mg and potassium perchlorate in daily dose 800 mg. Improvement has shown 1 week following the start of therapy. Patient has became euthyroid after 2–3 month of the therapy.

Conclusion

Amiodaron is often used in cardiology for the treatment of severe ventricular and supraventricular arrhythmias. In some cases can cause a thyroid dysfunction. Recognition of AIT form is useful for the best treatment of disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P473

**Thyreotoxic periodic paralysis in a Caucasian man after corticosteroid administration**

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Nicaea Hospital 'Ag. Panteleimon', Piraeus, Greece.

Introduction

Thyreotoxic periodic paralysis (TPP) is a very rare complication of hyperthyroidism in Caucasians. Furthermore there have been reported only a few cases that paralysis was induced by corticosteroids in patients with TPP.

Case presentation

We report the case of a 35-years-old Greek sailorman who had a spider bite in his right hand during a trip in Mexico. He was admitted to local hospital due to the significant swelling of his arm, where he received a single i.m. dose of a corticosteroid. When he woke up next morning he was almost totally paralyzed and able to move only his eyes. He was admitted to hospital again and severe hypokalemia was found. He received a KCl infusion and he recovered completely. A month later he had a second episode of morning paralysis. He had had a heavy carbohydrate meal the previous night. He was admitted to our hospital with severe hypokalemia which was carefully corrected with KCl. The clinical and laboratory examinations revealed hyperthyroidism due to Grave's disease. He took methimazole for several months without thyroxin replacement. Total thyroidectomy was performed and the patient received thyroxin replacement therapy with slow and careful titration. He never got paralyzed again and all potassium measurements are normal.

Conclusion

TPP is a very rare complication of hyperthyroidism in Caucasians, but it has to be considered especially when there's a need for corticosteroid administration.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P474

**Adrenal crisis due to unusual cause: case report**

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King's Mill Hospital, Sutton in Ashfield, UK.

Introduction

A 40-year-old woman, despite previously well controlled Hypothyroidism and Addison's disease, presented with adrenal crisis due to extremely rare cause.

Case Report

In 2002, age 31 years, she presented with symptoms of anorexia, weight loss and postural hypotension. She was subsequently diagnosed to have Addison's disease and primary hypothyroidism (confirmed with positive adrenal and thyroid peroxidase antibody). She was started on Hydrocortisone, Fludrocortisone and 5 days later Levothyroxine was initiated. She was regularly reviewed in endocrinology clinic.

Nine years later, she presented with 2 month history of excessive sweatiness, restlessness, heat intolerance, dizziness, weight loss and increased skin pigmentation despite doubling the dose of Hydrocortisone (20 mg am and 10 mg pm) and taking regular Fludrocortisone and Levothyroxine.

On examination, she had tachycardia (heart rate – 123 bpm), marked postural hypotension, increased tremulousness of outstretched hands and small diffuse goitre.

Investigation confirmed biochemical thyrotoxicosis; Free T4 – 47 (ref 11–23 pmol/l), FT3 – 25.9 (4.1–7.9 pmol/l), TSH $<$ 0.02 (0.3–5.5 mU/l), Cortisol $-$18 nmol/l, Adjusted Calcium 2.50 mmol/l and TPO Antibody $>$ 1300 (ref $<$ 60 IU/ml). Her TSH receptor antibodies were positive.

Levothyroxine was stopped and antithyroid medication was initiated. Gradually her TFT normalised.

Conclusions
This case highlights few important points:
1. When patient with Hashimoto's hypothyroidism present with hyperthyroidism, over-replacement with Levothyroxine is the likely cause, but possibility of endogenous hyperthyroidism should be considered.
2. Transformation of Hashimoto's hypothyroidism to Graves' hyperthyroidism is extremely rare, but does occur. This is due to presence of different types of TSH Receptor Antibodies which recognise different epitopes of TSH receptors and modify the patients' thyroid functions, resulting in hyperthyroidism or hypothyroidism.
3. Development of thyrotoxicosis in a patient with otherwise well controlled Addison's disease can result in hypoadrenal crisis as thyroid hormones accelerate glucocorticoid turnover and increase glucocorticoid requirement.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P475

**Persistently abnormal thyroid function in a 26 year old Afro-Caribbean man: a diagnostically challenging case**

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Introduction
While most common aetiologies accounting for hyperthyroidism are straightforward and respond predictably to treatment, a subset provides diagnostic or therapeutic challenge. We report such a case.
Case report
A 26-year-old Afro-Caribbean male presented with symptoms of thyrotoxicosis. FT4 was 50 (12–20 pmol/l), FT3 11.5 (3–8 pmol/l) and TSH 5.38 (0.27–4.20 mU/l). He had diffuse non-tender thyromegaly without eye signs. Thyroid antibody was negative. Three months later he felt better on Carbimazole 30 mg but complained of lethargy and weight gain. FT4 was now 15, FT3 12.3, TSH 69.6 mU/l. Carbimazole was therefore discontinued.
10 months later he presented with tremor and palpitations on 20 mg of Carbimazole. He insisted good compliance with medication. FT4 was 36, FT3 was 12.3 and TSH was 11.87 μ/l. Carbimazole was increased to 30 mg.
Short synacthen test and pituitary function was unremarkable. MRI of the pituitary ruled out TSH-secreting adenoma. 1 year after presentation FT4 was 22, FT3 11.7 and TSH 20.58 μ/l. Carbimazole was withdrawn.
Differential diagnoses considered were assay artefact, interfering antibodies to FT4 and Resistance to Thyroid Hormone (RTH). The former two were excluded by testing in multiple laboratories. Genetic testing revealed heterozygosity for a mutation in the thyroid receptor (TR)-β gene (targeted to exons 7, 8, 9 and 10). Patient later disclosed about an aunt in Jamaica with persistent hyperthyroidism on high doses of carbimazole.
Discussion
RTH is a rare, inherited condition of reduced responsiveness of target tissue to thyroid hormone. Incidence is estimated to be 1 case per 40 000 live births and clinical presentation is highly variable. Characteristically FT4 and FT3 are raised with an unsuppressed TSH. Diagnosis can be challenging and other common differentials need to be excluded. It is important to consider this diagnosis in a young person with family history and persistently unsuppressed TSH with raised FT3/FT4.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P476

**Asymptomatic hypocalcemia in elderly**

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Introduction
Hypocalcemia varies from asymptomatic biochemical abnormality to life-treating disorder, depending on the duration, severity and rapidity of development.
Case report
A 77-year-old woman was admitted for study of asymptomatic hypocalcemia discovered in routinely laboratory test. Past medical history, left thyroidectomy by goiter 53 year ago without follow-up. She denied any symptoms. Physical examination. Actinic keratosis in right cheek, scar of thyroidectomy. Cardiac and respiratory exam were normal. Neurologic examination discards abnormalities, Chvostek and Trousseau signs were negatives. Laboratory test; calcium 5.72 mg/dl, phosphate 5.67 mg/dl, ionized calcium 3.1 mg/dl, magnesium 1.60 mg/dl, albumin 3.7 g, PTH 2.6 pg/ml, urinary calcium 12.2 mg/24 h, D3, TSH, cortisol, ACTH, hepatic and renal test normal. Thyroid Ab negatives. EKG normal. CT of the neck an brain, normal thyroid remnant, calcifications of basal ganglia and the corona radiata. Skeletal X-ray degenerative lesion age related. Treatments with calcium carbonate 2.5 g. TID and calcitriol 0.25 μg BID were initiated. Follow-up, one month later calcium level 8.5 mg/dl and phosphate 3 mg/dl. Currently patients remain asymptomatic with calcium 2.5 g and calcitriol 0.25 μg daily.
Discussion
The incidence of hypocalcemia varies from 0.4–13.8% after total thyroidectomy to 0.2–1.9% after subtotal thyroidectomy. Chronic or moderate hypocalcemia may be asymptomatic. Clinical signs are observed only with decreases in ionized calcium concentration in our patient despite low level ionized calcium was asymptomatic probably due to duration of hypocalcemia. Chronic hypocalcemia is treated by calcium oral and vitamin D supplementation. The serum calcium level should be targeted to about 8.0 mg/dl. Cerebral calcifications are not reversible.
Conclusion
Periodical routine checks of calcium levels in patients undergoing thyroid surgery would allow early diagnosis of asymptomatic hypocalcemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P477

**Thyroid nodule: dormant, but lethal**

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We report two patients with thyroid nodules where thyroid cancer has unexpectedly been found. The first patient presented at the age of 58 years with a lump in her neck. She was clinically and biochemically euthyroid. Ultrasound revealed a solitary left hypoechoic nodule (23 × 17 mm), with no other suspicious sonographic features. She had two FNA's over a two year period which were reported as showing no malignant cells (THY2). Three annual ultrasounds showed no change in the size of the nodule and no suspicious features. However, despite these investigations, the patient was keen for surgery to provide a definite histological diagnosis. She underwent a left lobectomy and the histology unexpectedly revealed a 21 mm medullary thyroid carcinoma.
The second patient is a 42 year old lady with Graves' disease who presented with a 6 month history of palpitations. Clinically she had a moderate, smooth, non-nodular goitre, audible bruit and biochemically she was hyperthyroid with a free $T_4$ 52.8 pmol/l (10.0–19.8) and TSH $<0.01$ μ/l. A radionucleotide scan revealed features consistent with Graves' disease. She was initially commenced on carbimazole 40 mg daily. However, 14 months later despite being on carbiomazole 60 mg daily, with variable compliance, her free $T_4$ was 31.1 pmol/l. She underwent a total thyroidectomy due to poor compliance and possible resistance to medical therapy. Histology unexpectedly revealed a 4.5 mm papillary carcinoma in the right thyroid lobe; the left lobe was normal.
In summary we report two patients who have undergone thyroid surgery where a malignancy was not suspected on clinical or radiological investigations. The first case illustrates that surgery should be considered in patients with a thyroid nodule, even with a normal FNA. The second case demonstrates that thyroid cancer can still occur in a patient with Graves' disease, and in our patient the variable compliance has 'worked in her favour'.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P478

**Morbid obesity: what can be and should be done?**

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Patient C Caucasian male, 41 years old was admitted to the inpatient department of endocrinology with newly diagnosed $T_2$ DM, morbid obesity on the 03.03.2011.
Anamnesis: Overweight since childhood, but in the past few years he started to gain weight rapidly (because of smoking cessation). Arterial hypertension since 2003, with maximum increase up to 210/125 and he took antihypertensive drugs regularly. In 2006 painless myocardial ischemia was first diagnosed. He had a history of impaired glucose tolerance since 2009, and was administered diet with metformin 1700 mg/daily, but he soon stopped taking the drug. In 2009 he was endoscopically installed gastric band. His weight decreased up to 30 kilos in 5 month, but not for long. His parents had a history of T2DM.
Objective
Height – 182 cm, Weight – 190 kl, BMI – 57 kl/m$^2$. No signs of Cussing disease. BP 150/90 mm Hg, pulse rate – 80 per min. Medical examination revealed: thyroid hormones, prolactin and cortisol levels were normal, but there was a significant increase in C-peptide – 2.55 nmol/l (0.37–1.47). A1C–8.5%. Glucose levels (04/03/11) 10.3 – 13.6 – 10.4 – 9.7 – 8.9 – 7.7 mmol/l. He was administered metformin 2550 mg/daily and a hypocaloric diet. After his blood sugar became normal (5.2 – 7.0 mmol/l) he was transferred to the Department of Surgery and 30.03.11 he was performed bilio pancreatic bypass in modification of Hess–Marceau. Postoperative period was well. He was recommended calcium supplements, iron and multivitamin complex.
Follow up 09.2011 Weight 141 kl. No complains.
12.2011 Weight – 129 kl, BMI – 42.59 kl/m$^2$, A1C – 5.6%, BP – 120/80 mm Hg. Surgery is a method of choice in treatment of obesity when diet and drugs are no longer effective. It improves quality of life, glycemic control and metabolic disorders and give's patient hope for a better future.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P479

**Sever Grave's disease and diabetes melitus**

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March 2011. Patient P. Caucasian female, 53 years. Diagnosis: severe Grave's disease (GD), recurrent course. Endocrine ophthalmopathy (EO) NOSPECS – 4, CAS – 7. Thyrotoxic cardiomyopathy. Paroxysmal atrial fibrillation. Thyrotoxic proximal myopathy of the lower limbs. Thyrotoxic hepatitis. Type 2 diabetes mellitus. Chronic obstructive pulmonary disease.
Anamnesis: GD was first diagnosed in 2001, when she started to complain of weight lost (20 kilos in a couple months), tachycardia and general fatigue. Clinical examination revealed thyrotoxicosis. For 10 years she had been taking Thiamazole from 30 to 10 mg/daily depending on the level of hormones, because of fear of thyroidectomy and radioiodine therapy. In 2009 she was admitted to the inpatient Department of Endocrinology with ketoacidosis and blood glucose level – 27 mmol/l. She was administered intensive insulin therapy. 2010 – new complains of double vision, lacrimation and dry eyes appeared.
Clinical examination: TSH – 0.01 ME/ml (0.4 – 4), $fT_3$ – 20 pmol/l (2.3–6.3), $fT_4$ – 25 ng/dl (6.8–15.0), Ab-rTSH – 38 (0–0.99). C-peptide – 3 pmol/l (258–1758), A1C – 11%. Ultrasonography of the orbits (U.S.orbits): the value ofthe retrobulbar tissue (RBT) OD\OS = 18.3/17.5 mm ($n < 16$).
Blood glucose (BG): 17.3–20.0–5.5–18.9–17.6–13.4 mmol/l.
Treatment
Thiamazole 40 mg/daily, oral dexamethasone 0.1 mg/kg on alternate days, dose correction of the insulin, potassium preparations, omeprazole.
After normalization of $fT_3$, $f_4$ levels and BG, the patient was transferred to the surgical department for thyroidectomy.After that she was administered L-thyroxin 125 mkg/daily.
Follow up: November 2011. TSH, $f_4$, $fT_3$ – normal.U.S. orbits reveals no signs of edema. BG: 7.8– 9.0 –8.0 – 7.6 – 8.5 – 9.0 mmol/l, A1c – 7.5%.
After removing the source of the autoimmune aggression we've managed to achieve stable remission of EO, GD's complications and good glycemic control.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P480

**Secondary hypertension: a case presentation**

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Introduction
Secondary causes of hypertension exist in 10% of hypertensive subjects. Hypertension refractory to antihypertensive treatment must prompt the physician to screen for secondary causes.
Case presentation
A female 53-year old patient from Libya married and has three children. She works in a factory.
Complaints: Headache in attacks 1 year and 4 months, palpitations 4 months. She was discovered to be severely hypertensive and was given captopril 25 mg bid, with poor control of her ABP. No blurring of vision or diminution of visual acuity. No muscle weakness. No polyuria or polydipsia. No dysuria. Normal bowel habits. Normal sleep.
Past history: No past medical or surgical history.
Family history: No family history of hypertension, diabetes mellitus, dyslipidemia or ischemic heart disease.
Menstrual history: Patient is menopausal since 15 years on no HRT.
Social history and special habits
Nonsmoker. No special habits (no licorice intake, no coffee, tea once per day).
Drug history: Captopril 25 mg bid. No diuretics.
Clinical Examination
Radial pulse = 65 beats/min with 10 dropped beats/min, apical pulse = 73 beats/min with 12 dropped beats/min, A.B.P. = 170/100 mmHg, BMI = 30.20 kg/m$^2$, waist circumference = 102.5 cm, hip circumference = 99.5 cm, and WHR = 1.03.
Heart: Except for the frequent dropped beats and increased A2 over the aortic area, no other abnormalities detected.
Lower limbs: Dorsalis pedis felt bilaterally, soft pitting edema bilaterally ++.
The rest of the clinical examination was unremarkable.
Laboratory and radiological investigations were done.
The various causes of secondary hypertension will be reviewed laying particular stress on the patient's laboratory and radiological findings.
Conclusion
Making a diagnosis of a secondary disorder for hypertension is gratifying, because it may lead to significant amelioration or in some instances even cure of the elevated blood pressure like what occurred with the presented case.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Developmental endocrinology

## P481

**Regulation of trophoblast 11β-hydroxysteroid dehydrogenase type 2 expression and role of corticotropin-releasing hormone**

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The formation of the placental syncytiotrophoblast through trophoblast differentiation is a key cellular event, essential for successful embryonic implantation, oxygen/nutrient transport and secretion of placental hormones necessary for fetal development. Trophoblast differentiation alters expression of various molecules with important roles in placental biology, including

11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), the major component of the placental glucocorticoid (GC) barrier that limits exposure of the fetus and placenta to maternal GC. The endocrine effectors and molecular signaling pathways involved are not fully characterised. To investigate 11β-HSD2 regulation of expression, we employed the BeWo choriocarcinoma cell line, able to differentiate upon forskolin treatment and stimulation of district signaling cascades including the cAMP/PKA and ERK1/2 and P38 MAPKs[1]. Our results showed that BeWo trophoblasts express low levels of 11β-HSD2. Protein expression was significantly enhanced when specific inhibitors were used to inhibit activity of the PI3K-Akt-eNOS pathway that involves molecules recently identified as negative regulators of forskolin-induced BeWo fusion[2]. In contrast, inhibition of ERK1/2 and P38 MAPK attenuated 11β-HSD2 mRNA and protein expression. BeWo trophoblast 11β-HSD2 expression was also regulated by corticotrophin releasing hormone (CRH): interestingly, CRH treatment up-regulated 11β-HSD2 protein without affecting mRNA levels, raising the possibility of non-transcriptional effects of CRH targeting protein stability. Moreover, similar effects of CRH were demonstrated in experiments employing placental explants and appear to involve regulation of the ERK1/2, β38 MAPK and Akt pathways. Thus, this data identified in the BeWo trophoblasts novel signaling regulators of placental 11β-HSD2 expression and placental actions of CRH that might be important in the control of cellular sensitivity to GC with potentially important pathophysiological implications.

Delidaki *et al*. 2011 *Mol Cell Endocrinol.* 2011 **332** 213–20.
Vatish *et al*. 2012 *PLOS One*, in press.

Declaration of interest

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## P482

### Dimensional profiles of male to female gender identity disorder: an exploratory research

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Introduction

Male-to-female gender identity disorder (MtF GID) is a complex phenomenon that could be better evaluated by using a dimensional approach.

Aim

To explore the aggregation of clinical manifestations of MtF GID in order to identify meaningful variables describing the heterogeneity of the disorder.

Methods

A consecutive series of 80 MtF GID subjects (mean age $37 \pm 10.3$ years), referred to the interdepartmental Center for Assistance Gender Identity Disorder of Florence and to other Italian centers from July 2008 to June 2009, was studied. Diagnosis was based on formal psychiatric classification criteria. Factor analysis was performed.

Main outcome measures. Several socio-demographic and clinical parameters were investigated. Patients were asked to complete the Bem Sex Role Inventory (BSRI, a self-rating scale to evaluate gender role) and Symptom Checklist-90 Revised (SCL-90-R, a self-rating scale to measure psychological state).

Results

Factor analysis identified two dimensional factors: factor 1 was associated with sexual orientation, and factor 2 related to behavioral and psychological correlates of early GID development. No correlation was observed between the two factors. A positive correlation between factor 2 and feminine BSRI score was found, along with a negative correlation between factor 2 and undifferentiated BSRI score. Moreover, a significant association between SCL-90-R Phobic subscale score and factor 2 was observed. A variety of other socio-demographic parameters and clinical features were associated with both factors.

Conclusions

Behavioral and psychological correlates of Factor 1 (sexual orientation) and Factor 2 (gender identity) do not constitute the framework of two separate clinical entities, but instead represent two dimensions of the complex MtF GID structure, which can be variably intertwined in the same subject. By using factor analysis, we offer a new approach capable of delineating a psychopathological and clinical profile of MtF GID patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P483

### GH replacement therapy in patients with primary paediatric brain tumours

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Aim

Endocrinopathies are frequent complications in paediatric patients with primary brain tumours (PBT) and among them GH deficiency (GHD) is of particular relevance. In this study we evaluated the incidence of GHD and the impact of GH therapy in a large series of PBT bearing patients or adult patients previously treated for PBT.

Methods

From January 2001 to December 2011 more than 700 PBT patients were screened for endocrine complications. GHD was explored by provocative tests (arginine and clonidine) in a subgroup of patients on the basis of laboratory (IGF1 levels), auxological (growth failure, low height velocity, delayed skeletal maturation) and clinical findings (anti-neoplastic therapies adopted life expectancy). Patients with proven GHD were treated with recombinant GH at initial dosage of 0.22 mg/kg per week for children and 0.3 mg/die for adults. For each patient the GH dose was tailored on the basis of circulating IGF1.

Results

110 PBT patients displayed GHD. Of these patients, 47 had diagnosis of medulloblastoma, 21 germ-cell tumours, eight low-grade gliomas, eight ependymoma, five supratentorial PNET and 21 other histological PBT. They received chemotherapy (conventional and/or high-dose chemotherapy) and/or radiotherapy according to our institutional protocols and to the clinical course. In all but five children (with precocious puberty and cranio-spinal irradiation) growth recovery was observed. Improvement of bone mineral density was attained either in children or in adults. A particularly interesting observation was the improvement in the neuropsychological performances of several patients secondary to GH therapy. A temporary discontinuation of GH therapy was necessary in three patients due to worsening of cerebral radionecrosis. Disease recurrence was found in seven and this relapse rate is lower than in non-GH treated patients.

Conclusions

In our experience GH replacement therapy is safe and effective in the correction of GH deficiency and in improving quality of life of PBT patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P484

### The effect of educational program based on BASNEF model on diabetic (type II) control blood sugar

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Introduction

For prevention of diabetic disease complications,educational interventions by using health education models are performance. The purpose of this study was to

determine the effects of educational program based on the BASNEF model on diabetic (type II)) control blood sugar.
Material and methods
This is a perspective and quasi-experimental intervention study,. 100 diabetic patients type II (50 case and 50 control), between the ages of 40 and 65 years, having had diabetes for over 5 years, participated in the study. The instruments for data collecting were a questionnaire established based on the BASNEF model, a check list related to patient practice on the basis of self reporting, a check list for recording the patients' HbA1c and FBS levels report as well. All groups completed the questionnaires and check lists results were documented before and three months after intervention. The patients of the experimental group participated in 6 educational session classes during the one month of intervention and again two months after, with two session meeting classes as the follow up of intervention. The data were collected and analyzed by SPSS computer software.
Results
Our findings indicated that mean scores of BASNEF model variables (beliefs, attitudes, subjective norm, enabling factors) were significantly increased in the experimental group compared to the controls after intervention. Also, behavioral controlling blood sugar, rate of HbA1c (before intervention as 8.65% after three months 7.47%) and FBS levels (before intervention 207.08, after three months 124.2) improved significantly among the experimental group, compared to control group.
Conclusion
Applying the BASNEF model is very effective for developing an educational program for diabetics, in order to control their blood sugar and enhancing behavioral controlling blood sugar. Besides such programs, follow up education on controlling and monitoring is highly recommended.
Keywords: Type 2DM, blood sugar, Educational BASNEF model.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P485

**The timing of the onset of adrenarche in Pakistani boys**

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Adrenarche is a normal maturational event that in humans occurs at around 6–8 years of age. During adrenarche, a new zone of the adrenal cortex, the zona reticularis, is developed, resulting in increased production of adrenal androgens, androsteindione, DHEA and DHEAS. The increase in the secretion of adrenal androgens causes the appearance of axilliary and pubic hair, a brief increase in linear growth velocity and bone maturation, and the development of the brain. The premature and/or exaggerated adrenarche results in future onset of diseases and thus has a clinical relevance. The present study was aimed at unraveling the age of the onset of adrenarche in Pakistani boys. The history of the boys of 1–10 years of age with their body weight and height was obtained through a questionnaire. Blood samples were collected from antecubital vein of each boy, plasma was separated and concentrations of androsteindione, DHEAS and testosterone were determined by using specific assay systems. The data were analyzed using Student's *t*-test, ANOVA, and Pearson correlation *r*. The boys gained normal weight and height with the corresponding body mass index. The concentrations of androsteindione started to rise at 3rd year and reached peak levels at 7th year of age. The concentrations of DHEAS started to increase at 4th year and reached first peak at 7th year and continued to increase by 10th year of age. The levels of T remained very low throughout the study period but progressively increased and showed a peak at 6 years and then continued to rise non-significantly from 7th to 10th year of age. We observed strong positive correlations between androsteindione and DHEAS, androsteindione and T, and DHEAS and T at adrenarche. We report here that the age of adrenarche in Pakistani boys is 7 years.
Declaration of interest
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## P486

**Post prandial gut hormones: novel activators and regulators of innate immune function**

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Invariant natural killer T (iNKT) cells are key innate immune cells implicated in the pathogenesis of many diseases. Glucagon-like peptide-1 (GLP-1) is an incretin hormone implicated in regulating blood glucose and body weight. We recently demonstrated that GLP-1 is a regulator of iNKT cell function and that inflammatory conditions such as psoriasis, rheumatoid arthritis and ulcerative colitis improve following GLP-1 therapy. GLP-1 is one of several post-prandial gut hormones, including peptide YY (PYY), cholecystokinin (CCK) and ghrelin, each with a distinct metabolic function.
We investigated iNKT cell lines for expression of PYY, CCK and ghrelin receptors by PCR and examined receptor activation impact on cell function by ELISA and flow cytometry.
A PYY receptor (PYY-R) was identified and, as was previously demonstrated with GLP-1, PYY-R activation induced the anti-inflammatory transcription factor CREB and inhibited the pro-inflammatory transcription factor NFκB. This translated into reduced production of the pro-inflammatory cytokine IFN-γ (600–450 pg/ml, $P<0.01$) but not the anti-inflammatory cytokine IL4 (350–349 pg/ml). We also demonstrated increased tumour cell lysis by PYY-treated iNKT cells (6 vs 12% of tumour cells lysed ($P<0.001$).
Stimulation of the CCK receptor resulted in activation of the transcription factor NFκB but had no effect on CREB. This resulted in an increase in cytokine production of both IFN-γ and IL13 (300–400 and 280–370 pg/ml, respectively). We identified a ghrelin receptor on the iNKT cell, but activation had no impact on cell function or the CREB and NFκB transcription factors.
We conclude that post-prandial gut hormones differentially impact and regulate innate immune cell function and may have therapeutic potential as immunomodulatory agents in autoimmunity and malignancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P487

**Opposite effect of LXR on myelination process in the central and peripheral nervous systems and interplay with Wnt pathway**

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Oxysterols are reactive molecules generated from the oxidation of cholesterol. Few data are available about their functions in myelination of nervous system. Our aim was to study the influence of oxysterols on myelin gene expression and myelin sheath formation by oligodendrocytes (central nervous system), and by Schwann cells (peripheral nervous system). We show by gas chromatography/mass spectrometry that oligodendrocytes and Schwann cells contain 24(S)-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol, and that they express their biosynthetic enzymes and receptors (liver X receptors: LXRα and LXRβ). We demonstrate that oxysterols activate the expression of central myelin genes (PLP and MBP) in oligodendrocytes, but inhibit peripheral myelin genes expression (MPZ, PMP22) in a Schwann cells.
In the CNS, we showed that the activating effects of oxysterols were restricted to the cerebellum and dependant of LXR presence as myelin gene expression was drastically reduced in this structure in LXR-KO mice. By, using organotypic cultures of cerebellum slices, we showed that oxysterols were able to stimulate myelin gene expression after lysolecithin-induced demyelination and enhance remyelination process.
In the PNS, the down-regulation of myelin genes is mediated either by LXRα or LXRβ, depending on the promoter context. Importantly, the knockout of LXR in mice results in thinner myelin sheaths surrounding the axons of the nerves. Oxysterols repress peripheral myelin genes via two mechanisms: by binding of LXRs to myelin gene promoters and by inhibiting the Wnt/beta-catenin pathway that is crucial for the expression of myelin genes.
Altogether our results reveal new mechanisms of action of oxysterols and open new perspectives for the treatment of demyelinating diseases by targeting LXR.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P488

**Increased oxidative stress associated with angiogenic growth factors in preeclampsia**

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Hyperperhomocysteine has been implicated in vascular changes and oxidative stress contributing to endothelial dysfunction in preeclampsia. Our earlier studies have shown increased oxidative stress leading to reduced docosahexaenoic acid (DHA) levels in pre-eclamptic women. The present study examines the levels of angiogenic factors (VEGF, PlGF sFlt-1), homocysteine and oxidative stress marker malondialdehyde (MDA) levels, in 90 normotensive (control) and 137 preeclamptic women (90 women delivering at term (term PE) and 48 women delivering preterm (PT–PE)). Maternal homocysteine and MDA levels were higher ($P<0.01$ for both) in preeclampsia groups than control. Maternal plasma VEGF levels were lower ($P<0.05$) in the term-PE group, while it was higher in the PT-PE group ($P<0.05$) than the control group. In contrast, cord plasma VEGF levels were higher ($P<0.05$) in preeclampsia groups than the control group. Maternal plasma PlGF levels were lower ($P<0.05$) in preeclampsia groups as compared to control group. Maternal plasma sFlt-1 levels were higher ($P<0.05$) in preeclampsia groups than control group. Cord sFlt-1 levels were similar in term-PE and control groups while it was lower in the PT-PE group. sFlt-1:PlGF ratio was increased ($P<0.05$) in the preeclampsia groups than the control group. Maternal plasma MDA was positively associated with maternal plasma sFlt-1 levels ($P<0.05$) in the preeclampsia groups. Cord plasma DHA levels were negatively associated with cord plasma sFlt-1 levels ($P<0.05$ for both) in oth preeclampsia groups. Maternal plasma sFlt-1 levels were negatively associated with birth outcome parameters (baby weight, head circumference and chest circumference) in the term-PE group ($P<0.01$ for all). This study for the first time suggests that, levels of angiogenic factors may be differentially regulated in mother and cord. Dysregulation of angiogenic factors may be associated with maternal oxidative stress. Increased oxidative stress may reduce cord DHA levels and increase sFlt-1 levels, leading to poor birth outcomes in preeclampsia.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P489

**Congenital hypothyroidism influences hepatic gene expression of suppressors of cytokine signaling (SOCS) in adulthood**

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Thyroid hormones (TH) are required for normal postnatal growth development in mammals. The physiological importance of TH becomes evident under the conditions of congenital–neonatal hypothyroidism (CH). If not treated immediately, CH has a profound impact on physiology and can permanently imprint neurological and endocrine systems, which, in turn, led to mental retardation, growth arrest, and metabolic disturbances. A delayed growth development or long-lasting influence of CH on somatotropic-related liver functions could be related with GH resistance. GH resistance has been shown in rat models of sepsis, uremia or in small rats for gestational age. In these models, GH resistance has been associated with increased expression of suppressors of cytokine signalling (SOCS) proteins which contribute to impair GH–JAK–STAT signaling. Here, we studied expression of SOCS genes in liver from rats previously exposed to CH. Pregnant rats were given anti-thyroid drug methimazole (MMI) from Gestational Day 12 until postnatal day (PND) 30 to induce CH in male offspring. Growth defects due to CH were evident as a reduction in body weight and tail length from the second week of life. Once the growth inhibiting condition (MMI) was discontinued on PND30, significant catch-up growth was evident in CH rats. On PND80, significant reduction in body mass, tail length, and circulating IGF1 remained in CH rats. Serum levels of thyroid hormones, cholesterol, and triglycerides showed no significant differences. However, we observed down-regulation of female-predominant (e.g. CYP2C7) together with induction of male-predominant genes (e.g. CYP2C11) which suggested that a male pattern of gene expression was enhanced in CH rat liver. Finally, we observed an impaired somatic growth followed by catch-up growth in rats previously exposed to CH that was associated with increased expression of SOCS (SOCS2 and CIS), a phenomena that requires further research.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P490

**Characterization of a mouse model of paediatric combined hormone deficiency**

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To better understand the molecular and cellular nature of paediatric combined pituitary hormone deficiency diseases, our laboratory has recently generated a novel mouse model of the severe paediatric diseases caused by mutations in the *LHX3* transcription factor gene. Through gene targeting, we have produced an *Lhx3* W227Ter mouse modeling the *LHX3* W224Ter (loss of carboxyl terminus) human disease. Patients with the W224Ter mutation are of short stature and have deficiencies of GH, PRL, TSH, LH, and FSH. The homozygous *Lhx3* W227Ter knock-in mice show symptoms including marked dwarfism, lethargy, reproductive problems, and pituitary hormone deficiencies. To examine the developmental time course of these deficiencies, wild-type and W227Ter knock-in mouse embryos were preserved, cryosectioned and analyzed for pituitary hormone and marker gene expression via immunohistochemistry at embryonic days 13.5, 15.5 and 17.5. Knock-in embryos had a smaller developing anterior pituitary, as well as lower expression levels of ACTH, GH, PRL, TSH, LH, α-GSU, and the pituitary transcription factor PIT1. In addition, structural defects were often noted. To examine the effects of genetic background on the disease, the W227Ter mutation was separately backcrossed six generations into the C57BL/6 and 129/Sv mouse strains. Matings of sixth generation C57BL/6 animals produced viable homozygous knock-in dwarf mice that were able to survive after weaning. By contrast, matings in the 129/Sv background have to date only produced one live knock-in pup, which died before weaning. These differences suggest that genetic background has a significant impact on *LHX3*-associated diseases and that the effects of modifier genes may explain the varied disease outcomes seen in human patients. Supported by NIH HD42024 to SJR and NIH F32HD068113 to KLP.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P491

**DHEA attenuated mature adipocyte proliferation**

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Back ground

Numerous researches indicate that DHEA administration decreases fat mass in human and rodent. We evaluated the effects of DHEA treatment on adiposity. Male Otsuka Long evans fatty (OLETF) rats, hereditary obese type 2 diabetic animals derived from long evans tokushima (LETO) rats. These rats were fed with or without (control) 0.4% DHEA containing food for 52 weeks. Telomere length as a marker of whole cell division in adipose tissue were assessed. Genomic DNA isolated from fat tissue was digested with restriction enzyme, Hinf 1/Rsal, and then Southern analysis was performed to measure telomere length. Treatment with DHEA for 52 weeks in LETO and OLETF decreased fat mass. Furthermore, reduced telomere length in fat tissue isolated from OLETF and LETO rats was restored after treatment with DHEA. These results suggested that accelerated cell division in obese adipose tissue was prevented with DHEA administration.

Aim

We examined whether DHEA administration inhibited mature adipocyte proliferation in this study.

Methods
*In vivo* cell proliferation was estimated with 5′-bromo-2′-deoxyuridine (BrdU), a thymidine analog, uptake in adipose tissue. Male Wistar rats were fed with 0.4% DHEA or 0.005% pioglitazone, a thiazolidinedione, containing food for 4weeks, 200 mg/kg. BrdU was i.p. administered three times before sacrifice, then s.c., epididymal and periintestinal fats were collected. Immunohistochemical study using anti-BrdU antibody revealed that BrdU positive cells were identified both in stromal vascular fraction (SVF) and mature adipocyte.
Results
In visceral fat, BrdU positive cells were detected mainly in mature adipocyte. However they were observed predominantly in SVF area in subcutaneous fat. Treatment with DHEA mainly decreased BrdU positive cells in mature adipocyte. On the other hand, treatment with pioglitazone increased BrdU positive cells in SVF and mature adipocyte.
Conclusion
Considering the fact that DHEA reduce the expression of PPARg in adipocyte as reported previously, these results suggest that DHEA administration inhibited mature adipocyte proliferation, and PPARg may affect on mature adipocyte proliferation.
Declaration of interest
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## P492

**Oestradiol and its biochemical precursor levels decline in men around the age of their late twenties, what may determine the conclusion of bone maturation**

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Introduction
Longitudinal bone growth ceases by the end of puberty, and the induction of epiphyseal maturation and closure is thought to be a result in both sexes of the action of increased pubertal oestrogen concentrations. However, bone mass accrual progresses beyond puberty and we related sex steroid hormones and sex hormone binding globulin (SHBG) levels to calcaneal quantitative ultrasound index (QUI) in young adult men.
Methods
Eighty men aged between 18 and 39 years were invited by letter to attend for an interviewer-administered questionnaire, body mass index (BMI) measurement, blood sample and QUI of the calcaneus (Hologic – SAHARA). Blood was assessed for testosterone, oestradiol ($E_2$), DHEAS, SHBG, LH and FSH. The following bioactive fractions of testosterone and $E_2$ were calculated: free (non-bound to SHBG and albumin) and bio-available (non-bound to SHBG).
Results
While total $E_2$, total testosterone, DHEAS, LH and FSH levels were not related, a positive correlations with QUI ($P<0.05$) were found for the levels of free $E_2$, $\beta=6.30$ (CI: 1.14, 11.47), bio-available $E_2$, $\beta=0.21$ (CI: 0.02, 0.41), free testosterone, $\beta=0.04$ (CI: 0.005, 0.08) and bio-available testosterone, $\beta=1.56$ (CI: 0.84, 3.04). SHBG level was related negatively, $\beta=-0.55$ (CI: $-1.00$, $-0.095$). However, after dichotomisation for age, the associations remained significant only for younger subjects (18–28 years $n=41$). Older subjects (29–39 years, $n=39$) revealed in turn lower ($P<0.01$) serum concentrations of total $E_2$ ($-20.1\%$), free $E_2$ ($-25\%$), bio-available $E_2$ ($-25.7\%$), DHEAS ($-17.7\%$) and bio-available testosterone ($-12.8\%$) than the younger. QUI did not change.
Conclusions
In healthy men the levels of all fractions of $E_2$ and their biochemical precursors DHEAS and bio-available testosterone decrease substantially around the age of late twenties. This may determine the conclusion of bone maturation.
Declaration of interest
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## P493

**The origins and lineages of somatic cells during the formation of the mammalian ovary**

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The origins and lineages of somatic cells in mammalian ovaries are poorly understood and not universally agreed upon. By clonally-isolating different phenotypes of bovine fetal stromal cells and conducting microarray analysis, we identified markers of different somatic cells. Antibodies to these markers, markers of other known cell types in fetal ovaries and to extracellular matrix were used to examine bovine ovaries (60–300 days of gestation, $n=51$) by immunohistochemistry. We show that the gonadal ridge is initially formed by replication of surface epithelium of the mesonephros giving rise to a cluster of cells expressing only some epithelial markers (abbreviated to gonadal ridge epithelial like (GREL) cells). Primordial germ cells migrate into the gonadal ridge and form oogonia and replicate. Stroma from the mesonephros also penetrates into the gonadal ridge forming alternating areas of stroma and irregularly shaped ovigerous cords, composed of oogonia and GREL cells. The stroma brings with it capillaries from the mesonephros. Importantly the stroma is always continuously surrounded by a basal lamina, separating it initially from the ovigerous cords. Stromal cells also migrate to just under the outer layers of GREL cells on the surface and spread laterally, establishing a basal lamina under the cells on the ovarian surface and an epithelial/stromal interface and hence a mature ovarian surface epithelium. Stroma continues to partition the ovigerous cords, still with a basal lamina at the interface, to form follicles containing an oogonium/oocyte and GREL cells that form the granulosa cells. This region is the ovarian cortex. The ovarian medulla below is largely residual mesonephric stroma containing some rete ovarii derived from mesonephric nephrons. In summary we have identified the cell lineages and the cell fate decisions needed for maturation of all the different somatic cells in the adult ovary.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P494

**Childhood maltreatment in subjects with male to female gender identity disorder**

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Introduction
Childhood maltreatment is quite common and constitutes a non-specific risk factor for a range of different emotional and behavioural problems during lifespan. It has been demonstrated that sexual minorities are at higher risk of maltreatment and abuse, and a high proportion of transsexual subjects reports childhood maltreatment.
Aim
To evaluate the prevalence of reported childhood maltreatment in a clinical sample of patients with male to female gender identity disorder (MtF GID), and to explore the relationship between these early life events, body image, and different psychopathological and clinical variables.
Methods
A consecutive series of 162 patients with male genotype was evaluated for gender dysphoria in different dedicated centers from July 2008 to May 2010. One hundred-nine subjects (mean age $36\pm10$ years) meeting the criteria for MtF GID, were considered.
The occurrence of childhood maltreatment experiences was evaluated through a face-to-face clinical interview. Patients were asked to complete the body uneasiness test (BUT, a self-rating scale exploring different areas of body-related psychopathology) and Symptom Checklist-90 Revised (a self-rating scale to measure psychological state).
Results
More than one fourth of patients reported childhood maltreatment. Maltreated subjects reported a higher body dissatisfaction and display a worse mental health lifetime. They also reported a higher BUT depersonalization score.

Conclusions
An history of childhood maltreatment is associated with body image dissatisfaction, worse psychosocial adjustment during childhood and worse lifetime mental health in subjects with gender identity disorder. The presence of reported childhood maltreatment in these patients has relevant psychopathological implications, and therefore should be carefully investigated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P495

### Post prandial responses of insulin and free fatty acids following consumption malaysian vs mediterranean-like meals among healthy subjects

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Introduction
Refined carbohydrates which substitute the staple diet in most cultures may exaggerate postprandial responses in term of insulin and free fatty acid levels. These changes may herald the onset of metabolic syndrome and diabetes among those at high risk of these diseases.
Objectives
To determine the postprandial insulin and free fatty acids levels in healthy subjects after the consumption of two different breakfast meals, Mediterranean-like vs Malaysian meal each with different carbohydrate, glycaemic index and glycaemic load content.
Methods
Twenty subjects were made to take two different meals, Mediterranean-like representing low glycaemic index (GI) and low glycaemic load (GL) diets vs Malaysian meals (high GI and high GL) a week apart. Blood parameters including fasting serum lipid, serum insulin, serum non esterified free fatty acid were taken at 0, 30, 60, 90, and 120 min after each meals. Blood glucose was also taken at baseline and 120 min postprandially.
Results
Twenty subjects were randomized to either Mediterranean-like meal or Malaysian meal first, followed by the other meal a week later. Baseline clinical and demographic parameters were comparable for both meals. With both meals there was an increased in serum triglyceride (Mediterranean-like 0.91 vs Malaysian 1.09; $P=0.565$) with a corresponding drop in HDL and LDL levels throughout the 2-h postprandial period (1.47 vs 1.49; $P=0.844$) and (2.86 vs 2.75; $P=0.647$). There is a significant increase in the serum insulin with Malaysian meal compared to Mediterranean-like meal (16.95 vs 1.99; $P=0.001$). However, the non esterified fatty acid levels were significantly lower in Malaysian meal compared to Mediterranean-like meal (0.18 vs 0.28; $P=0.003$)
Conclusion
Malaysian breakfast meal which is characterised by high GI and GL content, resulted in significant increase in insulin responses with concurrent drop in free fatty acid compared to Mediterranean-like meal.
Keywords: Insulin, serum non esterified free fatty acid (NEFA).
Declaration of interest
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## P496

### Evaluation of cognitive, affective and relational skills of adolescents treated with testosterone for 47XXY klinefelter syndrome

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The early diagnosis of 47XXY- Klinefelter syndrome (KS) associated with infertility confronts the parents with concerns about their child outcome on cognitive, social skills and sexual identity. Few studies relate the weakness of intelligence, mainly verbal, and relational inhibition. Androgen may be offered to adolescents to normalize anatomic and psychic puberty processes. This has never been evaluated in psychological terms.
Subjects and methods
Eight adolescents aged from 11 to 17 with 47XXY-KS were recruited. The protocol included a semi-directive interview, a Wechsler intelligence scale and projective tests (Rorschach and TAT) offered before (t1) and after 12 months of testosterone treatment (t2).
Results
Interviews underline four themes in the narratives: i) insufficient knowledge of the disorder and medical history ii) problems in socio-educational integration iii) inhibition of psychic conflicts and relational withdrawal iv) shame and secrecy regarding the diagnosis and infertility inside the family. The small cohort only allows for descriptive cognitive results. At t1, the global cognitive abilities are clearly below the standard (average IQ $=75$) with a significant dispersion between verbal and logical/practical skills. At t2, a slight improvement in the global level is observed (average IQ $=79$) with an important homogenization of the scores. Besides, projective tests revealed inhibition in affective life underlined in representations of human relationship (phobic avoidance) and expression of sexual identity. At t2 improvement of relational skills is observed.
Conclusion
Inhibition of cognitive, affective and relational skills is understood as a specific way of expression of the processes of adolescence. The benefits of this research-action are observed at the cognitive and relational levels. More than normalization, it offers to these adolescents the opportunity of a psychic work. This allows the appropriation of their medical history which supports the development of psychic processes of adolescence.
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Declaration of interest
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## P497

### The development of endocrinologic dysfunction after allogeneic HSCT in children.

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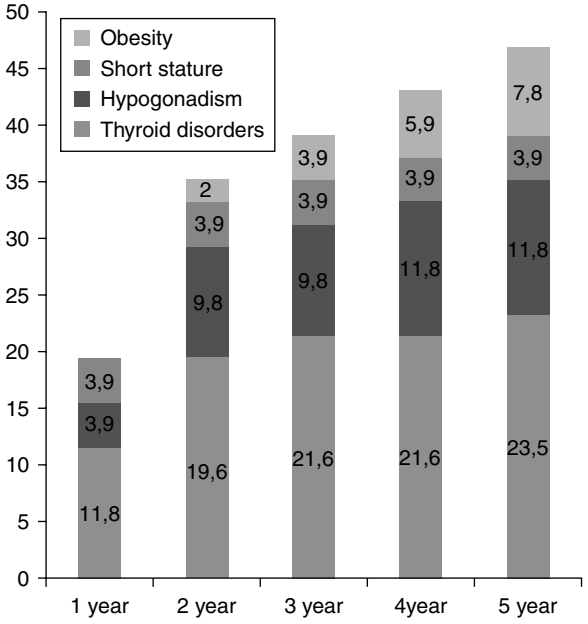
Introduction
Endocrinologic dysfunction is a well-known complication after hematopoietic stem cell transplantation (HSCT) in children. The mechanisms of thyroid damage developing after transplantation remain not completely recognized. Toxicity of the conditioning regimen, especially total body irradiation, is most commonly postulated cause of endocrine abnormalities; immunological mechanisms (e.g. graft vs host disease) may also contribute to thyroid dysfunction.
Patients and methods
We prospectively evaluated the function of thyroid, gonads, pituitary and adrenals in 51 children, aged 1–18 years, who survived more than one year after transplantation. The reasons for bone marrow transplantation were: severe aplastic anemia (SAA), acute lymphoblastic anemia, inborn errors, acute myeloblastic leukemia and others. The conditioning regimen included chemotherapy in 27 children and TBI in eight patients.
In the Endocrinological Dept. the high velocity and sexual maturation of the children were observed. Every year we evaluated TSH, $fT_4$, anti-tyreoglobuline antibodies (a-TG) and anti-peroxidase antibodies (a-TPO), FSH, LH, prolactin, estradiol and testosterone, GH, glycemia, lipidogram, insulin and glucose levels and circadian rhythm of cortisole secretion.
Results
In eight children, autoimmune hypothyroidism was diagnosed; the children needed L-thyroxin replacement therapy. In three patients this feature was transient, while four required thyroxin supplementation. One patient was diagnosed as having Graves' disease. Transient delay of growth velocity was observed in all the children in the first year after HSCT, but only in 2 boys they were permanent; however the growth hormone secretion in sleep was normal. In six children hypogonadism was developed – in four hypergonadotropic hypogonadism and in two hypogonadotropic hypogonadism. Three children were treated for obesity without adrenal and pancreatic disorders.
Conclusions
1. Abnormalities of autoimmunological thyroid function are a most frequent complication in children after HSCT.
2. The frequent complication is hyper- and hypogodotropic gonad disorders.
3. The children after HSCT need permanent observation in the Endocrinological Outpatient Clinic.
The percentage of endocrine disorders after allogeneic HSCT.




Declaration of interest
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## P498

**46 XX Male syndrome: is there any relationship with dysembryoplastic neuroepithelioma?**

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Introduction
De la Chapelle syndrome (46XX male syndrome) is a rare anomaly with the characterstics of discordant chromosomal and gonadal sex. Individuals with classical 46XX male sex reversal syndrome have an apparently normal male phenotype and presents with infertility or sub-fertility.
Case report
Twenty five year old male, married 2 years back, came to take opinion regarding his Infertility. He is a healthy looking male with well developed secondary sexual characteristics and normal external male genital phenotype with a normal sized penis and both testis in scrotum and are of normal consistency and volume. Testicular biopsy shows no active spermatogenesis. He was operated at the age of 14 years for an intracranial mass, the histological analysis shows dysembryoplastic neuroepithelioma, a rare benign neoplasm, cytogenetic analysis of which shows mosiac cell line. Sex karyotype in the mosiac and normal cell line was 46XX.
Conclusion
It is a rare disorder of sexual differentiation where the testes and male genitalia develop in the absence of Y chromosome and possibly without the SRY gene. Usually it is caused by unequal crossing over between X and Y chromosomes during meiosis. Is this condition is associated with increased incidence of neoplasm is still a question, needed to be answered. Klinefelter's syndrome, another similar condition causing infertility, is known to have increased incidence of neoplasms.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Diabetes

## P499

**Selective suppression of slow wave sleep does not affect insulin sensitivity in patients with type 1 diabetes**

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Background
Human sleep is composed of different sleep stages, of which slow wave sleep (SWS) is an important modulator of glucose regulation. Curtailment of sleep duration in general and of slow wave sleep (SWS) in particular reduces glucose tolerance in healthy subjects. Previously, we have reported that a single night of partial sleep deprivation reduces insulin sensitivity by 15–20% in patients with type 1 diabetes. We hypothesized that this effect could be a consequence of reduced SWS.
Objective
To determine the effect of selective suppression of SWS on insulin sensitivity in patients with type 1 diabetes.
Methods
We studied insulin sensitivity in seven patients with type 1 diabetes on continuous s.c. insulin infusion therapy without any known sleep disorders after a normal night of sleep and after a night of suppressed SWS. Objective sleep parameters were determined by polysomnography. SWS was suppressed by delivering acoustic tones of varying frequency, to replace SWS with shallow sleep without awakening the patients. The following morning, glucose metabolism was studied by hyperinsulinemic euglycemic clamp studies with infusion of (6.6-2H2) glucose.
Results
The amount of SWS was suppressed by 75% ($P<0.001$) without a reduction in total sleep duration (471 vs 480 min, $P=0.63$). SWS suppression did not affect endogenous glucose production in the basal state nor during clamp conditions compared to normal sleep. During clamp conditions, SWS suppression did not result in differences in glucose infusion rates ($22.6\pm3.1$vs $22.3\pm3.2$ μmol/kg LBM-1 per min, $P=0.95$) or glucose disposal rate ($31.6\pm2.9$ vs $30.0\pm2.7$ μmol/kg LBM-1 per min, $P=0.65$). SWS suppression did not affect plasma free fatty acid levels.
Conclusions
Selective suppression of SWS sleep during a single night does not influence insulin sensitivity in patients with type 1 diabetes. Therefore, the reduction in insulin sensitivity by partial sleep deprivation can not be explained by a reduced amount of SWS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P500

**Evidence that abscissic acid participates in the response to hyperglycemia in humans**

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Abscissic acid (ABA) is a hormone best known for its role in plants. We previously showed that it is released by pancreatic beta-cells upon glucose stimulation, and autocrinally enhances insulin secretion.
Here we additionally report that ABA promotes glucose uptake by rat L6 myoblasts and murine 3T3-L1 cells differentiated to adipocytes, at least partly by inducing GLUT-4 translocation to the plasma membrane.
To determine whether ABA is involved in the response to hyperglycemia *in vivo*, we assessed its plasma concentrations (ABAp) in eight healthy volunteers and eight type 2 diabetes mellitus (T2DM) patients during an oral glucose tolerance test (OGTT). In healthy subjects, ABAp increased two to ninefold over basal values, starting 15–60 min after glucose administration. There was a significant positive correlation between ABA and glucose areas under the curve (AUC) ($r$ 0.905, $P<0.01$). In contrast, ABAp did not increase during OGTT in T2DM patients. An intravenous GTT (IVGTT) was also performed in six of the eight volunteers. Only two showed an increase in ABAp similar to that observed during

OGTT. Overall, median and range ABAp AUC were lower during IVGTT than OGTT.
In rat insulinoma cells INS-1 and human pancreatic islets, GLP-1 potentiated ABA secretion by ~10 and 2 times in low- and high-glucose respectively ($P<0.01$), an effect abrogated by silencing of the GLP-1 receptor.
Beta-cells are likely the major source of circulating ABA, since fasting ABAp was significantly lower in seven type 1 DM patients ($0.17\pm0.10$ nM) than in 25 healthy subjects ($1.51\pm1.29$ nM, $P<0.01$) and in 21 T2DM patients ($1.45\pm1.30$ nM, $P<0.01$). However, human adipose tissue also released ABA when incubated with glucose.
In conclusion, our results suggest that ABA is a new hormone involved in glucose metabolism and particularly in the incretin response to oral glucose in humans.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P501

**Mitochondrial biogenesis is not significantly impaired in non obese asian indians with type 2 diabetes**

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Introduction
Deranged mitochondrial biogenesis has been linked to obesity with or without type 2 diabetes but this association has not been reported in non obese type 2 diabetes subjects. This study attempts to identify the various alterations of mitochondrial biogenesis in adipose tissue in obese and non obese type 2 diabetes subjects.
Methods & Design
Since type 2 diabetes is associated with elevated serum leptin and depressed serum adiponectin levels, these parameters were measured in four different groups of subjects- non obese non diabetic (C), non diabetic obese (OB), non obese type 2 diabetes (NOT2D) and obese type 2 diabetes (T2D+OB). Mitochondrial biogenesis was assessed in subcutaneous adipose tissue by estimating the content of several mitochondrial proteins e.g. cytochrome c, ND4L (subunit of complex I), COX 2 (subunit of complex IV etc.) and transcription factors in whole adipose tissue lysate and isolated mitochondria. A cell based model has been designed to examine the role of mitochondria in adipokine secretion and synthesis.
Results
There was a linear increase in serum leptin levels and decrease in serum adiponectin levels from OB to T2D+OB but remained unchanged in NOT2D compared to C. Mitochondrial biogenesis was significantly impaired in adipose tissue of OB and more remarkably in T2D+OB but remains unaffected in NOT2D. This aspect is being investigated in our cell based model.
Conclusion
High serum leptin levels and hypoadiponectinemia were not observed in NOT2D patients. This may account for the relative ineffectiveness of insulin sensitizers in NOT2D subjects as reported previously. Similarly, the absence of impaired mitochondrial biogenesis in NOT2D is an important finding which indicates the critical importance of obesity in mitochondrial pathology of type 2 diabetes. Other pathogenic aspects need to be explored in NOT2D subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P502

**Iron overload induces visceral adipose tissue insulin resistance**

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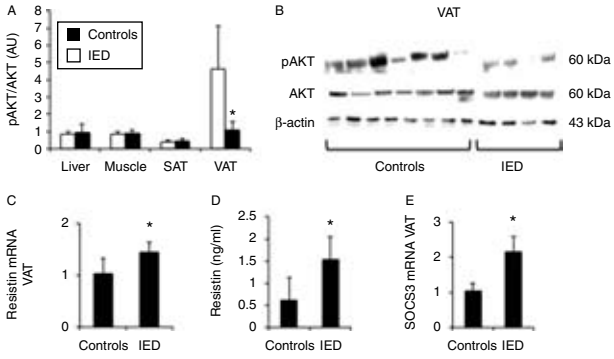
Excess body iron is associated with the metabolic syndrome, but whether it has a causal role in the pathophysiology of insulin resistance (IR) is not well understood. Aim of this study was to assess (a) the effect of dietary modulation of iron status on IR in Wild-type or ob/ob C57Bl/6 male mice fed a standard iron concentration (8 mg/kg) or an iron enriched diet (30 g/kg; IED), as well as in high-fructose diet (HFD) mice and (b) to investigate the mechanisms whereby. IED determined a progressive increase in glucose levels due to IR, as confirmed by the insulin tolerance test, associated with a reduction in visceral adipose tissue (VAT) mass (perigonadal fat pad: −60%, $P=0.02$), whereas in ob/ob mice IED led to overt diabetes. Moreover, in HFD fed mice increased IR by about 100%, was associated with decreased VAT despite no changes in total body mass, thus suggesting VAT-IR. IED induced iron accumulation in VAT, which was associated with IR, as shown by decreased phospho-AKT/AKT ratio (−80%, $P=0.03$). Gene expression analysis of VAT showed that IED upregulated iron-responsive genes (ferritin and hepcidin) and adipokines associated with IR (resistin and visfatin, both $P=0.005$), whereas downregulated inflammation. This resulted in hyper-resistinemia ($P=0.01$) and in increased levels of SOCS3 ($p<0.05$), a target of resistin and hepcidin implicated in adipose tissue IR. Furthermore, iron treatment induced hepcidin and inhibited the differentiation of human mesenchymal stem cells towards adipocytes. In conclusion, iron overload may induce IR and hyperglycemia by affecting VAT iron metabolism, endocrine function, and differentiation.




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P503

**Comprehensive evaluation of type 2 diabetes susceptibility loci in the Japanese population by using 1000 Genomes Project data**

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Although over 50 type 2 diabetes (T2D) loci have been identified through genome-wide association studies (GWAS), the vast majority of the genetic predisposition to T2D still remains to be clarified. One explanation is that despite the use of high-throughput genotyping arrays, only a small proportion of genetic variants in the human genome are actually surveyed, especially in non-European populations. We explored the comprehensive catalog of genomic variations provided by the 1000 Genomes Project to identify variations conferring susceptibility to T2D in the Japanese population that were not detected in previous scans. We imputed 10,524,368 variants derived from 194 East Asian subjects (June 2011 release) into 5,976 cases and 20,829 controls genotyped by 610K single-nucleotide polymorphism (SNP) array. Overall concordance was good, although imputed SNPs with minor allele frequency (MAF) below 1% apparently contained poorly imputed SNPs. We then tested associations for T2D before and after adjusting for age, sex, and body mass index. We found that in addition to variants of the previously reported loci there were 16 loci harboring multiple variants with a p value lower than 10–5 at 1q32, 5p13, 6p12, 7p12, 7q32, 8p11, 9q31, 9q34, 10p13, 10q23, 11q13, 11q24, 12p11, 15q26, and 17p13. The MAF range was 0.01 to 0.45, and the odds ratios were between 1.10 and 1.48. We are conducting a replication study to confirm the association in another 7,000 cases and 3,500 controls. We also sought to define the most relevant SNPs for susceptibility to T2D in the previously identified genes. We did not find any stronger association with T2D than the originally reported SNPs in our population. However, there is a tendency that lower frequency variants were enriched in those with large effect size. Our study highlights the benefit of using

data derived from next-generation sequencing of the human genome such as the 1000 Genomes Project to explore T2D loci more comprehensively.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P504

**Glycoalbumin is the best indicator for glycemic variability assessed by continuous glucose monitoring**

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Aims
We investigated the interrelationship between HbA1c, fructosamine, glycoalbumin (GA), 1,5-anhydroglucitol (1,5-AG) with glycemic variability data from continuous glucose monitoring system (CGMS).
Methods
Seventy diabetic patients (mean age of 54 years including seven type 1 diabetes mellitus subjects) were enrolled, and all wore a CGMS for 72 hours. For the parameters of glucose excursion, mean glucose, standard deviation (SD) of glucose, the area under the curve for glucose levels >180 mg/dl (AUC-180), and continuous overlapping net glycemic action calculated (CONGA), mean of the daily differences (MODD) and mean post meal maximum glucose (MPMG) were calculated from the CGMS data.
Results
Spearman's correlation coefficients were significant between all glycemic markers and various parameters of glucose excursion. Among them, GA displayed highest correlation ($r$ between 0.581–0.684) with all the parameters (i.e. AUC-180, SD, MAGE, MODD, CONGA-1, CONGA-24, MPMG) except for mean glucose. Mean glucose was best correlated with fructosamine ($r=0.676$, $P< 0.01$). In patients with HbA1c $< 7.5\%$ ($n=36$), 1,5-AG and GA showed better correlation with glycemic parameters for variability than HbA1c and fructosamine. In patients with HbA1c $\geqq 7.5\%$ ($n=34$), GA showed a statistically significant correlation with just AUC-180 and MODD among glycemic variability parameters. Furthermore, GA was significantly correlated to only SD among 3 independent parameters (mean glucose, SD, AUC-180) in multiple regression analysis but the other glycemic markers were significantly correlated with mean glucose.
Conclusion
Our results suggest that GA was the most representative maker for glycemic variability. 1–5 AG was also able to reflect glucose excursions only in well-controlled patients.
Spearman's correlation coefficient between glycemic markers and CGMS data
Table 1

**Table 1** Spearman's correlation coefficient between glycemic markers and CGMS data

| N=70 | HbAlc | Fructosamine | Glycoalbumin | 1,5-AG |
|---|---|---|---|---|
| Mean glucose | 0.600** | 0.676** | 0.636** | −0.493** |
| AUC-180 | 0.608** | 0.628** | 0.630** | −0.550** |
| SD | 0.549** | 0.567** | 0.646** | −0.559** |
| MAGE | 0.505** | 0.526** | 0.608** | −0.540** |
| MODD | 0.525** | 0.594** | 0.684** | −0.501** |
| CONGA-1 | 0.463** | 0.570** | 0.581** | −0.455** |
| CONGA-24 | 0.451** | 0.553** | 0.583** | −0.473** |
| MPMG | 0.540** | 0.602** | 0.656** | −0.565** |

**$P<0.01$, *$P<0.05$ (two-tailed).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P505

**Nonalcoholic fatty liver disease is associated with atherosclerosis in middle-aged and elderly chinese**

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Objective: To evaluate the associations between nonalcoholic fatty liver disease (NAFLD) and atherosclerosis.
Methods and Results: A total of 8971 participants aged 40 years and older from Baoshan district, Shanghai were included in the present study. The presence of NAFLD was evaluated by ultrasonography. Carotid intima-media thickness (CIMT) and brachial-ankle pulse wave velocity (ba-PWV) were measured in each participant. The prevalence of NAFLD was 30.0% in the total population, with that of 30.3% in men and 29.8% in women, respectively. Subjects with NAFLD had remarkably higher CIMT and ba-PWV as compared with those without NAFLD ($0.594\pm0.105$ mm vs. $0.578\pm0.109$ mm, $P<0.0001$; $1665\pm425$ cm/sec vs. $1558\pm430$, $P<0.0001$). Subjects with both NAFLD and metabolic syndrome had significantly higher CIMT and ba-PWV as compared with those with neither of or either of these two diseases after adjustment for age and sex ($P$ all $<0.05$). Logistic regressions also revealed that NAFLD conferred 29% and 20% increased odds ratios of elevated CIMT and ba-PWV independent of conventional risk factors and the presence of metabolic syndrome.
Conclusions: NAFLD was associated with elevated CIMT and ba-PWV independent of conventional CVD risk factors and metabolic syndrome. The effects of NAFLD and metabolic syndrome on atherosclerosis might not fully overlap.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P506

**Altered Mitochondrial Bioenergetics Does Not Explain Increased Ros Production In Non-Obese Asian Indians With Type 2 Diabetes (T2D)**

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Introduction
Deranged Mitochondrial bioenergetics is a key feature of type 2 diabetes and obesity but its status in non-obese type 2 diabetes patients is not known. The aim of the present study was to assess the status of mitochondrial oxidative phosphorylation and ROS metabolism in subcutaneous adipose tissue isolated from 4 groups of subjects: non-obese subjects with type 2 diabetes (NOT2D), non-obese non diabetic (C), obese non-diabetic (OB) and obese type 2 diabetes subjects (OB + T2D).
Methods & Design
Mitochondria were isolated from subcutaneous white adipose tissue of four groups of subjects under study: C, NOT2D, OB, and OB + T2D. The activities of respiratory complexes, the trans-membrane potential and the phosphate utilization capacity of adipose tissue mitochondria from these groups were measured. The rate of ROS (reactive oxygen species) formation, the levels of protein and lipid oxidation markers and the activities of SOD and catalase were also measured.
Results
There was a progressive decrease in mitochondrial transmembrane potential, phosphorylation capacity and the activities of respiratory chain complexes from OB to OB + T2D, whereas such parameters remained unaffected in NOT2D, compared to C. However, markers of oxidative stress and damage increased in adipose tissue mitochondria in a progressive manner from NOT2D to OB + T2D.
Conclusion
Mitochondrial bioenergetics was unaffected in NOT2D subjects, whereas a linear increase in ROS production and metabolism were observed from NOT2D to OB + T2D. Therefore, alteration in mitochondrial bioenergetics cannot fully explain the ROS production in all three groups. This raises the possibility of existence of some extra-mitochondrial pathway accounting for increased ROS production in the affected subjects. Our results may also possibly be explained by altered anti-oxidant enzyme status in the patient population under study.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P507

### QRFP43 and its fragment 26RFa both promote β-cell survival but differently regulate insulin secretion and glucose uptake in pancreatic β-cells and human pancreatic islets

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RFamides are a family of peptides containing arginine-phenylalanine-amide at their C terminus. A novel 43-amino acid RFamide, named QRFP43, and a shorter endogenous peptide 26RFa, were discovered and identified as ligands of the G protein-coupled receptor GPR103. Different studies have shown that RFamides, such as neuropeptide FF and QRFP43 and 26RFa, play a role in food intake, thermogenesis and energy homeostasis. Here, we investigated the effects of QRFP peptides on survival, proliferation, apoptosis, insulin secretion and glucose uptake of INS-1E pancreatic β-cells and human pancreatic islets. The signaling pathways involved in these actions were also determined. GPR103 expression was evaluated by immunofluorescence and RT-PCR; cell survival by MTT and proliferation by BrdU incorporation; apoptosis was assessed by Hoechst 33258 nuclear staining and caspase-3 activity; insulin secretion by RIA; activation of signaling pathways by Western blot analysis; intracellular cAMP production by ELISA; glucose uptake by [3H]2-deoxyglucose incorporation. The results showed that both INS-1E β-cells and human pancreatic islets express GPR103. Moreover, either QRFP43 or 26RFa promoted survival and proliferation and reduced apoptosis, in both serum-free conditions and under treatment with the cytokines TNF-α, IFN-γ and IL-1β. QRFP43 also stimulated both basal and glucose-induced insulin secretion in β-cells and human islets, enhanced glucose uptake, increased intracellular cAMP levels and ERK1/2 and PI3K/Akt phosphorylation. Conversely, 26RFa reduced insulin secretion and cAMP levels, not exhibited effects on glucose uptake and only increased ERK1/2 phosphorylation. The opposite metabolic effects of QRFP43 and 26RFa involved activation of the G proteins Gαs and Gαi respectively, as demonstrated by the use of specific inhibitors. In all, these findings indicate that both QRFP43 and 26RFa promote β-cell survival; however, they display opposite effects on β-cell function, suggesting a novel and differential role in glucose metabolism.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P508

### Influences of the 2011 Tohoku Earthquake on glycemic control in patients with diabetes mellitus in Tokyo

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Objectives
Huge 9.0 magnitude quake and tsunami attacked eastern areas of Japan, on March 11th 2011. They claimed more than 20,000 lives and destroyed the nuclear power plants. Although the property damage was very little in Tokyo, about 250 miles apart from the epicenter, the people in capital faced a lot of hard situations. For example, unusual food supply, the lack of the daily necessities, the limitation of the electricity supply, and a fear of hundreds of aftershocks and radioactive contamination. Therefore, we evaluated the influence of this disaster on the patients with diabetes in Tokyo.
Methods
We evaluated the changes of glycemic control of 99 diabetic out-patients in our hospital from the results of blood examination before and after the earthquake (PreE / PostE). Furthermore, self-administered questionnaires were applied to assess their changes of the life style, especially about the medical nutrition therapy, the physical activities, and the mental state.
Results
The mean HbA1c levels at one month PostE in 99 patients were significantly higher than those of PreE. However, HbA1c levels at 4 months PostE decreased into the PreE levels. There was further significant decrease at 6 months PostE. Next we divided these 99 patients into two groups. In one group of patients whose HbA1c apparently increased one month PostE, the increase of the food intakes and the decrease of the physical activities were significant. In this group, a fear of the aftershocks and the radioactive contamination must be the main reason for the decrease of the physical activities.
Conclusion
These results show us that the self-management of diabetic patients can be highly disturbed in case of such serious disasters even if they lives apart from disaster areas.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P509

### The impact of pioglitazone administration on circulating endothelial progenitor cells, inflammation and endothelial function of diabetic patients with and without coronary artery disease

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Introduction
Glitazones' admisitration has been proved to affect EPCs in peripheral circulation in endothelial function and inflammatory process. The purpose of this study was to further investigate if the administration of pioglitazone in diabetics can modify the number of EPCs in the peripheral blood and alter the inflammatory state and endothelial function of these patients.
Methods
Twenty five diabetic patients were recruited and pioglitazone was administered to all patients for a one-month period. In parallel 10 non-diabetic control subjects were treated with placebo. Blood samples were drawn on admission and after one month of drug's administration in order to count EPCs and inflammation markers such as CRP, vascular endothelial growth factor (VEGF) and asymmetric dimethylarginine (ADMA) were also measured. Circulating EPCs were defined by the surface markers CD34+ (CD34 expressing cells) and analyzed by flow-cytometry. Moreover the endothelial function was evaluated using the technique of flow mediated dilation (FMD).
Results
In the group of diabetic patients, no significant difference was observed in the number of endothelial progenitor cells before and after the administration of pioglitazone ($3.22 \pm 1.96$ vs $3.55 \pm 1.88$, $P$=NS), either in plasma concentrations of CRP ($2.998 \pm 3.35$ vs $1.962 \pm 1.57$, $P$=NS), ADMA ($0.78 \pm 0.51$ vs $0.67 \pm 0.51$, $P$=NS) or in endothelial function ($0.362 \pm 0.075$ vs $0.38 \pm 0.074$, $P$=NS). The plasma concentrations of VEGF in the group of diabetics showed a significant increase after the administration of pioglitazone ($128.14 \pm 144.85$ vs $177.91 \pm 126.26$, $P<0.05$). The number of circulating EPCs was compared between the groups of diabetic patients and control patients there was a significant difference ($P<0.01$).
Conclusions
Based on the existing data, there is no significant difference in the number of circulating EPCs, CRP and ADMA plasma concentrations and endothelial function before and after the administration of pioglitazone. Nevertheless, the administration of pioglitazone seems to increase plasma levels of VEGF playing a possible role in the stimulation of angiogenesis in diabetes.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P510

### The redution hypothalamic PP2A expression improve peripheral insulin sensitivity

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Protein phosphatase 2A (PP2A) is a multimeric serine/threonine phosphatase which has multiple functions. However, the potential involvement of PP2A in insulin's metabolic signaling pathway is presently unknown. Brain insulin resistant state is characterized by disturbances in transducing the signal from IR/IRS/AKT, changing feeding behaviour and body weight. We showed that diet induced obese rats (DIO) have a marked increase in PP2A protein expression in the hypothalamus. Thus, we generated a selective, transient reduction in PP2A by infusion of an antisense oligonucleotide designed to blunt the expression of PP2A

in rat hypothalamic areas surrounding the third ventricle in control and obese rats. The selective decrease in hypothalamic PP2A resulted in decreased food intake, reduced body weight, reduced adiposity after high-fat feeding, improved insulin action and signaling in hypothalamus. Central insulin signaling was increased by phosphorylation of AKT in PP2A ASO treated rats. To assess the impact of hypothalamic PP2A down-regulation on the peripheral action of insulin, we performed hyperinsulinemic-euglycemic clamp studies. The insulin action on peripheral glucose uptake was increased. In conclusion, we have demonstrated an important role of hypothalamic PP2A in the modulation of energy balance, insulin action, and glucose metabolism. Thus, the reduction in hypothalamic PP2A should be sufficient to promote an appreciable weight reduction and access to the brain may also be necessary to optimally improve insulin sensitivity and glucose homeostasis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P511

### The new combination of risk factors determining a high risk of gestational diabetes mellitus

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Introduction

Gestational diabetes mellitus (GDM) is a common complication of pregnancy. Identification of early determinants of GDM is necesary to designate possible preventive strategies. The aim of this study was to reveal the most significant risk factors for GDM.

Design

A total of 209 women with singleton pregnancies were included in the study. Maternal fasting glucose, anthropometric parameters and blood pressure were measured in the first trimester of pregnancy. Pregnant women were screened for GDM between weeks 24 and 28 of gestation, as defined by WHO criteria. Classification Tree Method was used to identify combination of early pregnancy risk factors that predict the highest risk of the development of GDM in later pregnancy.

Results

An age ≧ 35 years in combination with a fasting glucose level ≧ 4.8 mmol/L and a body mass index (BMI) ≧ 28.5 kg/m$^2$ in the first trimester was associated with 89% risk of two-hour glucose ≧7.8 mmol/L following the 75-g oral glucose tolerance test (odds ratio [OR] 15.7, 95% CI 1.8 - 130.6, $P=0.002$). In women aged less that 35 with the combination of a first trimester BMI 28.5 kg/m$^2$ with a level of fasting glycemia 4.8 mmol/L and hypertension (blood pressure ≧140 and/or ≧90 mmHg or use of antihypertensive medications) the absolute risk of the development of GDM was 75% and OR was 6.3, 95% CI 1.9- 20.6, $P=0.001$.

Conclusions

Advanced maternal age, higher BMI, higher fasting glycemia and hypertension in the first trimester predict increased GDM risk. Taking these combinations into consideration may facilitate the identification of women at particular risk for GDM and suggest potential strategies for reducing this risk.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P512

### Glycemic control influences CA19-9, CEA and ferritin levels in type 2 diabetes.

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Objective

Diabetes mellitus is associated with various types of cancer. Exocrine pancreas function is also influenced by diabetes.

Materials/Subjects and Methods

In this study we aimed to investigate the effect of glycemic control on tumor and inflammatory markers. Sixty eight type 2 diabetic patients, and 70 healthy controls comprised the study population. We measured fasting blood glucose (FBG), postprandial blood glucose (PPBG), serum total cholesterol (TC), triglyceride (TG), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), γ-glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate amino-transferase (AST), creatinine, akaline phosphatase (ALP), amylase, lipase, high sensitive C-reactive protein (hsCRP), ferritin, carcinoembryonic antigen (CEA), CA19-9, HbA1c concentrations with an automatic analyzer in diabetic and control subjects.

Results

Diabetic group had significantly higher body mass index (BMI), FBG, PPBG, HbA1c, VLDL, TG, ALT, GGT, ALP, CA19-9, CEA, ferritin, hsCRP and erythrocyte sedimentation rate (ESR) values. AST, HDL-C, LDL-C, amylase and lipase levels were significantly lower in the diabetic group. HbA1c showed significant correlation with ferritin (r: .335, $P$: < .0001), CA19-9 (r: .415, $P$: < .0001), CEA (r: .485, $P$: < .0001), ESR (r: .252, $P$: .003), LDH (r: .369, $P$: < .0001) and amylase (r: -.309, $P$: < .0001) levels. FBG was correlated with both CA19-9 (r: .386, $P$: < .0001) and CEA (r: .465, $P$: < .0001). PPBG was also correlated with both CA19-9 (r: .394, $P$: < .0001) and CEA (r: .560, $P$: < .0001).

Conclusions

We propose to consider the effect of hyperglycemia on CA19-9, CEA, amylase, lipase, ESR and ferritin measurements.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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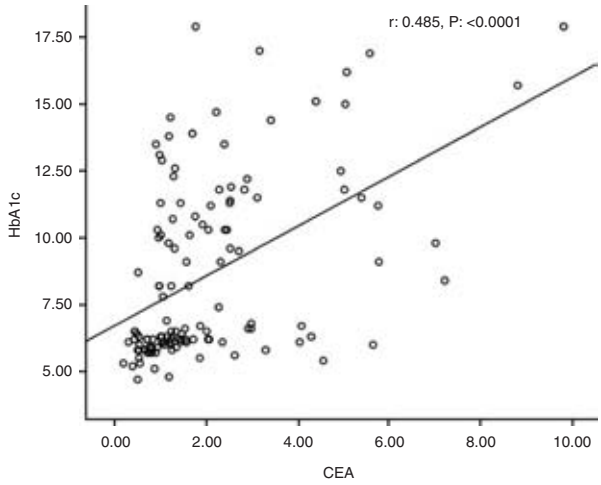


**Figure 1.** Correlation between HbA1c and CEA.

## P513

### Effects of perindopril on circulating endothelial progenitor cells, inflammation and endothelial function of diabetic patients with and without coronary artery disease

A. Kampoli[1], D. Tousoulis[1], G. Paterakis[3], Z. Pallantza[2], N. Papageorgiou[1], A. Miliou[1] & C. Stefanadis[1]

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Introduction

Endothelial progenitor cells (EPCs) seems to be reduced in patients with diabetes mellitus, however it is unknown whether treatment with ACE-I may modify the number of EPCs. Therefore, the purpose of this study was to investigate if the administration of perindopril in diabetic patients with and without coronary artery disease can modify the number of circulating EPCs and alter their inflammatory state and endothelial function.

Methods

Twenty five diabetic patients were recruited and perindopril was administered to all patients for a one-month period. In parallel, ten non-diabetic control subjects

were treated with placebo. In both groups blood sample were drawn on admission (baseline) and after one month of drug's administration. CRP, vascular endothelial growth factor (VEGF) and asymmetric dimethylarginine (ADMA) were measured by standard techniques. Circulating EPCs were defined by the surface markers CD34+ (CD34 expressing cells) and analyzed by flow-cytometry. Moreover the endothelial function was evaluated both on admission and after treatment using the technique of flow mediated dilation (FMD).
Results
The number of circulating EPCs was compared between the groups of diabetic patients and control patients there was no significant difference in their variation ($P>0.05$). In the group of diabetic patients, no significant difference was observed in the number of endothelial progenitor cells before and after the administration of perindopril ($3.72\pm1.98$ vs $4.02\pm2.03$, $P=$NS), either in plasma concentrations of CRP ($2.472\pm2.74$ vs $1.782\pm1.99$, $P=$NS), VEGF ($126.36\pm99.79$ vs $163.19\pm121.52$, $P=$NS) or in endothelial function ($0.368\pm0.073$ vs $0.388\pm0.073$, $P=$NS). Nevertheless, the plasma concentration of ADMA in the group of diabetics showed a significant increase after the administration of perindopril ($1.473\pm0.899$ vs $0.939\pm0.883$, $P<0.05$).
Conclusions
Administration of perindopril seems to increase plasma levels of ADMA. However, no significant difference in the number of circulating endothelial cells, CRP and VEGF plasma concentrations and endothelial function before and after the administration of perindopril was observed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P514

**Glycemic control in type 1 diabetic patients experience of a university hospital in tunis**

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Recent years have been marked by numerous advances in the quality of type 1 diabetes care. However, glycemic control remains suboptimal for many patients even in developed countries.
The aim of this study was to assess glycemic control in a cohort of Tunisian type 1 diabetic patients and to identify factors associated with poor glycemic control.
Methods
One hundred and eighty-eight type 1 diabetic patients admitted to the Endocrinology - Diabetology department of the university hospital "La Rabta" then followed up in the consultation for at least one year, were studied in a retrospective manner during five years.
Results
Mean age of patients was $28\pm12.3$ years (102 males and 86 females). One hundred and thirty two patients (70.2%) had newly diagnosed diabetes and 56 (29.8%) had previously diagnosed diabetes with a mean duration of $7\pm6.3$ years. Mean HbA1c during the follow-up period was $9.7\pm3\%$. It was less than 7% in 16.8% of cases. There was a reverse correlation between age at diabetes onset and HbA1c value ($P=0.02$). Adolescents had higher HbA1c value than adults ($10.8\pm2.9\%$ vs $9.2\pm2.8\%$, $P=0.02$). No relationship was found between number of daily insulin injections and mean HbA1c value. Mean HbA1c was higher in patients who did not adhere to their insulin treatment ($11.1\pm3.3\%$ vs $8.9\pm2.4\%$, $P<0.0001$), in those with less than 3 clinic visits per year ($10.7\pm3.5\%$ vs $9.0\pm2.2\%$, $P=0.001$), in patients with lipohypertrophy ($10.9\pm2.5\%$ vs $9.2\pm3.4\%$, $P=0.008$) and those with known celiac disease ($14.5\pm5.2\%$ vs $9.6\pm2.9\%$, $P=0.005$).
Conclusion
The overall glycemic control of our type 1 diabetic patients is poor. Several factors are associated with this. We have to target these factors, in particular by intensifying education strategies, to improve the prognosis of these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P515

**Progression to impaired fasting glucose, impaired glucose tolerance and diabetes in urban population during 8 years follow up**

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Objective
To determine the progression rate to impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes (DM2) in normal glucose tolerant (NGT) people during 8 years follow up using WHO 1999 criteria and new criteria (IFG-fasting glucose 5.6–6.9 mmol/l).
Research design and methods
This is an eight year prospective study in a randomly selected urban population aged $>=40$ years living in Krakow, Poland. 1752 persons had NGT.based on WHO 1999 criteria, 564 of them (32.2% of invited, (209 men and 355 women, aged 60.7, S.D. $=9.2$) attended the follow-up assessment. Subjects underwent a physical examination including weight/height, waist circumference, biochemical examination including glucose, insulin in 0', 120' OGTT and questionnaire examination concerning CVD health history and family history of type 2 diabetes.
Results
The prevalence of DM2, IFG and IGT according to WHO 1999 criteria in examined population with baseline NGT was 4.43%, 3.37% and 9.93% respectively. The prevalence of IFG using new criteria, was 13.48%. Lowering cutoff point for IFG caused 10.11% increase in the prevalence of IFG.
Among people with diagnosed diabetes, 56% had newly diagnosed diabetes during the control study, in 44% participants diabetes was diagnosed in the period between the baseline and control study.
The prevalence of DM2 and IGT/IFG was increasing with increasing age and BMI categories ($P<0.05$). The lowest obesity prevalence both baseline and after follow up was found in those who remained NGT.
Conclusion
In the studied baseline NGT population after 8 years of follow up high progression rate to impaired glucose metabolism was found. The implementation of new IFG diagnostic criteria increased the prevalence of IFG by 10.1%. The prevalence of impaired glucose metabolism was increasing with age and BMI categories. The lowest obesity prevalence both baseline and after follow up is found in those who remains NGT. Therefore prevention of diabetes initiatives should focus on normal body weight preservation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P516

**The role of sex hormone-binding globulin in the risk of incident metabolic syndrome: further evidence from a longitudinal population-based sample of adult men.**

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Background
In the classic paradigm, the primary function of sex hormone-binding globulin (SHBG) was thought to passively bind and transport steroids and estrogens in the blood to access target tissues and to determine their bioavailable fraction. Now there is increasing evidence that SHBG plays an active role in steroid actions and is an actively contributes to many age-associated comorbidities including type 2 diabetes, obesity, and metabolic syndrome (MetS).
Objective
To further evaluate the suggested independent association of SHBG with incident MetS in men.
Research Design and Methods
We used data from 956 men aged 20–79 years from the population-based Study of Health in Pomerania (SHIP). Cross-sectional and longitudinal logistic regression models with sequential adjustment for 1) age and smoking, 2) SHBG or total testosterone (TT), respectively, 3) body mass index, and 4) glycated hemoglobin were performed.
Results
In model 1) we found lower TT (odds ratio [OR] per SD decrement: 1.48 (95% CI: 1.27–1.73) p-value, $<0.001$) and SHBG (OR: 1.65 (1.36–2.00) p-value, $<0.001$) associated with incident MetS. After additional adjustment for the respective sex hormone, this association was abolished for TT (OR: 1.20 (0.99–1.46) p-value, 0.057), but not for SHBG (OR: 1.47 (1.17–1.86) p-value, 0.001).

Furthermore, p for trend analyses in fully-adjusted models showed a progressive inverse dose-response relationship for quartiles of SHBG ($<0.001$) and risk of incident MetS, but not across TT (0.468) or free T quartiles (0.438).
Conclusions
SHBG is a predictor of incident MetS independent of testosterone in men, but not vice versa.
Multiple logistic regression models for the risk of incident metabolic syndrome.

**Table 1**

| | | | Odds ratio per SD decrease (95% CI), p-value | | |
|---|---|---|---|---|---|
| | | Age & smoking | Age, smoking, and SHBG | Age, smoking, SHBG, and BMI | Age, smoking, SHBG, BMI, and HbA1c |
| Total testosterone | Cross-sectional | 1.74 (1.53; 1.98), <0.001 | 1.50 (1.30; 1.73), <0.001 | 1.34 (1.16; 1.56), <0.001 | 1.28 (1.10; 1.50), 0.002 |
| | Longitudinal | 1.48 (1.27; 1.73), <0.001 | 1.20 (0.99; 1.46), 0.057 | 1.14 (0.93; 1.39), 0.201 | 1.14 (0.93; 1.39), 0.205 |
| Free testosterone | Cross-sectional | 1.35 (1.19; 1.53), <0.001 | 1.48 (1.29; 1.69), <0.001 | 1.35 (1.17; 1.56), <0.001 | 1.29 (1.11; 1.51), 0.001 |
| | Longitudinal | 1.02 (0.88; 1.19), 0.780 | 1.12 (0.96; 1.32), 0.160 | 1.07 (0.90; 1.27), 0.430 | 1.07 (0.90; 1.27), 0.439 |
| | | Age & smoking | Age, smoking, and TT | Age, smoking, TT, and BMI | Age, smoking, TT, BMI,and HbA1c |
| Sex hormone-binding globulin | Cross-sectional | 1.68 (1.47; 1.93), <0.001 | 1.32 (1.15; 1.52), <0.001 | 1.12 (0.98; 1.27), 0.102 | 1.13 (0.98; 1.30), 0.083 |
| | Longitudinal | 1.65 (1.36; 2.00), <0.001 | 1.47 (1.17; 1.86), 0.001 | 1.30 (1.03; 1.65), 0.029 | 1.30 (1.03; 1.65), 0.028 |

SD, standard deviation; TT, total testosterone; SHBG, sex hormone-binding globulin; BMI, body mass index; HbA1c, glycated hemoglobin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P517

### Fracture predictors in diabetic postmenopausal women without hyperparathyroidism

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Introduction
The diabetes mellitus (DM) is the known risk factor of fractures. The aim was to determine predictors of fractures in type 2 diabetic postmenopausal women without hyperparathyroidism.
Design
We analyzed local trauma clinic data for 3 sequential years from 2 662 diabetic persons who were entered The National Register of diabetes mellitus patients (133 subjects with type 1 diabetes mellitus and 1995 ones with type 2 DM). Afterward we examined 62 postmenopausal women with type 2 DM, 32 of them had bone fracture within last month. The control group included 54 non-diabetic women, 25 within a group were recently fractured. Groups were comparable in body mass index, age, duration of menopause and calcium intake. Diabetic subgroups were comparable in glycosylated hemoglobin level. None of them had hyperparathyroidism. Tests involved blood and urine calcium and phosphorus, serum parathyroid hormone (PTH), insulin-like growth factor-1 (IGF-1), 25-hydroxyvitamin D, C-terminal telopeptide of type I collagen (sCTX), osteocalcin levels and data of dual-energy X-ray absorptiometry of lumbar spine and proximal femur. For determining predictors ($P<0.05$) we conducted unidimensional ANOVA.
Results
In epidemiological study main risk factor of fractures was vascular complication of diabetes. Independent risk factors in cohort study were relatively low level of ionized calcium, alkaline phosphatase, phosphorus excretion, vitamin D and osteocalcin, as well as decrease of lumbar spine (L1-4) and femur (trochanter and total hip) bone mineral density.
Conclusion
Fracture risk is elevated in postmenopausal women with type 2 DM but without hyperparathyroidism in whom diabetic influence on bone turnover primary involves bone formation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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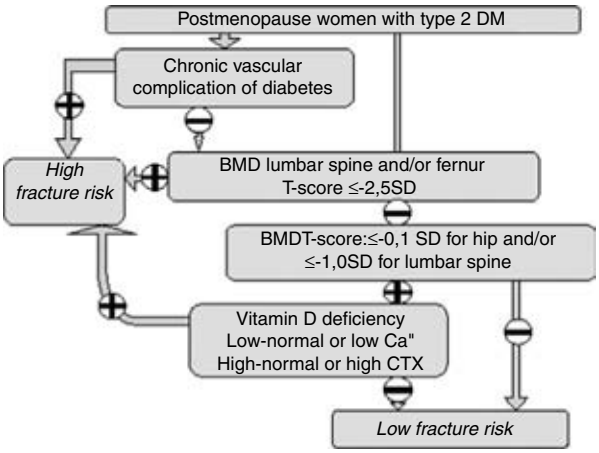


**Figure 1** High fracture risk algorithm for postmenopausal women with type 2 diabetes mellitus without hyperparathiroidism

## P518

### Characteristics and prevalence of latent autoimmune diabetes in adults (LADA) in a diabetic population

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Diabetes, one of the most commonly seen metabolic disorders, is affecting a major area of population in many developing as well as most of the developed countries and is becoming an alarming concern for the rising cost of the healthcare system. Latent Autoimmune Diabetes in Adults (LADA) is a form of diabetes which is less recognized and under diagnosed type of diabetes which appears to have characteristics of both type 1 (autoimmune in nature) and type 2 diabetes (adult age at onset and initial response to oral hypoglycemic agents). An epidemiological study was carried out on 500 patients in the western region of India. Various parameters such as age at onset, duration of diabetes, gender, basal metabolic index (BMI), type of diabetes, family history, HbA1c levels, cholesterol levels and current treatment regimen were evaluated and correlated with Type 1 and Type 2 Diabetes. Moreover, diagnostic markers for LADA, viz. GAD auto antibodies and C-peptide levels were determined for 80 patients selected from the epidemiological study. Some of the results obtained were found to be consistent with the literature whereas some results were found to be contradictory to the existing data.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P519

### Non Governmental Organizations [NGO's] & Diabetes- Advocacy for support services in resource-poor nations

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Background
Diabetes- support services facilities available only in few city hospitals. Especially diabetics in rural India lack comprehensive diabetes care plan.

NGO's play key role in psychosocial-support/Counseling/rehabilitation in remote towns of india. Our Project aimed to formulate policy for trained personals to give better & cost-effective Diabetes- support services.
Methods
We mobilized training resources from local primary Health-centers. Training in Counseling & diabetes care imparted to nurses. Team consisted 2 social worker, 4 nurse & one physician. Local traditional faith-healers & community leaders involved for more effective diabetes awareness/education programs. Aim was to provide physical-comfort to patient, improve relationship with diabetics family members, gradually we prepared patient/family for long term diabetes care needed by patients. Discomfort/anxiety decreases overall treatment efficacy. 51 Patients enrolled during community out-reach-programs. Data collected on feedback-questionnaire. Most difficult tasks is discussing cost of long term therapy & non availability of newer insulin preparations in rural/tribal areas.
Results
Diabetes Counseling/support services must be made more accessible in rural-areas. Our NGO's approach is also very cost-effective. Due to non-availability of trained-personal in rural areas this approach crucial in resource poor nations. We noted 86% responded favorably to counseling/nursing care programs, 79% showed willingness to motivate fellow patients to facilitate supportive-care-program of NGO-volunteers. Infact 17 patients themselves became regular active facilitators in our NGO's Diabetes-care workshops. Our Holistic approach helped overcome hopelessness/fear depression. supportive care emerged very serious issue affecting QOL in diabetics. NGO's need to Improve access to drugs by collaboration with national diabetes societies.
Conclusion
In no extra cost our NGO's performed good job of Counseling/rehabilitation for diabetics. Restricted resource-limitations didnot permit us to take study large-sample-size, but we can collaborate with other NGO's & diabetic associations like ICE/ECE at 2012 Florence congress venue for larger effort. Our approach most suitable to resource-poor-settings.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P520

**Mesenchymal stem cells-derived microvesicles modulate cellular immune response to islet antigen GAD in type 1 diabetes.**

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Background and aims
Mesenchymal stem cells (MSCs) exert an immunosuppressive effect on immune system and can abrogate *in vitro* the pro-inflammatory Th1 response to islet antigen GAD in type 1 diabetes by impairing the production of IFN-gamma. The mechanism may involve paracrine factors. Microvesicles (MVs) released from MSCs may account for this paracrine mechanism through a horizontal transfer of mRNA and microRNA. In the present study we evaluated whether MSC-derived-MVs exert immunomodulatory effect similar to that of MSCs on T cell responses against GAD in type 1 diabetes.
Methods
MVs were purified from supernatants of human MSCs and were characterized by cytofluorimetic and gene array analyses. Peripheral blood mononuclear cell (PBMCs) were obtained from 4 type 1 diabetic patients at disease onset with a positive IFN-gamma response to GAD65 stimulation by ELISPOT assay. PBMCs GAD65-pulsed were incubated with or without MV followed by IFN-gamma ELISPOT. Levels of PGE2, TGF-beta, IL-10, IFN-gamma, IL-6 in supernatants were measured by ELISA.
Results
When determined by Nanosizer, the size of MVs has a mean value of 158 nm. MVs showed adhesion molecules expressed on MSC-membrane and contained mRNA and in particular mRNA related to immune regulation. MVs labeled were incorporated by PBMC, as shown by confocal microscopy. Incubation of PMBCs obtained from the GAD-responder patients with MVs, resulted in a significant decrease in the number of IFN-gamma spots. Levels of IFN-gamma, IL-6, in supernatants of GAD65-pulsed PBMCs incubated with MVs were decreased compare to GAD65-pulsed PBMCs alone. Levels of PGE2, TGF-β and IL-10 were significantly increased compare to GAD65-pulsed PBMCs alone.
Conclusions
These results provide evident that MSC-derived-MVs inhibited *in vitro* pro-inflammatory Th1 response to an islet antigenic stimulus, in diabetic patients, possibly mediated by PGE2 and TGF-β. MVs induce IL-10 secretion, suggesting a possible switch to an anti-inflammatory Th2signalling of T cells.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P521

**Prevalence of autoimmune diseases in a Type 1 diabetic population**

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Background
Patients with type 1 diabetes (T1DM) are known to have a higher risk of developing other autoimmune diseases. The most common associated disorders are thyroid autoimmunity (Graves' disease or Hashimoto's thyroiditis) and celiac disease.
The aim of this study was to investigate the prevalence of other autoimmune disorders in T1DM patients of our clinic.
Methods
We reviewed 269 files of children and adolescents with T1DM. Autoantibodies to thyroperoxidase (anti-TPO), thyroglobulin (anti-Tg), transglutaminase and endomysial were investigated in all patients. Thyroid function (TSH and fT4) and thyroid receptor antibodies (TRAbs) were determined when appropriated. Statistic study was made in IBM SPSS Statistics, Version 19.
Results
The patient's mean age was 12.1 years, with a similar distribution between sex. The average duration of diabetes was 4.7 years.
Anti-TPO and anti-Tg antibodies were detected in 15.6% of our population, with significant female preponderance. Prevalence of thyroid dysfunction among diabetic patients was 5.3%, with one case of hypothyroidism and 12 subclinical hypothyroidisms. This risk of thyroid dysfunction was higher in cases with both positive antibodies compared with the presence of only one of them. The mean age of positive thyroid autoantibodies onset was 2.4 years after diabetic diagnosis. 10.2% of the population with T1DM had positive transglutaminase or endomysial autoantibodies titers. The prevalence of biopsy-confirmed celiac disease was 4.1%. Positive antibodies for celiac disease were detected after a mean of 2.3 years of diabetic diagnosis.
Graves' disease was present in 3 cases with positive TRAbs. There was 1 case of Addison disease and 4 children presented at least 3 autoimmune diseases simultaneously.
Conclusion
Autoimmune thyroid disease and celiac disease occurs more frequently in children with T1DM than in general population, therefore screening at onset of the disease and repeated measurements of autoantibodies is recommended. Our results are comparable to the published data.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P522

**Relation of glycemic control to Easter and summer holiday times**

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Objective
Poor glycemic control in type-2 diabetes mellitus is a risk factor for the development of diabetes complications. The aim of this study was to evaluate the effect of the holiday (Easter time) and the season (summer) on the glycemic control in Greek diabetic subjects.
Methods
50 type-2 diabetic patients participated in the study. Each participant was followed up in primary care settings (Health Centre of Soxos) and underwent a physical exam, including a brief medical history. Of the diabetics, 8 were using insulin, 31 were taking oral hypoglycemics and 11 were using both. Venus blood samples from diabetics were drawn in EDTA tubes and HbA1C was measured using the turbidimetric inhibition immunoassay (Tina-quant HemoglobinA1c, Roche HITACHI 902). These measurements were performed three times, at intervals of 10–12 weeks: pre-Easter holiday period (in the middle of March), at the beginning of summer (in the middle of June) and post-summer period (in the middle of September).

Results
The mean age of the diabetics and controls was 64.5 and 55.9, respectively. Median duration of diabetes was 14.5 years. The first measurement demonstrated a poor glycemic control of the patients, with mean level of HbA1C at 7.16%. The mean HbA1C increased significantly (increase:1.245, $P<0.05$) during Easter holidays and pre-summer period. The patients who were taking oral hypoglycemics demonstrated the poorest control (increase:1.512, $P<0.01$). During summer, the mean HbA1C decreased, but not significantly (decrease:0.404) among diabetics in total and among all patient groups.
Conlusions
Our data indicated a negative influence of the Easter holidays on the glycemic control of type-2 diabetic subjects. This poor glycemic control was not totally reversed during the summer months. More efficient interventions focusing on lifestyle changes and dietary habits during specific holiday (Easter) period are needed in order to improve care for diabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P523

### Change in quality of life by multiple education program in type 1 diabetic patients

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The aim of study was to recurrently follow quality of life in type 1 diabetes patients after multiple educations and counseling. Ninty six patients of the age group of "between" 10 to 25 were initially examined in 2004 and Eighty eight patients of them were reexamined in 2006 after multiple counseling with diabetologist and trained diabetes educator at dia care, Ahmedabad, Gujarat, India. Quality of life was defined as perceived well being and life satisfaction, globally as well as within key domains and functions. Various status and retrospective change ratings were repeatedly performed by patients and significant others. For nine patients quality of life was fairly stable between 2004 and 2006. Further 79 patients had shown very significant and consistent improvement in their quality of life. Delivery of the insulin injection, self monitoring of blood glucose and management of illness, hypoglycemia, hyperglycemia and further topics related to type 1 diabetes were part of education. The study underscores the benefit to other parts of the country that quality of life of type 1 diabetic patients can be improved by continuous and multiple educations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P524

### Cystatin levels in diabetic and prediabetic patients

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Background and aim
Serum Cystatin C has been shown to be associated with the progression to prediabetes and type 2 diabetes mellitus. High cystatin levels were also found to be associated with body mass index. The aim of this study was to evaluate cystatin C levels in diabetic and prediabetic patients ant its association with anthropometric measurements and insulin resistance.
Methods
Twenty four patients with diabetes, 17 patients with prediabetes and 24 healthy controls were included in the study. Age, weight, body mass index, waist circumference (Wc), fasting plasma glucose (FPG), post pyrandial glucose (PPG), lipid profiles, blood urea nitrogen (BUN) and creatinine levels were measured. None of the patients had any diabetic complications.
Results
Diabetic patients had higher BMI than controls ($P=0.01$). Serum cystatin C levels were not statistically different between the three groups ($P>0.05$) and were similar in men and women. Cystatin C levels were correlated with body mass index and HOMA IR in prediabetic patients ($P=0.039$ and $P=0.05$, repectively)) but not in diabetic patients and controls, when adjusted for age and gender.
Conclusions
Serum cystatin C levels are associated with body mass index and insulin resistance in prediabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P525

### Psychoemotional state of diabetic women with fracture

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Background and aims
The aim was to determine psychoemotional state in diabetic and non-diabetic patients with or without fractures and its correlation with bone condition.
Materials and methods
We examined 62 postmenopausal women with type 2 diabetes mellitus (DM), 29 of them had bone fracture within last month. The control group included 56 non-diabetic women, 26 within a group were recently fractured. Groups were comparable in body mass index, age, duration of menopause. Diabetic subgroups were comparable in glycosylated hemoglobin level. We measured depressive symptoms using the Beck depression inventory, Zung Self-Rating Depression and Center for Epidemiological Studies Depression Scales. There were also analyzed correlation with lifestyle factors, data of bone densitometry (lumbar spine and femur) and vitamin D level.
Results
As expected in patients with DM frequency of depression were significantly higher compared to women without DM ($P=0.01$–$0.04$). There were no rising of depression rate in groups with fracture. The direct correlation between Zung Scale total score and diabetes duration ($R=0.513$) was found. Only in non-diabetic group there were inverse correlations of depression score with both bone mineral density ($R=-0.50$ to $-0.62$) and lifetime weight gain ($R=$-0.44). In this (non-diabetic) group there were also found direct relationship of depression symptoms with coffee consumption ($R=0.43$–$0.54$) and smoking intensity ($R=0.46$). No correlation of vitamin D level and depression score was detected.
Conclusion
Depression is more common in diabetic women. Frequency of depression among patients with fracture was the same or even a bit lower. Depression was associated with low bone mineral density only in non-diabetic group.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P526

### Hypoglycemic and hyperglycemic patients in emergency room

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Aims
To evaluate the process of patients from emergency room (ER) admittance until discharge from the endocrinology service (ES) with hypoglycemia or hyperglycemia.
Methods
Two hundred twenty two patients either with hypoglycemia or hyperglycemia were evaluated. Hyperglycemic patients were divided into three groups: Patients with type 1 diabetes, type 2 diabetes and patients without diabetic history. The complaints, laboratory findings, stay in ER, stay in the ES and mortality rates were documented.
Results
Fatigue like unspecified complaints were the most common finding in whole hyperglycemic patients ($n=206$, age $=59.7\pm16.1$ yrs, glucose $291\pm195.7$ mg/dl). Mean stay in ER was $2.91\pm1.77$ hours, $9.3\pm13.2$ days in ES. Mortality rate was

7.6% in whole hyperglycemics with the highest ratio in the patients without any diabetic history (13%). Hypoglycemic patients ($n=16$, 15 with type 2 DM, age = 66.5 ± 19 yrs, glucose 28.5 ± 7.5 mg/dl) were found to be mostly presented with conscious deterioration (80%). Their mean stay in ER was 2.5 ± 0.7 hours, 2.6 ± 0.7 days in ES. The frequency of type 2 diabetes was 76% and stress hyperglycemia was 17.5%.

Conclusion

1- The short duration for both ER and ES stay (2.5 -3 hours) is the result of our high hospitalization (70%) rates. 2- The guidelines for diabetes lead us to manage these patients with shorter stay. 3- The necessity for diagnose and defining management protocols cause to expend much time on patients without diabetic history regards of hospitalization process. The mortality rates for these patients are higher as well. 4- The high percentage (76%) of type 2 DM -either with hypoglycemia or hyperglycemia- who admitted to ER; reflects the increased frequency of the disease. 5- The increased admittance of critically ill patients to an university hospital seems to account for the increased rate of stress hyperglycemia (17.5%).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P527

**Diabetes risk in relation to weight loss, weight stability and degree of obesity - The Swedish Obese Subjects (SOS) study**

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Objective

Among severely obese individuals, surgically induced weight loss reduces the risk for type 2 diabetes. The aim of this study was to analyse the incidence and remission of type 2 diabetes among individuals with different degrees of obesity losing weight, and compare them with obese individuals remaining weight stable during follow-up.

Research design and methods

The ongoing non-randomized prospective controlled Swedish Obese Subjects (SOS) intervention study enrolled 2010 obese persons who received bariatric surgery and 2037 contemporaneously matched obese controls. Health examinations with anthropometric measurements and laboratory analyses were conducted at baseline and at 2- and 10-year follow-up. Diabetes was defined by fasting venous whole blood glucose 6.1 mmol/litre or more, and/or self-reported diabetes medication. Two- and 10-year diabetes risk was assessed by initial BMI and weight changes during follow-up. Incidence and remission rates were calculated for those without and with diabetes at baseline, respectively.

Results

Among weight stable obese individuals with BMI $<35$, 35–40, and 40–45 both at baseline and at 2-year follow-up, type 2 diabetes incidence rates were 6.5%, 7.7% and 9.3%, respectively. Among those with initial BMI of 35–40, 40–45 and $\geqq 45$ attaining five BMI-unit weight reduction, the corresponding rates were 2.4%, 2.0% and 3.4%, respectively. In patients with diabetes at baseline and who attained a 5 BMI-unit reduction at year 2, remission rates were independent of the initial degree of obesity. Similar results were observed with the 10-year follow-up data.

Conclusions

A BMI reduction of 5 units, independent of the initial degree of obesity, is associated with a reduction of diabetes risk compared with weight stability at any obese BMI-level.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P528

**The role of Insulin Receptor in Cancer**

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The IGF-I receptor (IGF-IR) and its ligands (IGF-I and IGF-II) play a physiological role in growth and differentiation and are also involved in cancer growth and progression. The insulin receptor (IR), which shares high homology with the IGF-IR, has a predominant role in glucose metabolism. However, several studies have convincingly shown that the IR pathway is more intimately involved in cancer development and progression than previously thought. Recent evidences showing that insulin resistance and hyperinsulinemia are important risk factors for cancer have renewed the interest for these studies. One mechanism explaining the increased sensitivity of malignant cells to hyperinsulinemia is the frequent IR overexpression in cancer cells. Moreover, cancer cells commonly show an altered IR gene splicing, with predominant expression of IR-A, one of the two IR isoforms. IR-A binds not only insulin but also IGF-II and, therefore, it competes with IGF-IR for IGF-II binding. IGF-II, via IR-A, stimulates subtly different signaling than insulin. IR-A overexpression and high IGF-II autocrine production are important factors for both normal and cancer stem cells expansion and are often present in dedifferentiated malignancies. Finally, IR overexpression enhances cell responsiveness to IGF-I and IGF-II by participating to the formation of hybrid IR/IGF-IR receptors, which bind both IGFs.

Until very recently, only IGF-IR, but not IR, has been considered a target in cancer therapy. However, the results of the clinical first trials employing selective anti-IGF-IR drugs have been disappointing, with only a small subset of malignancies showing an objective response. Recent data have indicated that resistance to anti-IGF-IR drugs may include upregulation of IR isoform A and/or increased secretion of autocrine IGF-II. Therefore, cotargeting of IR and IGF-IR may prevent adaptative resistance to selective anti-IGF-IR drugs, especially in malignancies with high IR-A:IGF-IR ratio and autocrine IGF-II production.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P529

**Local effects of cytokine TNF-alpha on glucose, lipid and protein metabolism in the placebo controlled bilaterally perfused human leg**

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Introduction

Cytokine TNF-alpha has widespread metabolic actions, including induction of insulin resistance, lipolysis and cachexia. Systemic TNF-alpha administration, however, generates complex hormonal and metabolic scenario. No studies employing regional, placebo controlled TNF-alpha infusion exist. Our study was designed to test the hypothesis that local leg perfusion with TNF-alpha directly induces insulin resistance, lipolysis and protein breakdown.

Methods

We studied eight healthy volunteers with bilateral femoral vein and artery catheters during 3-h basal period and 3-h insulin stimulation (hyperinsulinemic euglycemic clamp). One femoral artery was perfused with saline and the other with TNF-alpha ("Beromun", Boehringer-Ingelheim, 6 ng/kg/h). Amino acid metabolism was quantified with 15N-Phenylalanine tracer, lipid metabolism with 3H-Palmitate tracer and arterio-venous differences of free fatty acids and glucose metabolism was quantified by arterio-venous differences.

Results

TNF-alpha perfusion significantly increased local leg glucose uptake during the clamp. Basal glucose arterio-venous difference was unaltered, but substantially increased during the clamp ($P<0.001$), with 0.925 ± 0.49 mmol/1 in the TNF-alpha leg, and 0.744 ± 0.43 mmol/1 in the placebo leg.

Net phenylalanine release was increased by TNF-alpha perfusion ($P=0.023$). During the basal period there was an increased phenylalanine release ($P=0.003$), with 4466 ± 1390 μg/min in the TNF-alpha leg, and 3794 ± 1122 μg/min in the placebo leg, and an increased phenylalanine uptake ($P=0.023$) with 2951 ± 1134 μg/min in the TNF-alpha leg, and 2464 ± 708 μg/min in the placebo leg.

Free fatty acids and palmitate kinetics were not affected by TNF-alpha.

Conclusion

TNF-alpha directly increased muscle protein breakdown and local muscle insulin sensitivity in terms of increased muscle glucose uptake. The finding of increased muscle loss may contribute to general protein loss during severe illness. The finding of increased insulin sensitivity is unexpected and highlights the necessity of differentiation between direct local cytokine effects as in this study and those secondary to release of stress cascades including hormones such as epinephrine, glucagon and cortisol.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P530

**Increased nuclear translocation of GAPDH in peripheral blood mononuclear cells (PBMCs) of diabetic patients.**

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) not only functions as a central glycolytic enzyme but also is implicated in the process of apoptosis and gene transcription moving between the cytosol and nucleus. Oxidative stress modifies the activity of GAPDH.
The aim of the study was to determine whether oxidative stress (OS) in diabetics is associated with induction of the apoptotic process and higher GAPDH translocation to the nucleus in PBMCs.
GAPDH activity and -SH group content were determined in both the cytoplasmic and nuclear fraction of PBMCs collected from 20 diabetic and 20 control subjects. NF-kB p65 subunit and Bcl-2 levels were estimated in the nuclear and cytoplasmic cell fractions respectively. Chromatin integrity was assessed by diphenylamine (DPA) reaction and DNA laddering assay.
Intracellular OS was significantly higher in PBMCs from diabetic patients compared to controls as it is suggested by the -SH groups depletion in both cytoplasmic ($P=0.030$) and nuclear ($P=0.003$) cell fractions. The higher OS in the diabetics was accompanied by a significant increase in the nuclear NF-kB p65 subunit ($P=0.015$) and a three-fold decrease in cytoplasmic Bcl-2 levels ($P=0.001$). DNA damage was more evident in diabetics compared to controls as it is suggested by the DPA reaction ($P=0.002$) and DNA laddering assay. Compared to controls, GAPDH activity in PBMCs was significantly lower in diabetics ($P=0.017$). In spite of this reduction a significantly higher fraction of GAPDH activity was observed in the nuclear compartment of patients ($P=0.001$). In conclusion, diabetic OS activates the pro-apoptotic process in PBMCs and simultaneously the NF-kB, which may acts as anti-apoptotic factor. The higher GAPDH fraction that translocates into the nucleus may facilitates the repair of DNA damage, inserting new insights that link metabolic enzymes with energy production and cell survival.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P531

**Conserved mitochondrial coupling and adaptive substrate control in inuit and caucasians: acclimatization to active life styles in the arctic winter**

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Mitochondria oxidize food substrates to generate energy for cellular work and heat is variably generated depending on the degree of coupling of electron transport to oxidative phosphorylation (OXPHOS). It has been hypothesized that climatic pressures exerted selection for mitochondrial haplogroup in arctic populations for a lower coupling of mitochondrial respiration to ATP production in favor of heat production. In this study muscle mitochondrial function was studied in Inuit from northern Greenland and sedentary Caucasian Danes who travelled to Greenland. Muscle biopsies were obtained from the vastus lateralis and deltoid muscles and mitochondrial function was assessed by high-resolution respirometry. OXPHOS capacity in the leg (vastus lateralis) was lower in Inuit compared to Danes ($70\pm4$ vs. $90\pm5$ pmol/mg/sec). Leak respiration was proportionate with OXPHOS such that mitochondrial coupling efficiency was equivalent between groups and across muscles of both arm and leg. The traditional Inuit had a higher OXPHOS capacity with lipid substrate compared to Danes ($27\pm3$ vs. $22\pm3$ pmol/mg/sec). In conclusion, this study refutes the hypothesis that mitochondrial uncoupling and metabolic heat production is higher in arctic haplotype populations, yet reveals acclimatization of mitochondrial substrate control in arctic inhabitants.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P532

**Association of adiposity trajectories with insulin sensitivity and glycemic deterioration: a longitudinal study of rural chinese twin adults**

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Objective
To evaluate associations between adiposity trajectories over time and insulin sensitivity and glucose deterioration in a Chinese twin cohort.
Research Design and Methods
This study focused on 341 males and 292 females who aged 20–50 years old at baseline and had physical, clinical examinations and oral glucose tolerance test(OGTT) at two time points with an average of 6 years apart. Body mass index (BMI), waist circumference (WC), percent body fat (PBF), and percent trunk fat (PTF) trajectories were classified into 5 track groups based on age- and gender-specific tertiles at each visit. We calculated the odds of the insulin sensitivity index(0.120) (ISI(0.120)) or glycemic deterioration at follow-up among 5 defined trajectories (tertile baseline to tertile follow-up) using generalized estimate Default (GEE). Additionally, we applied structural equation models to examine genetic and environmental influences on adiposity, adiposity change over time(ACO), ISI(0.120), and the inter-relationships.
Results
Participants with adiposity (BMI, WC, PBF and PTF) stable in the highest tertile or shifting to the highest tertile tended to have the lowest ISI(0.120) at follow-up or experience glycemic deterioration. Genetic factors exerted the major influence on adiposity, but environmental factors unique to each twin contributed more strongly to ISI and ACO. Correlations between adiposity/ACO and insulin sensitivity were mainly due to environmental influences.
Conclusion
When adiposity stays or becomes high, insulin sensitivity falls and risk of glycemic deterioration rises. Additionally, we found that genetic factors exerted the major influence on adiposity, while environmental factors played the principal role for ACO and insulin sensitivity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P533

**Direct metabolic effects of locally administered lipopolysaccharide in the placebo controlled bilaterally infused human leg; decreased insulin sensitivity and insulin signaling with unaltered lipid and protein metabolism**

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Introduction
In humans bacterial lipopolysaccharide (LPS/endotoxin) causes chronic low-grade and acute inflammatory responses, which decrease insulin sensitivity and may lead to chronic type 2 diabetes and acute stress diabetes. Systemic LPS invariably generates cytokine release, which triggers release of anti-insulin stress hormones and it is not known whether insulin resistance is secondary to stress hormones or LPS per se. Our study was designed to test whether local placebo controlled leg perfusion with LPS directly induces insulin resistance and affects protein and lipid metabolism.
Methods
We studied eight healthy volunteers with bilateral femoral vein and artery catheters during 3-h basal and 3-h hyperinsulinemic euglycemic clamp conditions. One femoral artery was perfused with saline and the other with LPS (0.025 ng/kg/h). Lipid metabolism was quantified with 3H-Palmitate and arterio-venous differences of FFA. Amino acid metabolism was quantified with 15N-Phenylalanine tracer and lactate and glucose by raw arterio-venous differences.

Results
Overall LPS perfusion significantly decreased leg glucose uptake ($P=0.015$); Basal glucose a-v differences were not significantly reduced ($0.02\pm0.05$ mmol/l vs. $0.06\pm0.06$ mmol/l in the placebo leg, $P=0.19$); during the clamp LPS decreased glucose a-v differences ($0.65\pm0.21$ mmol/l in the LPS leg, and $0.73\pm0.23$ mmol/l in the placebo leg, $P=0.021$). Akt308 phosphorylation was inhibited in the LPS leg both during the basal period ($P=0.021$) and clamp ($P=0.027$). Phosphorylation of AS160 was unaltered by LPS. Regional leg phenylalanine, palmitate, FFA and lactate kinetics were unaffected by LPS.
Conclusion
LPS directly inhibits insulin-stimulated glucose uptake in the perfused human leg without detectable effects on amino acid metabolism, lipolysis or lactate release. The mechanism in all likelihood involves inhibition of insulin mediated Akt308 phosphorylation. These data strongly suggest that a primary metabolic effect of LPS is muscle insulin resistance, which together with secondary insulin resistance caused by stress hormones may trigger diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P534

**Suppressive effect of hyperinsulinemia on serum interleukin 18 concentration in young healthy subjects**

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Introduction
Interleukin 18 (IL-18) is a proinflammatory and proatherogenic cytokine which is associated with obesity, insulin resistance and cardiovascular disease. It is supposed that insulin has anti-inflammatory action. The aim of the present study was to estimate serum IL-18 concentration in young healthy population, its regulation by hyperinsulinemia and relationship with insulin sensitivity and glucose and lipid oxidation.
Methods
We studied 36 healthy male subjects (mean age, $24.50\pm2.67$; mean BMI, $25.77\pm3.70$ kg/m$^2$). Serum IL-18 concentration was measured before and after 2 hour euglycemic hyperinsulinemic clamp. In 18 subjects, clamp was prolonged to 6 hours and at the end an additional measurement of serum IL-18 was taken. Respiratory quotient (RQ) and glucose and lipid oxidation (LOx) were assessed with indirect calorimetry in the baseline state and every 2 hours of the clamp.
Results
Hyperinsulinemia decreased serum IL-18, this effect was present both in 2 and 6 hours of the clamp (both $p<0.001$). Additionally, serum IL-18 decreased from 2 to 6 hour ($p=0.044$). Two-hour and 6 hour serum IL-18 values were positively related to plasma free fatty acids in the respective time-points ($r=0.43$, $P=0.033$ and $r=0.52$, $P=0.028$). Two-hour and 6 hour serum IL-18 were also negatively related to RQ ($r=-0.40$, $p=0.018$ and $r=-0.54$, $p=0.02$) and positively to LOx ($r=0.46$, $p=0.006$ and $r=0.66$, $p=0.004$) in the respective time-points. Six-hour IL-18 was negatively related to insulin sensitivity calculated for the 6th hour of the clamp ($r=-0.53$, $p=0.025$). The change in serum IL-18 in response to insulin was inversely related to the white blood cell count ($r=-0.52$, $P=0.027$) and the neutrophil cell count ($r=-0.56$, $P=0.016$), i.e., the higher the cell count, the lower the decrease in IL-18.
Conclusion
Serum IL-18 is negatively regulated by hyperinsulinemia, suggesting anti-inflammatory effect of insulin. IL-18 is related to decreased insulin sensitivity mainly through its association with lipid oxidation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P535

**The Association of the severity of nonalcoholic fatty liver disease with development of type 2 diabetes: A 4-year longitudinal study**

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Aims
Nonalcoholic fatty liver disease (NAFLD) is reported to contribute to the development of type 2 diabetes, but it is largely unknown whether such a relationship between NAFLD and incident diabetes relates to the severity of NAFLD or it is independent. We aimed to evaluate the association of the severity of NAFLD with development of type 2 diabetes in a retrospective cohort of Korean subjects.
Methods
This study included 7,849 individuals without diabetes who underwent health check-ups annually for 5 years. Based on the presence or absence of fatty liver on ultrasound and serum alanine aminotransferase (ALT) values at baseline, subjects were classified into controls, an increased ALT group without steatosis, a steatosis group with normal ALT, and a group with presumed nonalcoholic steatohepatitis (NASH) with steatosis and an elevated ALT.
Results
At baseline, there was a significant trend of worsening metabolic variables and index of insulin resistance across the groups from the control group to the increased ALT group, the steatosis group and the NASH group. Over 4 years, the incidence of diabetes was 3.5% in the control group, 4.6% in the increased ALT group, 7.3% in the steatosis group, and 11.8% in the NASH group. The hazard ratio (HR) [(95% confidence interval (CI)] of incident diabetes was increased in the increased ALT group, the steatosis group, and the NASH group in a stepwise fashion. Subjects with NASH had a significantly increased HR of 1.64 (1.27–2.13), even after multivariable adjustment.
Conclusions
The severity of NAFLD as well as the presence of NAFLD has an independent and additive effect on the development of type 2 diabetes. Thus, the evaluation of severity of NAFLD, especially through the clinical diagnosis of NASH, could be included in incident diabetes prediction algorithms.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P536

**Predictive ability of prediabetes defined by fasting plasma glucose and HbA1c for progression to diabetes in Koreans**

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Background
The predictive ability of prediabetes defined by HbA1c (5.7–6.4%) or fasting plasma glucose (FPG) criteria (5.6–6.9 mmol/l) for the development of diabetes may differ according to ethnic groups, but only limited longitudinal data are available in Asians. We examined the progression rate to diabetes in Korean adults diagnosed as prediabetes according to these two criteria.
Methods
We analyzed data of 9432 Korean adults (age 20–79 years) who underwent regular health check-ups in 2005 (baseline) and in 2010 (follow up). After excluding patients with previously diagnosed diabetes ($n=332$) and those with FPG $\geqq7.0$ mmol/l or HbA1c $\geqq6.5\%$ ($n=353$) at baseline, 8747 subjects (5706 men and 3041 women) were included for the analysis.
Results
Among the 8747 participants without diabetes at baseline, 3493 individuals (39.9%) were categorized as prediabetes. Among them, 1318 individuals (15.1%) met HbA1c criteria only (HbA1c only group), 1229 (14.1%) met FPG criteria only (FPG only group), and 946 (10.8%) met both criteria (both group). After 5 years, a total of 356 subjects (4.1% of total participants) converted to diabetes. Only 0.3% (17/5254) of subjects who had normal baseline FPG and HbA1c

developed diabetes during 5 years. The 5-year incidence of diabetes was 3.5% (43/1229) for the FPG only group, 4.9% (65/1318) for the HbA1c only group, and 24.4% (231/946) for the both group. Multiple logistic regression analysis showed that age, sex-adjusted odds ratios (ORs) for the FPG only, HbA1c only, and both groups were 10.7 (95% CI, 6.0–18.8), 15.8 (9.2–27.1), and 95.0 (57.3–157.4), respectively. After further adjustments for smoking, alcohol drinking, exercise habits, body mass index, waist circumference, systolic blood pressure, hemoglobin, cholesterol, HDL-cholesterol, and triglycerides levels, ORs for the FPG only, HbA1c only, and both groups were 7.7 (95% CI, 4.3–13.9), 12.9 (7.5–22.3), and 69.8 (41.7–116.7), respectively.
Conclusion
Individuals categorized as prediabetes by both of the FPG and HbA1c criteria have substantially high risk for progression to diabetes, while those classified as normal by both criteria have very low risk in Koreans. Those who discordantly met only one criterion have intermediate risk, but HbA1c only group tends to have higher risk than FPG only group.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P537

**Relationship between serum levels of 1-84 parathormone (PTH1-84) and obesity, HOMA insulin-sensitivity or beta-cells function (HOMA-B) in type 2 diabetic patients**

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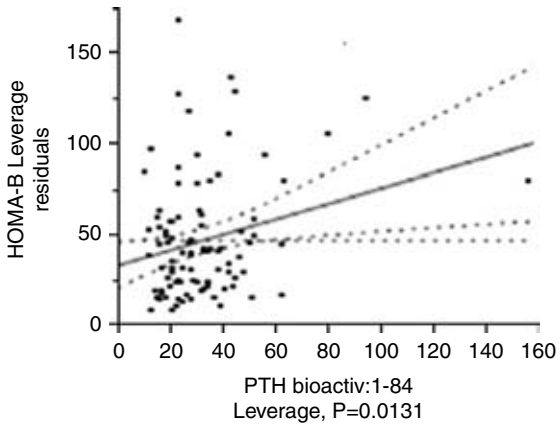
Introduction
PTHrP (Parathyroid hormone-related protein) is known to enhance beta cell function and proliferation. PTH levels predict myocardial and coronary heart disease in non-diabetic patients with normal calcium levels and no kidney failure. We aimed at determining in type 2 diabetes patients without kydney failure the relationship between serum levels of 1-84 parathormone (PTH1-84), obesity, HOMA insulin-sensitivity or beta-cells function (HOMA-B), but also between PTH1-84 and confirmed myocardial arterial disease (MAD).
Research design and methods
106 adult patients with type 2 diabetes were divided into two groups: group A - absent myocardial arterial disease (n=64) and group B- confirmed myocardial arterial disease (n=42). The groups were matched regarding age, HbA1c, body mass index (BMI), glomerular filtration rate, microalbuminuria, vitamin D.
Results
We found a positive correlation between PTH1-84 and abdominal perimeter ($P=$ 0.007), BMI ($P=<0.0001$), fibrinogen ($P=0.008$), beta-cells function ($P=$ 0.01), C-peptid ($P=0.0003$), and a negative correlation between PTH1-84 and HOMA insulin-sensitivity ($P=0.008$) or HbA1C ($P=0.01$). All type 2 diabetic patients had normal levels of PTH 1-84 and calcium, but low levels of 25(OH)D. Serum PTH1-84 were significantly increased in group B (MAD) vs group A (absent MAD) ($P=0.02$). There was a significant correlation between PTH level and systolic left ventricular diameter (p=0.01).




Conclusion
In type 2 diabetic patients, obesity, insulin resistance and poor control of diabetes are positively associated with increased levels of PTH. Parathormone may contribute to the development of systolic dysfunction and myocardial heart disease. In this regard, treating the hypovitaminosis D in type 2 diabetic patients can improve the cardiovascular protection. In the same time, we found a positive correlation between PTH1-84 and beta-cells function suggesting a role for PTH1-84 in insulin secretion.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P538

**The burden of disease in elderly people with IFG**

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Introduction
The prevalence of impaired fasting glucose (IFG) in the elderly approaches 50% in some community-dwelling cohorts. We examined whether IFG is associated with a higher burden of disease, cardiovascular risk factors and circulating low-grade inflammation than in normoglycemic elderly people.
Methods/Design
Cross-sectional data of 929 participants of the Sydney Memory and Aging Study (MAS) were examined. MAS is a population-derived cohort of community-dwelling adults aged 70–90 years. Normoglycemic (NG) and IFG participants were compared using contingency tables and the Chi-square test. Logistic regression and ANCOVA analyses were performed adjusted for age, sex and Body Mass Index (BMI).
Results
Mean age was 78.6 ($\pm$4.7) years; 47% had IFG, 12% had diagnosed type 2 diabetes and 4% previously undiagnosed type 2 diabetes. There were proportionately more males with IFG compared to NG (49% vs 37% males, $P<0.001$). As expected, BMI was higher in IFG compared to NG subjects (27.4 kg/m$^2$ vs 26.1 kg/m$^2$, $P<0.001$). Hypertension and hyperlipidemia was equally distributed (62% and 60% in IFG vs. 57% and 56% in NG), although lipid-lowering drugs had been more frequently prescribed in IFG (55% vs 42%, $P<0.001$). After adjustment for covariates, rates of stroke, cardiac disease, myocardial infarction, kidney disease or any cancer were similar between participants with IFG and normal fasting glucose. However, PAI-1 (83.7 vs. 79.8 ng/ml, $P<0.05$), IL-8 (21.8 vs. 18.6 pg/ml, $P<0.01$), IL-12p70 (3.64 vs 3.40 pg/ml, $P<0.05$) and products of oxidative metabolism (urate [0.35 vs. 0.32 mmol/l, $P<0.001$] and malondialdehyde [13.4 vs. 12.8 μmol/l, $P<0.001$]), were all significantly higher in IFG.
Conclusion
In this cohort of community-dwelling elderly, IFG was not associated with an increased burden of disease cross-sectionally. Prospective data are required to elucidate whether the moderately increased markers of inflammation accompanying IFG in this population adversely affect health outcomes during residual life span.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P539

### GABA A receptor gene regulatory networks in peripheral blood TCD4+, TCD8+ AND CD14+ cells of recently diagnosed type 1 diabetes mellitus

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Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter primarily produced by neurons and pancreatic beta cells. GABA functional receptors (GABR) are expressed in brain, pancreas and peripheral blood lymphomononuclear cells (PBMC). After evaluating the differential gene expression in PBMC of recently diagnosed type 1 diabetes mellitus patients (T1D), three GABRA networks were observed, modulating the expression dozen of genes. The study was conducted on T (CD4+ and CD8+) and monocyte (CD14+) cells obtained from 20 pre-pubertal patients and 20 age- and sex-matched healthy controls, using cDNA microarrays. Significant and differentially expressed genes, revealed using the SAM program, were studied for gene networks (GeneNetwork 1.2 algorithm). Three different GABR subunit class genes (alpha1, alpha6 and beta1) up-regulated the expression of the genes related to cell cycle (CNNB1-cyclin M1, STOM-stomatin), apoptosis control (FAS-TNF receptor superfamily, BAT3-HLA-B associated transcript 3), cellular receptor (IL1RAP- interleukin 1 receptor accessory protein, BCAP29-B cell receptor, IK cytokine-down-regulator of HLA II), complement control (CD93-C1q receptor), glucose metabolism (PFKFB2-6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2), insulin receptor (INSR), oncogenes (RHOG-ras homolog gene family, RAB6-member RAS oncogene family), ubiquitins (UBC-ubiquitin C, UBE2Z-ubiquitin-conjugating enzyme E2Z, UBR4-ubiquitin protein ligase E3 component n-recognin 4, and UBAP-ubiquitin associated protein 1), growth factor (COL6A2-collagen, alpha 2, FGF13-fibroblast growth factor 13, FGF9-Fibroblast growth factor 9) among others. Although the role of GABA in the pathogenesis of diabetes has not been established, some studies have shown that GABA down regulates many immune system functions. This study further reports that PBMC may reflect the ongoing diabetes pathogenesis, and further discriminates several other genes with potential interest for T1D.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P540

### Diagnosis of Metabolic syndrome in Saudi Arabia: implications for the importance of local criteria

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Background and Objectives

Different definitions for the metabolic syndrome (MS) exist.Established criteria for diagnosis includes: raised blood pressure, dysglycemia, dyslipidemia, and obesity, but the used thresholds differed. Individual patients with MS need to be identified to reduce future complications. We aimed to determine the characteristics of subjects identified from healthy population to have MS by NCEP-ATP III, IDF and the consensus definition by IDF and AHA/NHLBI, and to investigate the importance of demographic characteristics and other measures of obesity, dysglycemia and dyslipidemia.

Subjects and methods

233 healthy adults aged 18–50 years were recruited randomly from Jeddah. Anthropometric and demographic information were taken. Insulin, glucose, and lipids profile were measured in fasting blood. Individuals were identified using the three definitions of MS, and their characteristics were compared statistically to the rest of the population.

Results

18.9%, 16.7% and 20.6% of population were identified as having MS by IDF, ATPIII, and the consensus definition respectively. Subjects identified by the latter were also identified by either/ or both definitions. The most common feature was central obesity with IDF definition, and low HDL- cholesterol using NCEP-ATP III definition. The least common feature was high blood pressure in all cases. There was no significant difference between subgroups with and without MS with regard to demographic characteristics when any definition was applied.

Using regression analysis the strongest predictors of MS were blood glucose, LDL-C/HDL-C ratio and insulin when NCEP-ATPIII definition or the consensus definition were used, and blood glucose, Waist /Hip ratio and Plasma Atherogenic Index (PAI) when IDF definition was used.

Conclusion

In absence of local thresholds for components of the MS the use of waist: Hip ratio, LDL-C: HDL-C, PAI, and insulin level might help diagnosis. More studies are needed to reach accurate definition of MS in Saudis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P541

### Comparison of plasma osteoprotegerin and related factors in two groups of pregnant women

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Objective

The aim of the present study was to determine the plasma osteoprotegerin (OPG) levels in women with gestational diabetes mellitus (GDM) and normal glucose tolerance (NGT) during the last trimester of pregnancy, and to investigate the relationship between OPG and some related factors.

Method

In this study, 65 women with GDM and 65 women with NGT were enrolled. Plasma concentration of OPG was measured by enzyme linked immunosorbent assay (ELISA). The level of fasting plasma glucose (FPG), fasting insulin(FINS), glycated hemoglobin (HbA1c), lipid, high-sensitivity C-reactive protein (hs-CRP), blood cell count was also assayed, and the homeostasis model assessment of beta-cell function (HOMA-B) and insulin resistance (HOMA-IR) was calculated.

Results

The level of plasma OPG was not significantly different between women with GDM and NGT. In the group of NGT, OPG levels were positively correlated with FINS ($r=0.335$, $P=0.012$), HOMA-IR ($r=0.363$, $P=0.006$), platelet count ($r=0.333$, $P=0.009$) and apolipoprotein B ($r=-0.254$, $P=0.043$).

Conclusion

OPG may be involved in insulin resistance and inflammation during the pregnance in women with NGT, the effects were not obvious in the group of GDM. One possible explanation was that OPG tended to have a weaker effect on insulin resistance and inflammation and the role of OPG may be covered by other stronger factors in the pathogenesis of GDM.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P542

### Protein from Hevea brasiliensis latex rubber tree enhances wound healing in diabetic rats

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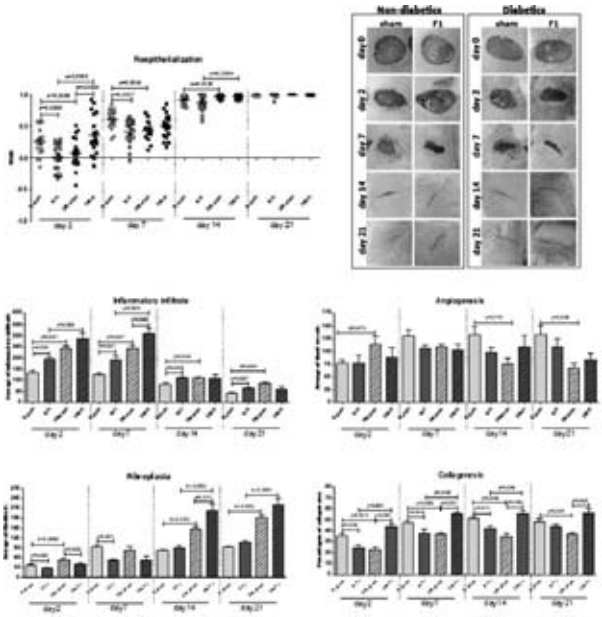
The healing effects of Hevea brasiliensis latex biomembrane has been widely cited in the literature, mainly regarding its protein fraction F1 in patients with diabetes. The purpose of this study was to evaluate the wound healing mechanism of F1 in diabetic rats. Eighty Wistar rats were used for the experiments, where: 40 rats were induced to diabetes by streptozotocin 45 mg/kg (DM) and 40 rats were left non-diabetic (N). Two wounds were created on the dorsal of each rat using a skin biopsy punch (1.5 mm diameter). The groups were assigned according to treatments: Carboxymethylcellulose (CMC) Gel 4% [DM sham, N sham ($n=20$)] and CMC +0.01% F1 [(DM F1, N-F1 ($n=20$)]. Wounds were treated daily, over 21 days. On the 2nd, 7th, 14th and 21st days, animals were euthanized and 10 biopsies/group/day were collected. Wounds reepithelialization was assessed and histological parameters (inflammatory cells, blood vessels, fibroblasts and the percentage of collagen area) were analyzed by HE and Gomori's trichrome

staining. DM-F1 group showed higher reepithelialization rates than DM-sham ($p=0.0026$) and all wounds were re-epithelialized on the 14th day, different from N-F1 group. In a similar way, DM-F1 group showed higher collagenesis then DM-sham ($p=0.0001$), different from N-F1; fibroplasia was more evident for DM-F1 ($p=0.0001$) on the 14th and 21st days. Interestingly, these results can be associated with higher reepithelialization of wounds treated with DM-F1. The metabolic status of diabetes induced chemotaxis of inflammatory cells to the skin, that increased with the injury up to the 7th day ($p=0.0026$), mainly when wounds were treated with DM-F1. Angiogenesis was more pronounced for DM-F1 group on the 7th an 14th days. The inflammatory process triggered by the metabolic stress of diabetes mellitus seemed to add and interact with the stimulus induced by F1, accelerating the cutaneous wound healing when compared to non-diabetic groups.

Distribution of variables reepithelialization by Wound Healing Rate (WHR), inflammatory infiltrate, angiogenesis, fibroplasia and collagenesis of wounds treated topically with F1 (F1 groups) and non-treated (sham groups) in non-diabetic (N-F1 ou N-sham) and diabetic (DM-F1 or DM-sham) rats, for 2, 7, 14 and 21 days of follow-up.



Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P543

**Prevalence of Microalbuminuria in Galicia (NW Spain) and its association with risk factors: Hypertension, Type 2 diabetes mellitus and Pre-diabetes.**

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Introduction
Microalbuminuria tends to appear in Type 2 diabetes (T2D) and reflecting underlying cardiovascular disease rather than diabetic nephropathy presented at diagnosis. The objective of this study was to evaluate the prevalence of microalbuminuria and its possible relations with T2D, pre-diabetes and hypertension, in a representative sample ($n=2860$) of the adult population ($>18$ years old) of Galicia (NW Spain).
Material and methods
Subjects were selected through a two-step cluster sampling procedure from the Galician public health service database, which covers more than 95% of the population. The criteria recommended by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2002) were used to establish the existence of T2D or pre-diabetes (presence of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or both). Chi-square and/or exact Fisher tests and odds ratio association measure were used for assessing the association of categorical covariates. Statistical significance was indicated by $p<0.05$.
Results
The overall prevalence of microalbuminuria in the adult Galician population, defined as persistent albumin excretion between 30–300 mg/day or 20–200 µg/min, was 4.7%, with significant differences by age ($p<0.001$), but without differences by gender ($p=0.095$).
14.9% of the diabetic subjects had microalbuminuria and it increased to 8.1% among hypertensive subjects. This prevalence increased with age, being higher in males than in females (10.2% versus 5.2%). Considering diabetes + hypertension, prevalence increased up to 19.4%. All of these pathologies showed a significant association with microalbuminuria ($p<0.001$ in all cases), Hypertension: OR = 2.393 (1.672–3.426); Diabetes: OR = 4.445 (2.914–6.781); Hypertension + Diabetes: OR = 5.869 (3.663–9.403). However, there wasn't association between pre-diabetes and microalbuminuria ($p=0.423$).
Conclusions
The prevalence of microalbuminuria in Galicia was 4.7%. Significant association was observed between microalbuminuria and T2D and HTA. No significant relation was observed with pre-diabetes, thus it's very important to prevent the transition from pre-diabetes to T2D or to early detection/control of high blood pressure.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P544

**Sarcopenic phenotype in patients with newly diagnosed type 2 diabetes**

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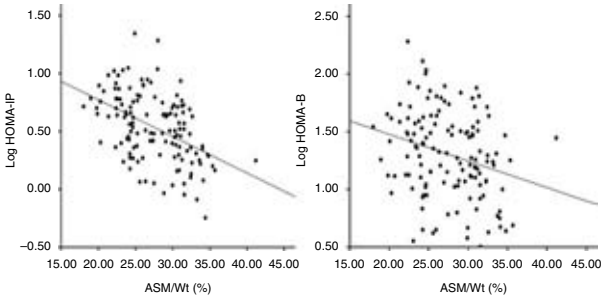
Objective - Sarcopenic phenotype is associated with the increased risk of insulin resistance and type 2 diabetes. We investigated the proportion of sarcopenia and sarcopenic obesity (SO) and their clinical characteristics in patients with newly diagnosed type 2 diabetes.
Research design and methods - We conducted a cross-sectional analysis of 133 (75 men, 58 women) patients with newly diagnosed type 2 diabetes. All patients underwent a 75 g oral glucose tolerance test. Sacopenia was assessed by appendicular skeletal muscle mass(ASM)/weight (%) using dual-energy X-ray absorptiometry and obesity was assessed by a visceral fat area using computed tomography.
Results - The mean age of the subjects was $47\pm12$ years, the mean HbA1c level was $10.59\pm1.94\%$ and the mean body mass index was $25.5\pm3.4$ kg/m$^2$. ASM/weight (%) was inversely associated with the log-transformed homeostasis model assessment of insulin resistance (HOMA-IR) and HOMA-β. The proportion of obesity, sarcopenia and SO was 23.3%, 16.5% and 35.3%, respectively. Sarcopenic phenotype was more prevalent in women than in men (Sarcopenia 29.3% vs. 6.7% and, SO 44.8% vs. 28%, respectively). In comparison to normal phenotype (neither obese nor sarcopenia), SO was significantly associated with higher degree of HOMA-β and the surrogate markers of insulin resistance (HOMA-IR and insulin sensitivity index composite). Notably, SO subjects were more insulin resistant compared to obese without sarcopenia subjects, although there was no significant difference in HbA1c between two groups.
Conclusions - We found the high proportion of sarcopenia and SO in patients with newly diagnosed type 2 diabetes. Among metabolic phenotype, SO was strongly associated with the degree of insulin resistance and compensated hyperinsulinemia in the early phase type 2 diabetes.

**Table 1** The proportion of each metabolic phenotype in patients with newly diagnosed type 2 diabetes

| | Male(%) | Female(%) | Total(%) |
|---|---|---|---|
| Normal | 24(32.0) | 9(15.5) | 33(24.8) |
| Sarcopenia | 5(6.7) | 17(29.3) | 22(16.5) |
| Obesity | 25(33.3) | 6(10.4) | 31(23.3) |
| Sarcopenic Obesity | 21(28.0) | 26(44.8) | 47(35.3) |
| total | 75(100) | 58(100) | 133(100) |




**Relationship of sarcopenic index with log HOMA-IR and log HOMA-B**

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P545

### Type 2 diabetes mellitus and Ramadan fasting: a continuous glucose monitoring (CGM) study of glucose excursions in a group of patients with good glycaemic control

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Introduction
Muslims including many patients with diabetes mellitus practice dawn to sunset fasting during the month of Ramadan. Using CGM recorded data we have investigated glucose excursions with Ramadan fasting in a group of patients with type 2 diabetes mellitus and good glycaemic control.
Methods
Thirty-four patients with type 2 diabetes mellitus (8 female, 26 male, age 46.5±11.1 years, HbA1c 6.9±1.0%, BMI 29.5±6.5 kg/m$^2$) underwent CGM for a minimum of three consecutive days during Ramadan fasting. Comparison with non-fasting CGM was made. Diabetes treatment ranged from none to a mixture of insulin and oral hypoglycaemic agents. Any change in treatment dose/timing during Ramadan followed ADA guidelines. Using CGM sensor values for average (avG), minimum (minG) and maximum (maxG) glucose, duration of time below lower limit (<80 mg/dl-DBL), within limit (80–150 mg/dl-DWL) and above higher limit (>150 mg/dl-DAL) for each patient, mean & SD values were obtained for the group as a whole during and outside Ramadan fasting periods. Comparison was made using paired t-test (SPSS 20).
Results
The group had good glycaemic control as indicated by HbA1c and mean CGM glucose. There were no statistically significant differences in avG, minG, maxG, DBL, DWL or DAL outside and during Ramadan fasting (Table 1). During Ramadan fasting period mean CGM curve showed a characteristic rapid rise in CGM recorded glucose at iftar time.
Conclusion
In this group of patients with good glycaemic control, overall CGM measures of glucose control and excursions show no significant changes with Ramadan fasting. However, timing and pattern of glucose fluctuations were different with a major rise at time of evening meal. Management during Ramadan should specifically address these areas.

**Table 1**

| | Non-fasting | Fasting (Ramadan) | *P* |
|---|---|---|---|
| avG (mg/dl) | 138.5±37.1 | 140.5±29.0 | 0.67 |
| minG (mg/dl) | 70.8±27.0 | 69.0±15.6 | 0.72 |
| maxG (mg/dl) | 232.8±72.0 | 246.8±72.2 | 0.37 |
| DAL (%) | 34.6±31.6 | 31.1±25.7 | 0.47 |
| DWL (%) | 58.7±30.1 | 65.9±23.7 | 0.11 |
| DBL (%) | 7.9±15.9 | 2.7±4.1 | 0.066 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P546

Abstract withdrawn.

## P547

### High GADA titre: a marker of insulin dependence in adult onset autoimmune diabetes

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Introduction
In latent autoimmune diabetes of adults (LADA), the progression to insulin-dependent diabetes is usually faster than in type 2 diabetes (T2DM) but the factors influencing this progression are not completely known. The aim of the present study was to determine whether GADA could be used as predictors of insulin dependence and whether GADA titre could define the risk of progression to insulin therapy in LADA patients. We looked for other biochemical and clinical parameters associated with early development of insulin dependence.
Methods
Adult onset GADA positive autoimmune diabetes subjects (n=191) were selected from the Non Insulin Requiring Autoimmune Diabetes (NIRAD) cohort of 4,250 T2DM subjects. GADA titre showed a bimodal distribution which identified two subgroups of patients with high (>32 GADA U) or low GADA titre (≤32 GADA U). One hundred ninety one GADA positive patients were followed for 6 years from diagnosis to evaluate the progression of patients started insulin therapy. Kaplan-Meir curves were plotted and log-rank test was performed to identify possible markers capable to influence the progression to insulin dependence. During the follow up 6/191 GADA positive patients dropped out from the study.
Results
The number of LADA patients who required insulin therapy was 93/191 (48.7%). We observed, that a significant higher number of high GADA titre patients 61/93 (65.6%) progressed to insulin dependence within 6 years of diagnosis compared to low GADA titre patients 32/93 (34.4%) (p=0.003). LADA patients with BMI<25 kg/m$^2$ could have a faster progression towards insulin therapy compared to patients with BMI ≧ 25 kg/m$^2$ (p=0.07).
Conclusion
We observed that neither TPO Ab titre neither gender were predictive markers of progression to insulin dependence. High GADA titre is a marker of progression towards insulin dependence in LADA patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P548

**Aerobic and resistance exercise have different short-term effects on glucose levels in type 2 diabetes subjects**

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Introduction
Aerobic (AER) and resistance (RES) exercise similarly improve HbA1c levels in type 2 diabetes subjects. However, it is still unknown whether exercise-induced acute changes in blood glucose differ according to exercise type. Our aim was to compare glucose level changes after a single bout of AER or RES exercise in trained diabetic subjects.
Methods
Twenty-five subjects participating in the RAED2 Study, a RCT designed to compare the effects of AER and RES training in diabetic patients, were submitted to a continuous glucose monitoring during a 60-min exercise and the following 48-h. Glucose concentration areas under the curve (AUC) during exercise, the subsequent night, and the 24-h period following the exercise, as well as the corresponding periods of the non-exercise day, and the low (LBGI) and high (HBGI) blood glucose indices, which summarize duration and extent of hypoglycaemia or hyperglycaemia, respectively, were assessed.
Results
AER and RES groups showed similar HbA1c improvements after training. However, comparison of glucose AUC during the 60-min exercise and the corresponding period of the non-exercise day showed different behaviors between groups (time-by-group interaction, $p=0.04$), glucose being lower during exercise in the AER but not in the RES group. Similar differences between groups were observed in the comparison of nocturnal periods of exercise and non-exercise days ($p=0.02$). Consistently, in the AER group nocturnal LBGI was higher in the exercise day than in the non-exercise day ($p=0.012$), whereas there were no differences in the RES group.
Conclusion
Although AER and RES training show similar long-term metabolic effects in diabetic subjects, the short-term effects of single bouts of these exercise types differ, with a potential increase in risk of late-onset hypoglycaemia after AER exercise. These findings suggest that, in diabetic subjects, medications and diet adjustments to exercise should take into account the scheduled type of physical activity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P549

**Regulation of somatostatin/cortistatin and ghrelin systems under diet induced obesity conditions at the mouse endocrine pancreas**

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Somatostatin (SST), Cortistatin (CORT), ghrelin, and their corresponding receptors (ssts and GHS-R) are closely interrelated systems involved in the regulation of essential hormones for metabolic homeostasis, like insulin, whose release is inhibited by SST/CORT and stimulated by ghrelin. To investigate the underlying mechanisms of this insulin regulation, we analyzed the expression of such systems at pancreatic level in wild type (WT) and CORT knock-out mice (CORT-KO) under both normal, low fat (LF) and high fat (HF) diets. Pancreatic tissue was processed for either direct RNA isolation or islet culture, and expression of distinct components of SS/CORT/ghrelin systems was analyzed by quantitative RT-PCR. Results reveal that SST, ghrelin and their receptors are expressed in both whole pancreas and endocrine islets of WT and CORT-KO mice, being their expression levels variable depending upon the peptide and receptor subtype analyzed. CORT expression was not detected in neither whole nor endocrine pancreas of WT-mice. SST levels remain unaltered in obese animals (HF), while ghrelin expression increased exclusively in WT obese mice. When the expression of the systems analyzed was compared between WT and CORT-KO mice, we found that expression levels of ghrelin, GHS-R, ghrelin o-acyl-transferase (GOAT) and insulin (In-2) were significantly reduced in obese mice. Interestingly, blood insulin levels were significantly lower in CORT-KO than those of WT animals. Taken together, our data reveal that key components of the SST/ghrelin/receptors systems are tightly regulated in the pancreas of WT and CORT-KO under normal and obesity conditions, suggesting that both systems may play a relevant role at the endocrine pancreas under normal physiological conditions and/or in response to extreme metabolic conditions (obesity). Moreover, the fact that circulating insulin levels are significantly diminished in CORT-KO mice strongly suggests that endogenous CORT is an important modulator of pancreatic function.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P550

**Influence of iron metabolism indices on HbA1c in non-diabetic pregnant women with and without iron-deficiency anemia: effect of iron supplementation**

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Aims
Condition that influence erythrocyte turnover also affect HbA1c. Although many forms of anemia are associated with lowering of HbA1c, iron-deficiency anemia (IDA) tends to increase HbA1c. In this study, we examined the relationship between HbA1c and erythrocyte indices in non-diabetic pregnancy and assessed the effect of iron supplementation on HbA1c.
Methods
150 women were studied (30 non-diabetic, non-pregnant, non-anemic women in child bearing women with varying parity as controls (Gp 1); 30 non-diabetic, non-anemic pregnant women in first trimester of pregnancy (Gp 2a); 30 non-diabetic, non-anemic pregnant women in second trimester of pregnancy (Gp 2b); 30 non-diabetic, non-anemic pregnant women in third trimester of pregnancy (Gp 2c) and 30 non-diabetic pregnant women with IDA (Gp 2d). HbA1c, OGTT, erythrocyte indices and iron metabolic indices were determined in Gp 2d subjects not supplemented with iron and repeated these indices after 3 months of iron-supplementation.
Results
The mean fasting and postprandial blood glucose levels ($79.9\pm8.0$ mg/dl, $108.1\pm14.1$ mg/dl) in Gp 1 were found to be significantly lower in first trimester among Gp 2a ($74.4\pm5.3$ mg/dl and $97.2\pm11.1$ mg/dl, in second trimester among Gp 2b ($76.2\pm5.2$ mg/dl and $103.4\pm7.9$ mg/dl) followed by increase in IIIrd trimester among Gp 2c ($82.3\pm5.7$ mg/dl and $112.5\pm8.5$ mg/dl) subjects. A significant difference in HbA1c was also observed among the groups (HbA1c $4.7\pm0.3\%$ in Gp 1; $4.6\pm0.4\%$ in Gp 2a; $4.5\pm0.3$ in Gp 2b; $4.7\pm0.3$ in Gp 2c). Among Gp 2d subjects, HbA1c was $5.2\pm0.3\%$ and the level decreased after iron supplementation to $5.1\pm0.3\%$. Significant correlation between erythrocyte indices, iron metabolic indices and HbA1c was also observed. We conclude that consideration should be given for performing glucose testing in patients with IDA to ascertain the reliability of HbA1c in the diagnosis of diabetes. HbA1c concentrations in diabetic patients with IDA should be interpreted with caution
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P551

**Both β-cell dysfunction and insulin resistance are primary determinants of diabetes mellitus in patients with liver cirrhosis candidate to organ transplant**

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Background
Impaired glucose (G) regulation (IGR) and diabetes mellitus (DM) are common in patients with liver cirrhosis. DM development has been associated to advanced

liver disease (LD) and HCV infection. The relative roles played by insulin secretion and action, advanced LD and HCV infection in DM are somewhat unclear.
Aim of the study
To assess in cirrhotic patients candidate to organ transplantation with no history of G abnormalities: i) prevalence of altered G homeostasis; ii) β-cell function (βF); and iii) insulin resistance.
Materials and methods
104 patients, 33M/71F, age 53±9 years, with liver cirrhosis (HCV +/HCV− = 44/60), with normal fasting G (5.2±0.11 mmol/l) and with HbA1c of 4.9± 0.08%. βF was assessed by state-of-art modeling of G/C-peptide curves during a 180′ sampled OGTT, thereby providing the β-cell responses to the rate of G increase (derivative control: DC) and to G concentration (proportional control: PC). Insulin resistance was estimated by the HOMA index (HOMA-IR).
Results
13 patients had normal G regulation (NGR), 35 had IGR, 56 had DM. HOMA-IR steadily increased with declining G tolerance ($P=0.065$), and it was higher in HCV+ than in HCV- patients (5.7±0.7 vs 4.5±0.5, $P=0.023$). Both DC and PC gradually declined from NGR to IGR to DM ($P<0.01$ for both). Child-Pugh score, HCV infection and alcoholic LD were neither related to alterations in G regulation ($P=0.13$–$0.99$) nor to βF ($P=0.55$–$0.88$). In a multivariate model, HOMA-IR ($P=0.014$) and betaF ($P=0.017$), but no LD related parameter ($P=0.40$–$0.78$), were independent 'predictors' of DM.
Conclusions
In cirrhotic patients, altered G homeostasis, including DM, is due primarily to alterations in both βF and insulin action, neither of which apparently is influenced by alcohol or severity of LD. HCV infection might play a role in deteriorating G homeostasis via insulin resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P552

### The effects of fat distribution and some adipokines on insulin resistance in prediabetic patients

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Introduction
In this study, we aimed to evaluate the effects of fat distribution and some adipokines on insulin resistance (IR) in prediabetic patients.
Materials and Methods
cases were divided into three groups according to 75 gr-oral glucose tolerance test (OGTT) results [27 cases impaired fasting glucose (IFG), 36 cases impaired glucose tolerance (IGT) and 24 healthy control subjects]. Serum fasting insulin levels and lipid parameters were measured. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated. Body fat mass measurements were assessed by TANITA. Abdominal fat distributions (visceral, subcutaneous, preperitoneal fat thickness) were evaluated by ultrasonography. The fasting serum levels of adipokines [adiponectin, leptin, resistin, vaspin, visfatin, retinol-binding protein 4 (RBP4), tumor necrosis factor-α (TNF-α)] were measured by ELISA method.
Results
There were no differences between groups in terms of the age, sex, body mass index (BMI), mean insulin, lipid parameters, vaspin, RBP4, leptin, resistin, TNF-α, abdominal fat distributions, fat mass measurements. There were significant differences between groups in terms of HOMA-IR values, adiponectin and visfatin (NGT-IFG groups $P>0.05$, $P<0.001$, $P<0.05$; NGT-IGT groups $P<0.05$, $P<0.01$, $P<0.001$ respectively). There was significant correlation between HOMA-IR and BMI values ($r=0.367$, $P<0.01$), waist circumference ($r=0.361$, $P<0,05$), OGTT 2nd hour-glucose ($r=0.282$, $P<0.05$), total fat percentage ($r=0.308$, $P<0,05$), total fat mass ($r=0.411$, $P<0.01$), visceral fat thickness ($r=0.395$, $P=0.01$), adiponectin ($r=-0.322$, $P<0.05$) and resistin ($r=0.245$, $P=0.05$) levels in the prediabetic group. In regression analysis, visceral fat thickness was found to be an independent risk factor for development of insulin resistance ($P<0.001$, B=0.438, 95% CI 0.01–0.039).
Conclusion
In this study, we concluded that adiponectin and resistin affected the insulin resistance in prediabetic patients and especially the increments in visceral fat thickness had negative effects on insulin resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P553

### New fast and reversible leptin antagonist-induced mice model of metabolic syndrome and T2DM

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Obesity and its major consequence, type II diabetes mellitus (T2DM), is epidemic throughout Western society. T2DM accounts for 95% of the diabetes worldwide. One limitation to the development of new diabetes treatments has been a lack of effective animal models to use in research. There are no rodent models that recapitulate the pancreatic β-cell lesions of humans with T2DM. Moreover, animal models of obesity either require overfeeding which is expensive and takes weeks to months to establish, or specific genetic mutations that cause lifelong metabolic dysfunction. Thus the ability to rapidly induce obesity in healthy rat and mouse strains would be a major advance that could enhance research in diabetes and obesity and the development of novel therapies. We have recently developed superactive mouse leptin antagonist (D23L/L39A/D40A/F41A). Mono-pegylated antagonist (PEG-SMLA) is orexigenic, and when given to mice every 24–48 h for 2–3 weeks leads to weight gain of nearly 50% with primarily fat accumulation. PEG-SMLA -induced weight gain is accompanied by elevated fasting glucose and glucose intolerance with hyperinsulinemia, higher plasma cholesterol and TGs, and a dramatic rise of corticosterone. These changes were fully reversible with cessation of PEG-SMLA injections, and disappeared within 10–14 days. 1 month exposure to PEG-SMLA altered the expression of key regulatory genes in adipose tissue, muscle and brain. These findings indicate that leptin antagonism induces systemic dysmetabolism in a rapid, practical manner, and provides a valuable tool for research in obesity and diabetes.

**Table 1** Relative expression of several genes in adipose tissue, brain and muscle.

| Gene | Adipose tissue | | Brain | | Muscle | |
|---|---|---|---|---|---|---|
| | Control | Peg-SMLA | Control | Peg-SMLA | Control | Peg-SMLA |
| Leptin | 1.28 | 5.57† | NT | NT | NT | NT |
| LPL | 1.05 | 2.29* | NT | NT | NT | NT |
| LeptinR | 1.05 | 0.23* | 1.00 | 0.94 | 1.06 | .88 |
| UCP2 | 1.06 | 2.42* | 1.00 | 0.58‡ | 1.08 | 1.40 |
| UCP3 | 0.82 | 1.85* | 1.00 | 0.58‡ | 1.12 | 3.48† |
| PPAR-gamma | 1.01 | 4.85* | NT | NT | NT | NT |
| PHB | 1.01 | 2.55 | NT | NT | NT | NT |
| CASP3 | 1.05 | 0.45* | NT | NT | NT | NT |
| GLUT4 | 1.03 | 0.45* | NT | NT | 1.00 | 1.83† |
| Rcan2 | NT | NT | 1.00 | 1.24* | 1.01 | 1.12 |
| NPY | NT | NT | 1.00 | 1.64* | NT | NT |

*$P<0.05$; †$P<0.01$; ‡;$P<0.001$; NT-not tested

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P554

### High-fructose diet, an animal model of insulin resistance, causes mitochondrial dysfunction by altering the activity of respiratory chain complex I

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Introduction
The current epidemic of Type 2 Diabetes Mellitus (T2DM) and obesity is positively correlated with a rise in consumption of high-fructose syrups and refined carbohydrates. Recent reports have showed that feeding rodent a high-fructose diet leads to insulin resistance, glucose intolerance and dyslipidaemia. Nevertheless, its mitochondrial effects has not been fully studied yet.
Aim
We decided to explore the alterations in mitochondrial bioenergetics induced in high-fructose fed rats, as well as the underlying mechanisms.
Materials and methods
Male Wistar rats were separated in two different groups and received either a standard diet or the high-fructose diet during 6 weeks. Liver mitochondria were isolated from fed animals by standard differential centrifugation. Oxygen consumption rates were measured at 30 °C using a Clark-type oxygen electrode whereas ROS production was assayed by incubating mitochondria in a stirred 2 ml volume with 10 UI horseradish peroxidase and 2 μM Amplex Red. Moreover, the activity of mitochondrial respiratory chain complexes I, II and III were spectrophotometrically determined.
Results
High-fructose diet inhibited oxygen consumption (JO2) when glutamate and malate (GM) or succinate and malate (SM) were used as energetic substrate. This inhibition affected the state three of respiration (in the presence of ADP) and the uncoupled state (after addition of dinitrophenol). On the other hand, after addition of rotenone that stimulates ROS production in presence of GM, mitochondria isolated from fructose-fed rats showed lower ROS production. By using SM as respiratory substrate, high-fructose diet also reduced ROS production. Finally, we found that complex I activity was significantly inhibited by feeding a high-fructose diet.
Conclusions
Our data show that high-fructose diet diminishes respiratory complex I activity leading to a reduction of both ROS production and mitochondrial respiration. This new finding could contribute to the knowledge of the mechanisms involved in mitochondrial dysfunction in type two diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P555

### Does the presence of thyroid antibodies affect the course and outcome of pregnancy in type1 diabetic women?

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Introduction
In the literature there are only three papers so far, addressing the impact of thyroid antibodies (Anti-TPO) on pregnancy in Type1 Diabetic Women (DM1) and these present conflicting results. The aim of the study is to evaluate the presence of Anti-TPO in DM1 pregnant women and whether these are related with differences in thyroid function, metabolic control and pregnancy outcome.
Methods
In 78 DM1 women with singleton pregnancies Anti-TPO, Anti-Tg, TSH, FT4I (T4/TBC) were measured each trimester. At each visit (every 1–2 weeks) blood glucose, HbA1c, BMI, units of insulin/kg were recorded, as were complications and pregnancy outcome.
Results
27/78 women (34.6%) presented with positive Anti-TPO Abs. Clinical data of Anti-TPO positive and negative women are as shown in the table.
First trimester TSH levels were statistically different between the two groups.
There were no differences in the prevalence of diabetic complications, gestational hypertension-preclampsia, abortions or preterm deliveries.
Conclusion
One third of DM1 pregnant women presented with positive Anti-TPO Abs. However their presence is not related with worse metabolic control or adverse pregnancy outcome. It seems that early treatment of thyroid dysfunction and stricter metabolic control a plays more important role than the presence of thyroid antibodies with regard to the pregnancy outcome.

**Table 1**

| | Anti-TPO positive ($n=21$) x±S.D. | Anti-TPO negative ($n=57$) x±S.D. | P |
|---|---|---|---|
| Age(years) | 29.1±4.5 | 28.8±4.9 | NS |
| Duration(years) | 11.2±8.2 | 11.6±7.7 | NS |
| BMI(kg/m$^2$) | 23.7±0.9 | 23.9±2.9 | NS |
| TSH 1$^{st}$ trimester(μIU/ml) | 2.4±1.6 | 1.3±1.0 | 0.005 |
| TSH 2$^{nd}$ trimester(μIU/ml) | 2.5±1.8 | 1.7±1.1 | 0.0058 |
| TSH 3$^{rd}$ trimester(μIU/ml) | 1.5±0.8 | 1.7±0.9 | NS |
| FT4I 1$^{st}$ trimester | 95.0±12.7 | 101.2±20.8 | NS |
| FT4I 2$^{nd}$ trimester | 112.6±24.5 | 110.3±24.1 | NS |
| FT4I 3$^{rd}$ trimester | 106.1±14.4 | 105.1±18.1 | NS |
| HbA1c 1st trimester(%) | 6.5±1.2 | 6.5±1.7 | NS |
| HbA1c 2nd trimester(%) | 5.3±0.9 | 5.4±0.9 | NS |
| HbA1c 3rd trimester(%) | 5.2±0.8 | 5.2±0.6 | NS |
| Ins 1$^{st}$ trimester(IU/kg) | 0.7±0.3 | 0.7±0.2 | NS |
| Ins 2$^{nd}$ trimester(IU/kg) | 0.9±0.5 | 0.8±0.3 | NS |
| Ins 3$^{rd}$ trimester(IU/kg) | 1.1±0.7 | 1.0±0.4 | NS |
| Birth weight(g) | 3215.0±408..3 | 3092.3±688.0 | NS |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P556

### The association between insulin-like-growth-factor I and insulin resistance: a general population study in danish adults

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Objective
IGF1 has almost 50% amino acid sequence homology with insulin and elicits nearly the same hypoglycaemic response. Studies showed that low and high IGF1 levels are related to impaired glucose tolerance and to a higher risk of type two diabetes. The aim of the present study was to evaluate the association between IGF1 level and insulin resistance in a Danish general population.
Research Design and Methods
From the cross-sectional Health2006 study 3354 adults aged 19 – 72 years were included. The homeostasis model assessment of insulin resistance (HOMA-IR) was used as the index to estimate insulin resistance. Serum IGF1 levels were determined by an immunoassay and grouped into quintiles (Q1–Q5). Linear or multinomial logistic regression analyses were performed.
Results
In the study population 520 subjects (15.5%) had increased HOMA-IR values above 2.5. After adjustment for age, sex, physical activity and waist-to-height ratio, an U shaped association between IGF1 and HOMA-IR was found. Low IGF1 [Q1: OR 1.64 (95% CI 1.15; 2.33), $P<0.01$] as well as high IGF1 [Q5: OR 1.96 (95% CI 1.38; 2.79), $P<0.01$] levels were related to a higher odds of increased HOMA-IR values compared to subjects with intermediate (Q3) IGF1 levels. These associations remained statistically significant after the exclusion of subjects with type two diabetes mellitus and by using the updated computer HOMA2-IR model.
Conclusions
In conclusion, low- as well as high-normal IGF1 levels, are both related to insulin resistance. The biological mechanism of this complex phenomenon has to be elucidated in more detail for future risk stratification.

Declaration of interest
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## P557

Abstract withdrawn

## P558

**Alterations in fecal lactobacillus and bifidobacterium species are associated with differences in cholesterol metabolism in type 2 diabetic patients**

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Background
Type 2 diabetic (T2D) patients have alterations in the gut microbiota composition, compared to healthy individuals. Such alterations may play a role in the pathogenesis of T2D and insulin resistance. Bifidobacteria and Lactobacillus are among the most predominant bacteria found in human gut. They can also easily be added in probiotic preparations, thus making them ideal agents for therapeutic interventions. In this study, we aimed to determine if there were alterations in a selected group of fecal Lactobacillus and Bifidobacterium species in T2D patients compared to a control group of patients, and to determine if there were metabolic measures associated with any of bacterial species in the T2D patients.
Methods
Fifty patients with T2D and thirty control individuals of similar BMI were recruited from Southern China. The bacteria species were chosen because of their relevance to probiotic preparations; their fecal amount was measured by real-time quantitative PCR. Insulin sensitivity was measured by an OGTT.
Results
T2D patients had significantly ($P<0.05$) more total Lactobacillus (+17%), L. bugaricus (+13%), L. Rahmnosum (+37%) and L. acidophilus (+17%). In contrast, they had less amounts of total Bifidobacterium (−7%) and B. adolescentis (−12%) ($P<0.05$ both). Cluster analysis showed that gut microbiota pattern of T2D patients is characterized by greater number of *L. Rhamnosus* and *L. Acidophillus*, together with lesser number of B. adolescentis ($P<0.05$). Several of these bacteria were significantly associated with total cholesterol levels ($P<0.05$).
Conclusion
The gut microflora in T2D patients is characterized by greater numbers of Lactobacillus and lesser numbers of Bifidobacterium species. The changes are associated with cholesterol levels suggesting that they play a role in influencing cholesterol metabolism in T2D patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P559

**Low testosterone is associated with decreased expression of glut-4 and hexokinase 2 in muscle of the testicular feminised mouse**

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Testosterone deficiency is a common in men with type two diabetes (T2D). We have shown testosterone replacement therapy (TRT) improves insulin resistance and glycaemic control. The mechanisms by which testosterone mediates this action are unknown but may be due to a combination of effects on muscle, liver and adipose tissues. This study investigates the expression of Glut4 and HK2, (two key proteins involved in insulin sensitivity) in muscle tissue of the testicular feminised (Tfm) mouse which exhibit non-functional androgen receptors and low circulating testosterone.

Tfm mice were fed a high-cholesterol diet ad libitum for 28 weeks and received either physiological testosterone replacement (mixed testosterone esters, Sustanon100) or placebo (saline) and were compared to wild-type littermates (WT). Striated muscles tissue was collected from the thigh. Expression of Glut 4 and HK2 mRNA and protein expression was analysed by qPCR and Western blotting.

There was a significant decrease in the relative mRNA expression of Glut−4 ($0.59\pm0.14$, $P=0.02$) and HK2 ($0.5\pm0.16$, $P=0.01$) in Tfm mice compared to WT. TRT did not significantly alter mRNA expression of Glut4 or HK2. Western blotting confirmed reduced protein expression of HK2 ($0.33\pm0.09$ vs $0.04\pm0.01$, $P=0.005$) and Glut4 ($1.05\pm0.22$ vs $0.57\pm0.07$, $P=0.037$) in Tfm mice compared to WT. TRT showed no change in HK2 protein expression compared to Tfm placebo ($0.05\pm0.02$ vs $0.04\pm0.01$, $P=0.627$), but an increase in Glut4 was observed ($1.09\pm0.16$ vs $0.57\pm0.07$, $P=0.005$).

Testosterone deficiency is associated with decreased expression of Glut4 and HK2. TRT did not have any effect on HK2 in the Tfm mouse suggesting that testosterone acts via the androgen receptor to stimulate HK2 expression. Although testosterone failed to increase Glut4 mRNA in the Tfm an increase in protein was observed indicating that post-translational mechanisms are involved. This study shows that testosterone has important actions on modulating glucose metabolism in muscle. This may explain part of the mechanism by which testosterone improves insulin sensitivity in T2D.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P560

**Serum visfatin is differentially regulated by insulin and non-esterified fatty acids in healthy men**

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Introduction
Visfatin is a protein secreted by adipose tissue which was discovered as a protein with insulin-mimetic properties. Experimental and clinical studies demonstrated that visfatin can be involved in the pathogenesis of insulin resistance. It was demonstrated that plasma visfatin is elevated in insulin resistant states i.e. obesity, type two diabetes mellitus, PCOS. *In vitro* study showed that insulin inhibits visfatin release from adipocytes. The aim of the present study was to evaluate serum visfatin concentration during hyperinsulinemia (6 h hyperinsulinemic euglycemic clamp) and than during insulin resistant conditions caused by an acute elevation of NEFA (6 h hyperinsulinemic clamp combined with Intralipid – heparin infusion).
Materials and methods
The study group consisted of 19 apparently healthy male volunteers (mean age $25.1\pm3.1$ years, BMI-$26.7\pm4.7$ kg/m$^2$). Clinical examination, anthropometric measurements, OGTT, plasma lipids and liver enzymes activity were measured. Subjects underwent 6 h euglycemic hyperinsulinemic clamp and after one week 6 h hyperinsulinemic euglycemic clamp combined with Intralipid – heparin infusion. Measurements of serum visfatin during both clamp studies were performed.
Results
6 h of insulin infusion during clamp resulted in significant decrease in serum visfatin concentration ($P=0.0057$), however after 2 h there was no change in

serum visfatin concentration ($P=0.097$). Concomitant Intralipid-heparin infusion which caused a significant increase in NEFA concentration ($P<0.0001$), resulted in marked increase in serum visfatin ($P=0.00035$) which was already observed after 2 h of Intralipid infusion ($P=0.00028$). Serum visfatin during clamp study after 2 h and 6 h of Intralipid infusion were significantly higher than respective values during clamp study without elevation of NEFA (both $P<0.0001$). The increase of serum visfatin during Intralipid infusion (δ visfatin) was positively related to body weight ($r=0.54$, $P=0.016$), %body fat ($r=0.48$, $P=0.036$) and GGTP ($r=0.56$, $P=0.011$).
Conclusion
Our data show that plasma visfatin is differentially regulated by insulin and NEFA. One might suggest that induction of insulin resistance by NEFA suppress insulin inhibition of visfatin production by adipose tissue, resulting in plasma visfatin increase in insulin resistant conditions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P561

**Mitochondrial impairment and oxidative stress in leukocytes from type 2 diabetic patients**

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Diabetes is associated with oxidative stress. This study evaluated the rates of oxidative stress and mitochondrial impairment in type 2 diabetes patients. The study population consisted of 182 diabetic patients and 50 body-composition- and age-matched controls. We assessed anthropometric and metabolic parameters and mitochondrial function by evaluating mitochondrial oxygen (O2) consumption, reactive oxygen species (ROS) production, glutathione (GSH) levels, GSH/GSSG ratio, mitochondrial membrane potential, and mitochondrial complex I activity in polymorphonuclear cells from diabetes type 2 patients. We found an increase in waist circumference and augmented serum levels of triglycerides, proinflammatory cytokines (IL-6 and TNF-α), homocysteine, glycated hemoglobin, ultrasensitive C-reactive protein, glucose, insulin, and homeostasis model assessment of insulin resistance score in diabetic patients versus controls. There was an impairment of mitochondrial function in diabetic patients, evidenced by a decrease in mitochondrial O2 consumption, an increase in ROS production, decreased GSH/GSSG ratio, a drop in GSH levels, and an undermining of the mitochondrial membrane potential. Furthermore, an impairment of mitochondrial complex I was detected. This study supports the hypothesis of an association of type 2 diabetes and the rate of impaired mitochondrial function. We also propose that one of the targets of oxidative stress responsible for diabetes is mitochondrial complex I.
Declaration of interest
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## P562

**High prevalence of autoimmunity in adult onset diabetes subjects with high GADA titre**

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Introduction
LADA is a heterogeneous population of diabetes subjects wherein subgroups can be identified based on their autoimmune status: the titre of autoantibodies to GAD.
The aim of the study was to correlate, in LADA subjects, GADA titre, with the presence of other organ and non organ specific autoantibodies.
Methods
LADA subjects ($n=191$) and type 2 diabetes (T2DM) subjects ($n=382$) were selected from the Non Insulin Requiring Autoimmune Diabetes cohort. GADA titre showed a bimodal distribution (high ($>32$ GADA U) or low GADA titre ($\leq 32$ GADA U). The following autoantibodies were measured: protein tyrosine phosphatase IA-2 (IA–2A), thyroid peroxidase antibodies, (TPO), steroid-21-hydroxylase (21OHAb), anti-parietal cell antibodies (APCA), tissue transglutaminase antibody IgA (tTGA). IA–2A, 21 OHAb, TPO, IgA, tTGA antibodies were measured by a radioimmunoassay and APCA by a ELISA method.
Results
Subjects with high GADA titer compared to low GADA titer showed a significantly higher prevalence of TPOAb (37.2 vs 16.5%; $P=0.002$), IA – 2AIC (25.5 vs 8.2%; $P<0.002$), APCA (23.3 vs 9.3%; $P<0.01$) and ZnT8 (29.7 vs 8.2%; $P<0.0003$). A significant decreasing trend was observed from high GADA titre to low GADA titer and to T2DM for the prevalence of TPO and tTGA ($P<0.001$ for both comparisons). 21OHAb showed a prevalence of 3.2% in high GADA titer and were not present neither in low GADA titer nor in T2DM subjects. The prevalence of tTGA was of 4% in high GADA titer, 1.2% in low GADA and 0.4% in T2DM ($P$ for trend $=0.001$, high GADA vs T2DM $P=0.03$).
Conclusion
High GADA titre, in LADA subjects, is associated with a profile of more severe autoimmunity. Previous studies indicate that the prevalence of multiple autoantibodies is a better index of disease progression than prevalence of antibodies directed at individual antigens. This may indicate intermolecular epitope spreading that can amplifies the autoimmune response.
Declaration of interest
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## P563

**Testing of type 2 diabetes risk locus rs7578597 in THADA gene in the czech population**

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Introduction
The SNP rs7578597 (Thr1187Ala) of THADA gene was identified in GWA studies as a novel type 2 diabetes risk locus. Nevertheless, subsequent studies of this SNP effect have been inconclusive. In some studies, rs7578597 has been associated with insulin secretion but no significant correlation with type 2 diabetes has been reported. Product of THADA gene is considered to be involved in apoptosis. Whether rs7578597 affects insulin secretion throught the apoptosis of pancreatic β-cells is, however, still unknown. We analyzed the genotype distribution of rs7578597 (TT/CT/CC) in 1084 individuals comprising 247 type 2 diabetes patiens (T2D), 275 women with gestational diabetes mellitus (GDM) and 562 non-diabetics (ND). The association of genetic variants in rs7578597 with anthropometric and biochemical parameters related to type 2 diabetes was examined.
Methods
The SNP rs7578597 was assessed by TaqMan SNP Genotyping Assay. Study cohort was anthropometrically and biochemically characterized, in subgroups GDM and ND 3 h OGTT was performed. For statistical analyses Mann-Whitney test, Chi-square test and ANOVA were used (NCSS 2004).
Results
The allelic frequencies did not differ among subgroups (minor C allele: T2D 23.5%; GDM 19.2%; ND 23.8%). C allele carriership in T2D and GDM subgroups was not associated with any of the studied parameters. Carriers of C allele in non-diabetic subgroup (ND) had decreased insulin levels: AUC insulin CC + CT vs TT $P=0.047$. Insulin secretion was significantly lower particularly in the late phase of OGTT: 90 min $P=0.039$; 120 min $P=0.019$.
Conclusion
We found out that the C allele carriership of rs7578597 is associated with lower insulin secretion in the Czech non-diabetic population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P564

**Impact of diabetes on length of stay and inpatient mortality for elderly patients presenting with proximal fracture of the femur**

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Aim
To compare inpatient length of stay (LOS) and mortality of diabetic and non-diabetic patients aged ≧65 years admitted in a single UK hospital with fractured proximal femur.
Methods
Patients with a fractured proximal femur at University Hospital of Birmingham (2007–2010) were identified from administrative data with ICD 10 discharge diagnostic codes S720, S721 and S722 as the primary diagnosis. A discharge diagnostic code for diabetes or a prescription for diabetes medication (excluding prescription for other clinical needs) was defined as diabetes. Univariate analysis (Mann-Whitney-U-test (LOS) and Chi2 (mortality)) and multivariate analysis (linear regression for LOS after log transformation and logistic regression for mortality) to adjust for age, gender, ethnicity, social class and Charlson co-morbidity score (excluding diabetes) were conducted to contrast and assess the impact of diabetes on fracture of proximal femur in elderly patients.
Results
Among 1468 admissions with fracture of proximal femur 197 had diabetes (13.4%). Diabetes patients had a higher median length of stay but this was not significant (20.2 vs 19.1 days; $P=0.17$). After adjustment for covariates diabetes patients had a significant impact on length of stay (regression coefficient β of log transformed data = 0.062; $P=0.05$). Inpatient mortality rates were higher in the diabetes patients (14.2 vs.12%) but it was not significant before ($P=0.37$) or after adjustment for covariates (Odds Ratio 1.11; 0.70–1.77). Increasing age and higher co-morbidity score were independently associated with increasing LOS and mortality ($P<0.001$).
Conclusion
Diabetes patients tend to have slightly longer length of stay compared to non diabetic patients presenting with fracture of proximal femur. Involvement of diabetes specialist team may have had a favourable outcome in reducing the length of stay and improving quality of care. Further study to understand the impact of diabetes on long term mortality (1 year) will be beneficial.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P565

**Screening of latent autoimmune diabetes in insulin requiring diabetic adults**

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Introduction
Latent autoimmune diabetes in adults (LADA) is a genetically linked, autoimmune form of type 1 diabetes mellitus (DM) that accounts for 2–12% of all cases of diabetes.
The goal of our study is to detect LADA among patients who were diagnosed as type 2 DM and who became insulin requiring.
Methods
Our study included 45 patients (15 men, 30 women) diagnosed as type 2 DM, who had a poor glycemic control on oral hypoglycemic drugs, and so became on insulin therapy.
These patients had systematically islet auto antibodies testing (anti glutamic acid decarboxylase 65 (antiGAD 65), anti islet cell cytoplasm (ICA) and anti tyrosine phosphatase like protein (IA–2A)).
Results
All our patients were aged more than 30 (mean age: 49 ± 11.3). They have been on oral hypoglycaemic drugs for at least one year (mean duration: 4 ± 2,7 years). They presented with poor glycemic control (mean HbA1c: 11.9 ± 2.36%) and they had an important weight loss (13 kg ± 9.4).
AntiGAD 65 were detected in 32.4% of our patients, ICA in 13% and IA–2A in 4,3%. 44% of patients with positive test presented with ketosis.
Conclusion
Approximately, 10 to 30% of adults diagnosed with type 2 DM are positive for islet auto antibodies, which means that they had in fact a LADA.
Single antibody positivity for GAD is more frequent than ICA and IA–2A, like in our study.
Our results are particularly interesting because they show a relatively high prevalence of LADA in our population. It is important to recognize these patients to prevent complications like ketosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P566

**Epidemiological study of patients attended the pediatric outpatient clinic of national institute of diabetes & endocrinology**

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Background
Diabetes mellitus is the most common endocrine metabolic disorder of childhood. It is widely spread all over Egypt as its prevalence was found to be 1.09 per 1000 among school aged children.
Aim of the work
The aim of this work was to do a retrospective epidemiological study of the records of diabetic children attended the outpatient pediatric clinic in NIDE.
Subjects & Methods
The files 0f 3000 diabetic children were examined retrospectively without any reference to the personal data.
Results
The results showed that there is no statistical differences between male, ($n=1528$) to female, ($n=1472$) distribution. The results of this retrospective study showed that the mean age of onset of diabetes in children attended the outpatient pediatric clinic of National Institute of Diabetes was (mean = 8.37 + 10.96 y). The present study showed also that there was a decrease of age of onset of diabetes among diabetic children as the age of onset between 5–<10 years were the highest percentage (46%). As regards the insulin regimen used by the diabetic children, 17.5% used conventional insulin therapy, 11% used modified insulin therapy as they used regular insulin before lunch, and 71.5% used basal-bolus insulin regimen. The mean percentage of insulin unites per Kg. was 1.00 + 0.38 U/kg./day. The mean BMI was = 24.54 + 6.42, while the BMI distribution was: 56% were with normal weight = < 25, 27% were overweight = 25: <30, 14% were obese = 30: < 40 and only 3% were with severe obesity where BMI = > 40. The results showed also that 34.8% of the diabetic patients were doing continues home blood glucose monitoring with glucose sensors, 25.8% were doing the monitoring only with visual strips, while 39.4% of the diabetic children were not doing home monitoring at all.
Conclusion & recommendations
The discussion of these results documented that it will be essential to follow the international guidelines of management of type 1 diabetes and it was recommended to do proper diagnosis of different types of diabetes among diabetic children and to study the prevalence and incidence of diabetes among Egyptian children as the prevalence and incidence still uncertain till now.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P567

**Clinical and histopathology tumor characteristics of patients with invasive breast carcinoma on insulin glargine compared with other types of basal insulin**

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Background and aims
*In vitro* studies on breast cancer cell lines showed that the serum of diabetic patients was stronger mitogenic when using glargine as compared to other types of insulin. The aim of this retrospective study was to examine if the patients with diabetes mellitus (DM) using insulin glargine have a higher tumor stage of breast carcinoma in comparison to patients using other types of insulin.
Patients and Methods
Altogether 174 patients were surgically treated because of breast carcinoma in our institution from 2006–2010. A chart review of 174 patients with DM was performed and the object of this study were 55 breast carcinoma female patients (mean age 67.4 years; range 38–86 years), who were on insulin. Insulin glargine was used in 10 patients, while the other 45 patients were on other types of insulin. The data on clinical and histopathology characteristics (age, BMI, TNM tumor stage, number of metastatic lymph nodes, presence of estrogen and progesterone receptors, HER-2 expression) were collected. Clinical and histopathology characteristics of patients on glargine versus other types of insulin were compared by chi-square test and non-parametric statistical analysis.
Results
Mean tumor size was 2.8 cm. TNM tumor stage at diagnosis was not higher among patients on glargine compared to patients on other types of insulin (T1/T2 87 vs 69%, T3/T4 13 vs 31%, $P=0.29$; N1 53 vs 50%, $P=0.85$; M1 2 vs 0% respectively). No significant differences between both study groups were found in age of patients, BMI, tumor histology, grade, number of metastatic lymph nodes, hormone receptors or Her-2 status.
Conclusion
We could not show that the patients with diabetes mellitus using insulin glargine have a higher tumor stage of breast carcinoma in comparison to those using other types of insulin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P568

**Evaluation of clinical care in young type 1 diabetes subjects: a nationwide multicenter study in brazil**

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Introduction
The increasing incidence of type 1 diabetes (T1D) is a matter of concern because it negatively impacts both the quality of life and its duration, due to the great morbidity and mortality caused by its chronic complications.
Objective
To evaluate clinical care provided to Brazilian youths with T1D in daily practice, according to the American Diabetes Association's guidelines.
Methods
This was a cross-sectional, multicenter study conducted between December 2008/December 2010 in 28 public clinics, in 20 Brazilian cities. Data were obtained from 1692 patients (55.3% female, 56.4% Caucasian), age 13 (1–18) years, age at diagnosis of $7.1 \pm 4$ years and diabetes duration of $5 \pm 3.7$ years. Overall, 75% of the patients were from a low or very low economic status.
Results
HbA1c goals were reached by 23.2%, LDL cholesterol by 57.9%, systolic blood pressure by 83.9% and diastolic blood pressure by 73.9% of the patients. In total, 20.2% of the patients were overweight and 9.2% were obese. Three or more daily shots of short-acting insulin were reported by 57.9%, and self-monitoring of blood glucose (SMBG) by 73.5% of the patients. A first-degree familial history of type 2 diabetes, obesity, cardiovascular disease or hypertension was reported by 7.6, 12.9, 6.0 and 10.6% of the patients respectively. The rate of annual screening for complications was 66.2% for retinopathy, 69.7% for nephropathy and 62.7% for foot alterations. On multivariate analysis, age, insulin dose, economic status, number of daily SMBG and gender were independently associated with HbA1c levels ($P<0.001$).
Conclusions
Screening for diabetes complications and reaching goals for glucose, lipids and blood pressure are difficult to achieve in Brazilian T1D youths. The low economic status found in the majority of our patients might represent a barrier to reach these goals. Further studies are warranted to address this issue.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P569

Abstract withdrawn.

## P570

**DNA methylation influenced β-arrestin 2 expression in type 2 diabetes patients**

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Background
Hyperglycemia and glucose intolerance are perhaps the most common pathophysiologies associated with diabetes. β-Arrestin 2, component of G-protein coupled receptor signaling, has been shown to be down-regulated in insulin resistant and type 2 diabetic mouse models. However, if this down-regulation also existed in diabetic humans, and the possible mechanisms of this differential expression in normal and type 2 diabetes were not clear.
Methods
Venous nucleus cells from normal glucose tolerance subjects and newly-diagnosed type two diabetic patients without any medications were investigated. β-Arrestin 2 mRNA expression were measured by RT-PCR, β-Arrestin 2 DNA promoter methylation was evaluated by MS-PCR. Clinical data of all the subjects were collected.
Results
In diabetic subjects with confirmed insulin resistance, β-Arrestin 2 mRNA was significantly down regulated compared to normal subjects. Tight methylation-regulated CpGs were identified within β-Arrestin 2 promoter area. β-Arrestin 2 promoter methylation were found in diabetic patients but not in the normal subjects.
Conclusion
DNA methylation might be related to the differential expression of β-Arrestin 2 in type 2 diabetes with insulin resistance, and β-Arrestin 2 DNA promotor methylation might contribute to the pathogenesis of type 2 diabetes.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P571

**Innervation and vascularization into islets of langerhans in the presence or absence of proglucagon-derived peptides**

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Introduction
Islets of Langerhans are well innervated and vascularized, however, the regulatory mechanisms for innervation and vascularization are not well clarified. In mice deficient for proglucagon-derived peptides (Gcg–gfp/gfp: GCGKO), islet cells that express green fluorescent protein (GFP) under the control of glucagon promoter ('α"-cells) display hyperplasia and number of islets is increased. The present study was aimed to clarify role of the proglucagon-derived peptides in innervation and vascularization into islets.
Method
Immunohistochemical analyses for pancreata of GCGKO and heterozygous Gcg–gfp/+mice were performed by using antibodies for platelet-endothelial cell adhesion molecule-1 (PECAM1), vascular endothelial growth factor (VEGF), tyrosin hydroxylase (TH), laminin, synapsin I, neuropeptide Y (NPY) and insulin.
Results
Neuronal fibers positive for NPY and/or TH were identified in and around islets of both Gcg-gfp/+ and GCGKO mice. Basement membranes positive for laminin were present around the islets and blood vessels. Synapsin positive spots were mostly identified nearby blood vessels and α-cells. Some of the non-α-cells were positive for TH. NPY was detected in some of α-cells, β-cells and cells negative for both glucagon and insulin. VEGF were expressed in both α-cells and β-cells. In GCGKO islets that contained mostly GFP positive 'α'-cells and β-cells, numbers of blood vessels and nerves, as well as signal intensities of VEGF, NPY, and TH were reduced.
Conclusion
Proglucagon-derived peptides are not an absolute prerequisite for innervation and vascularization into islets. However, reduced innervation and vascularization into malformed GCGKO islets consisted of mostly GFP positive 'α'-cells suggested that non-α populations play regulatory role in establishing networks of nerves and blood vessels in islets of Langerhans.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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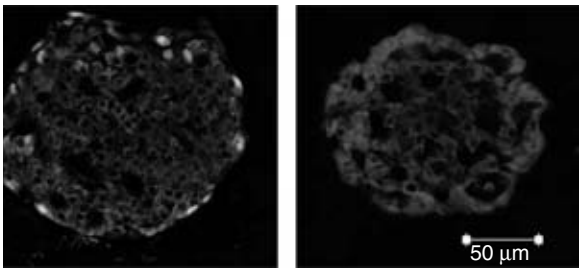


**Figure 1**. Islets of Langerhans in glucagon–GFP knock-in mice. Green: GFP autofluorescence, Red: Insulin.

## P572

**Expression of ghrelin and its receptor genes is tissue specific in streptozotocin treated piglets**

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Ghrelin is protein hormone originally isolated from the stomach and it is the endogenous ligand for the growth hormone secretagogue receptor (GSH-R 1a). It is well established that ghrelin plays roles in appetite regulation, gut function, metabolic disorders and inflammation. Ghrelin and its receptor is expressed in brain and peripheral tissues sensitive to inflammation such as heart and spleen. As a part of study dealing with the role of ghrelin in the development of inflammation the present study was carried out to assess the effect of streptozotocin (inflammatory agent) on the ghrelin concentration, expression of ghrelin and its receptor genes in piglets heart and spleen. Animal studies were performed on 6 weeks old piglets divided into control and experimental group treated with streptozotocin (STZ) 3 times every two days (75+50+25 mg, i.p.). Ghrelin concentration was estimated by RIA, expression of genes of ghrelin (ghrl) and its receptor (GHS-R1a) was measured by PCR-RT. Expression of ghrelin receptor protein was estimated by Western blot analysis. All tested parameters were higher in the heart than in spleen of control animals. Streptozotocin injections significantly increased the expression of ghrelin and its receptor genes as well as the receptor protein concentration in heart - by 7, 5 and 2 times, respectively. However, the concentration of ghrelin in piglets treated with STZ was lower in the heart and higher in the spleen. Unexpectedly, in spite of higher level of ghrelin receptor protein the expression of ghrelin gene was decreased in the spleen of STZ treated piglets.
The obtained results clearly showed that inflammation and diabetes type I induced by STZ changed the activity of the ghrelin-receptor system in the heart and spleen of piglets. The expression of ghrelin and its functional receptor genes was tissue specific.
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Declaration of interest.
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P573

**Empowering patients with diabetes: a qualitative study focusing on knowledge of self-care behaviors amongst asians in south florida**

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Background
South Asians have a higher prevalence of diabetes and increased complications compared to other ethnic minorities, leading to poorer health outcomes. There is a lack of evidence about knowledge of diabetes self-care behaviors amongst this group. Having such knowledge is crucial in preventing complications and providing effective health services.
Objectives
The aim of this study was to identify knowledge of self-care behaviors and factors complicating self-care among South Asians with Type II Diabetes.
Methods
A qualitative focus group analysis was conducted using randomly selected South Asians residing in two counties and receiving care at UHI, a free South Florida clinic. Group discussions were audio-recorded, transcribed, and analyzed using qualitative methodology to identify key themes.
Results
The main barriers to optimal self-care were time constraints, monitoring blood glucose, difficulty in adapting to dietary changes, and lack of understanding of severity and complications. Cultural demands that complicate self-care included the need to honor one's hosts by having otherwise unhealthy dishes and adhering to appropriate social roles, especially among South Asian women. Women reported little time in caring for their own health, in part due to responsibilities to their children and families. In addition to unhealthy dietary habits, they also reported lack of women-only exercise facilities.
Conclusion
Health professionals should strive to provide individually and culturally tailored health education to South Asian diabetics. The needs of women in this community should be addressed. This issue requires more research and should be recognized as a crucial factor in improving the health of communities.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P574

**Circulating ghrelin and leptin concentrations in metabolic disorders of obese and lean piglets**

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Ghrelin as well as leptin are involved into regulation of food intake, development of obesity, diabetes (DM I, DM II) and inflammation. However, in spite of many experiments, the correlation between their effects are not fully established. As a part of study dealing with the interaction of ghrelin and leptin in metabolic disorders the present study was carried out to assess the effect of statin, DMI, DMII on the ghrelin and leptin plasma levels in lean and obese piglets. Animal studies were performed on sixty 6 weeks old piglets of two genetically different strains – lean(L) and obese (F), divided into control and four experimental groups. DMI group was treated with streptozotocin 3 times every two days (75 + 50 + 25 mg, i.p.), ATOR group received statin for 5 days, 10 mg/day, per os), third group received streptozotocin and statin (STZ + ATOR) and DMII group was injected i.p. with glucocorticoid for three days in a manner of 30, 20, 10 mg/piglet. Ghrelin and leptin levels were estimated by RIA. The plasma levels of both hormones in control piglets were significantly higher in obese than in lean $-4.66 \pm 0.11$ vs $3.03 \pm 0.08$ nmol/l (ghrelin) and $27.01 \pm 1.1$ vs $23.2 \pm 0.9$ pmol/l, (leptin). Obese piglets were susceptible to streptozotocin, statin and glucocorticoid treatment and reacted by higher significant increase of ghrelin plasma level (by 18–60%) than lean animals. Unexpectedly, the increase of leptin plasma level was much higher in lean piglets treated with streptozotocin and statin than in obese animals. Molar ratio ghrelin/leptin was much higher in obese control and STZ + ATOR groups but was decreased by streptozotocin in lean and obese piglets. The results clearly showed involvement of both hormones into the development of DMI and DMII in piglets but their interaction in lean and obese animals was genetically dependent.

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P575

**Effective utilization of anti-GAD measurement**

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Introduction

Responsible use of healthcare resources dictates that judicious test ordering be encouraged. Although the appearance of anti-GAD antibodies is known to precede and predict the development of autoimmune diabetes, its use as a screen for risk of developing diabetes is not warranted. Results are useful in determining treatment, however, when it is difficult to distinguish type 1 from type 2 diabetes or when the probability of progression from type 2 to type 1 is high. Method: Anti-GAD measurement was initially made available only to Endocrinologists in the Edmonton Region. The correlation between C-peptide levels and anti-GAD positivity was examined to determine if, as suggested in the literature, there is a C-peptide level above which the probability of the presence of antibody was low. Results: An overall sensitivity of 93% and specificity of 70% was observed using a C-peptide cut-off of 0.80 nmol/l. The anti-GAD positive samples with a C-peptide $>0.80$ nmol/l were from pediatric patients. The laboratory then accepted orders from any physician but implemented criteria for processing requests which included identification of the patient as a diabetic based on HbA1c values or elevated glucose levels and C-peptide levels $<0.80$ nmol/l (normal 0.30–1.32 nmol/l). The Laboratory Information System was programmed to pull the required data and flag those specimens that did not meet the criteria. A positivity rate of 47% was observed when testing was confined to Endocrinologists. With requests accepted from all physicians but restricted using the given criteria the positivity rate was 73%. An audit of pediatric samples continues. Conclusion: Programming the LIS to identify appropriate requests for anti-GAD has been successful in ensuring the test is not used for screening but is available when the result may alter treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P576

**The relationship between the level of knowledge of physicians on guidelines and the degree of glucometabolic control in type 2 DM patients in Turkey**

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Introduction

Clinical practice guidelines on diabetes mellitus (DM) was created by The Society of Endocrinology & Metabolism, Turkey (SEMT). The ADMIRE Project is designed to evaluate the effect of implementation activities to increase physicians' awareness on guidelines.

Methods

A total of 180 physicians from national representative diabetes clinics kept medical records of 885 T2DM patients for 6 months, without any interference to their routine practice. Then, following awareness activities they kept medical records of 1616 T2DM patients for 6 months. Adherence to guidelines was scored to reach a total adherence to follow-up procedures (TOTAL_ADH_FU) for medical history (HISTORY), physical examination (PHY_EXAM) and laboratory evaluation (LAB_EVAL). Here, baseline results from pre-activity and post-activity periods (PRE and POST) are compared.

Results

PRE and POST data were similar (age 55 vs 57 years, women 62 vs 59% and DM duration 7.1 vs 7.4 years). Treatment preference in PRE and POST were OAD: 46 vs 55%, insulin: 11% in both, OAD + insulin: 25 vs 26%. In PRE, guidelines had been adhered fully for HISTORY in 41% of patients and increased to 85% in POST. The proportions of patients who had PHY_EXAM in full adherence were 9 vs 34% ($P<0.001$). Patients with LAB_EVAL in full adherence were 30 vs 22% ($P<0.001$). Only 22.9% of patients were at A1C target ($\leq 6.5\%$) in PRE, while this figure increased to 28.3% at POST ($P=0.014$). Proportion of patients at FBG (70–120 mg/dl) and PPBG targets ($<140$ mg/dl) changed from 17.5 and 12.6–20.7 and 12.8% in POST respectively. Mean A1C levels (%) in PRE and POST were $8.7 \pm 2.1$ vs $8.4 \pm 2.1$ ($P=0.002$). FBG (mg/dl) and PPBG levels (mg/dl) were $191 \pm 88$ vs $178 \pm 77$ ($P=0.001$) and $248 \pm 106$ vs $240 \pm 99$ respectively.

Conclusion

Awareness activities significantly increased the proportion of patients at A1C target and decreased both the level of A1C and FBG.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P577

**Previous history of gestational diabetes mellitus and flow mediated dilation of brachial artery**

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Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.

Background

Gestational diabetes mellitus (GDM) is a common pregnancy condition which is associated with long term complications. In this study we examined the levels of inflammatory mediators, adiponectin, hemocystein and insulin resistance index in women with and without previous GDM (pGDM). Also association between these factors and early atherosclerosis process was evaluated.

Methods

Serum levels of IL6, hs-CRP, adiponectin, homocystein and other biomedical parameters were measured in 40 women with and without pGDM. The average time passed from the index pregnancy was 4 years (range 1–5 years). Two groups were matched based on age, BMI and years after affected pregnancy. The existence of early atherogenesis process was evaluated by measuring both CIMT and FMD. FMD was measured according to guidelines of American College Cardiology. The Levene's test was used for assessing the equality of variances. Pearson correlation was used to investigate the correlation between quantities variables.

Results

Even not hypertensive, the mean of systolic and diastolic blood pressure in pGDM group was significantly higher ($P<0.04$). Lipid profile, fasting blood sugar, hs-CRP, IL6, adiponectin and homocyteine levels did not significantly different between two groups. However fasting insulin ($6.44 \pm 4.52$ vs $10.19 \pm 5.04$,

$P<0.01$) and insulin resistance index as was measured by the homeostatic model assessment (HOMA; $1.41\pm1$ vs $2.33\pm1.2$, $P<0.01$) were significantly higher in pGDM group. Women with pGDM had slightly, however, not significantly, higher CIMT and significantly lower percent of brachial FMD ($26\pm0.11$ vs $19.32\pm0.05\%$, $P<0.02$). FMD and CIMT, adjusted for age and blood pressure, still showed the same pattern. No correlation between inflammatory markers, adiponectin and homocystein and FMD was found.
Conclusion
Women with pGDM are at increased risk of developing insulin resistance and premature atherosclerosis process despite of any change in lipid profile or being hypertensive. Early follow up with FMD should be considered in this group of women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P578

**Frequency and outcome of follow-up testing after a positive gestational diabetes screen**

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Objective
In Edmonton, universal screening of all pregnancies is performed using a 50 g gestational diabetes screen (GDS). Canadian Diabetes Association (CDA) Practice Guidelines recommend a 75 g 2 h oral glucose tolerance test (OGTT) if the GDS glucose result is between 7.8 and 10.2 mmol/l. This study is designed to assess the frequency of OGTT requests in response to a positive GDS and the prevalence of normal OGTT in this population.
Methods
All GDS results reported from April 1 to June 30 2009 and 2010 were collected. The number of OGTT performed within 30 days of a positive GDS was determined. The diagnosis based on the OGTT is compared to that of the GDS.
Results
A total of 8801 GDS were performed, 22% of which produced abnormal results. About 3% met the CDA criteria for diagnosis of GDM (glucose $>10.2$ mmol/l). Although not recommended for these patients, OGTT was requested in 12 and 60% produced normal results. Of the 19% with GDS results between 7.8 and 10.2 mmol/l, 40% did not complete the OGTT within 30 days. Among those who completed the OGTT, 70% were normal.
Conclusions
Confirmatory testing of positive GDS results is not done consistently which may result in misdiagnosis of these patients. The diagnostic cut-off for GDM in the GDS may need further validation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P579

**Relationship vitamin D levels and glycemic control in type 2 diabetic patient**

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Objective
Vitamin D deficiency has been shown to alter insulin synthesis and secretion in both humans and animal models. However, less is known on the association between vitamin D and type 2 diabetes mellitus. In the current study we aimed to analyze the relationship between vitamin D levels with glycemic control in type 2 diabetic patients.
Material and methods
Three hundred and twenty diabetic patients who are follow up at least 6 months for diabetes and one hundred fifty five healthy volunteers were included in this study. Physical examination and anthropometric measurement were performed in all the patients. Metabolic and glycemic indicators such as fasting glucose, hemoglobin A1c (A1c), homeostasis model assessment-insulin resistance (HOMA-IR), high-density lipoprotein cholesterol, triglyceride and insulin levels were measured. Also vitamin D and parathyroid hormone (PTH) levels were evaluated in all patients.
Results
Plasma vitamin D levels were significantly lower in diabetic patients than in control group ($P=0.025$). PTH levels were significantly higher in diabetic patients ($P=0.001$). Serum calcium, phosphorus and alkaline phosphatase levels were similar between diabetic and control groups. No correlation was observed among plasma vitamin D levels, PTH levels and glycemic parameters. Compared with non-diabetic patients, diabetic patients had high plasma glucose, A1c, HOMA-IR, triglyceride levels as expected.
Conclusion
These results indicate that diabetic patients have low levels of vitamin D. Decreased vitamin D levels could influence the prevalence of diabetes mellitus in a population. To clarify these important points, future prospective studies conducted in larger patient groups.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P580

**Association between educational level with glycemic and risk factor control in type 1 diabetes: results from DIACAM 1 study**

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Objective and aims
This study was designed to investigate the clinical characteristics of a representative group of type 1 diabetic (T1D) population in Castilla La Mancha, a region in central Spain. The aim of this report is to evaluate the relationship between the educational level and cardiometabolic risk in adult patients with T1D.
Patients and methods
This is an observational, cross-sectional, prospective and multicentre study of 1465 patients who received attention along 2010. From this cohort patients aged $>25$ years were analyzed ($n$:1130, 48% women, mean age $42.9\pm11.6$ years and mean diabetes duration: $20.9\pm0.9$ years). Educational levels were classified as low (no studies or primary school, $n$:541 (47.9%)), middle (high school, $n$:347 (30.7%)) or high (university, $n$:242 (21.4%)). Diabetic patients underwent clinical and laboratory evaluation. A multivariate logistic regression analysis was used to asses the influence of educational status in good glycemic control (HbA1c $\geq7\%$).
Results
There was no difference between groups regarding gender, but patients with low educational level (LEL) were older and had longer duration of diabetes ($P<0.001$). The prevalence of cardiovascular risk factors was higher in patients with LEL (Table). The prevalence of metabolic syndrome (IDF criteria) was higher in patients with LEL versus high (39.7 vs 21.5%, $P<0.001$). Glycemic control was worse in patients with LEL (HbA1c $8.1\pm1.2$ vs $7.3\pm1.0\%$, $P<0.001$). There were no differences in lipid and pressure control between groups (except for HDLc and systolic blood pressure) but antihypertensive and lipid-lowering treatments were more prevalent in LEL group. After adjusting for diabetes duration, LEL was inversely associated with good glycemic control (OR 0.7 95% CI: 0.5–0.9, $P<0.01$).
Conclusions
In this cohort of adult TID patients, LEL is associated poor glycemic control and higher prevalence of cardiovascular risk factors, these patients should receive adecuate follow-up to reduce their risk of complications.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

**Table 1** Prevalence of classic cardiovascular risk factors according to educational level

| | Low | Middle | High | *P* |
|---|---|---|---|---|
| Hypertension (%) | 32.5 | 24.2 | 14.0 | <0.001 |
| Dyslipidemia (%) | 46.2 | 38.2 | 28.5 | <0.001 |
| Obesity (%) | 20.9 | 13.4 | 13.6 | <0.01 |
| Smoking (%) | 29.6 | 28.2 | 19.0 | <0.01 |

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## P581

**Basal and postprandial free fatty acid kinetics in normal and type 2 diabetic subjects: effects of various treatments**

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The pathophysiology of type 2 DM is complex and involves insulin resistance, pancreatic beta cell dysfunction and visceral adiposity. The levels of free fatty acids (FFA) increases in obese persons and diabetic patients. Elevated levels of FFAs may cause resistance and deficient insulin secretion. Our aim in this study was to investigate the change of FFA levels, glucose, insulin and triglyceride in fasting and postprandial state in 15 healthy controls and 45 diabetic patients under treatment of various agents.

Our study included four groups; 15 patients on diet or plus metformine, 15 patients using rapid acting insulin analogues, 15 patients having glinides, 15 healthy controls. Blood samples were withdrawn at fasting and following breakfast composed of foods proper for each person, at 60th, 90th and 120th min. In control group glycemia peak occured at 90th min and insulin peaked at 60th min, when the levels of FFA was lowest. In the group of patients using glinide, glycemia and insulin levels peaked at 90th min. In the group of patients on diet and metformin, glycemia and insulin levels peaked at 60th min, whereas glycemia has peaked at 60th and 90th min at insulin group. FFA levels were lowest at 120th min in both groups. In the group of patients using insulin analogues glycemia levels peaked at 60th min, FFA levels were lowest at 120th min. FFAs were decreasing postprandially in all groups.

Basal FFA and TG levels were higher in diabetic patients than controls but the difference was not statistically significant. At postprandial phase: 1-FFAs suppression was late in diabetic patients independent from the treatment modality comparing with the healthy individuals. 2-FFA level was inversly associated with insulin and positively associated with HbA1c and fructosamine.

Conclusion

A reduction in elevated plasma FFA should be an important therapeutic target in type 2 diabetes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P582

**Measles and new onset type 1 diabetes presented with bilateral facial paralysis: report of a case**

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Introduction

Measles virus infections generally occur in childhood, but infections in adolescence and adulthood can lead to complications.

Case report

A previously well 28-year-old woman had suffered from nausea, vomiting and generalized fatique for one day before being transferred to our emergency department in a confused state. A diagnosis of type 1 diabetes mellitus (T1D) complicated by ketoacidosis was made based on markedly decreased serum C-peptide level, antiGAD positivity, ketonuria and metabolic acidosis. She was treated by intravenous infusion of saline and insulin and eventually switched to intensive insulin therapy four times a day. On the second day of hospitalisation she developed bilateral facial paralysis. Serological testing for several viral antibodies revealed significant elevation of the measles IgM and IgG titers. One week later, the patient's facial weakness had improved spontaneously with no residual weakness.

Conclusion

Our case is interesting due to coexistence of bilateral facial paralysis, new onset T1D and measles. And there is not a similar case in the literature. Although there are limitations with respect to the true relation between measles and these two manifestations, this clinical picture should be kept in mind as a possible atypical presentation of measles infection in adults.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P583

**Identification of dipeptidyl peptidase-4 inhibitors among boioactive peptides derived from whey proteins**

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Preclinical and clinical studies suggest that whey protein intake can ameliorate postprandial glucose control and potentiate insulin release in both type-2 diabetic patients and healthy subjects.

Most likely, the insulinotropic effect of whey occurs by multiple pathways, including the potentiation of incretin activity through inhibition of DPP-4 in the proximal bowel, with the outcome of increasing intact incretin levels.

During last decades, it has been clearly shown that milk proteins, both caseins and whey proteins, are a source of short peptides with distinct biological activities which are generated by enzymatic hydrolysis during gastrointestinal digestion or during food processing.

Our aim was to identify DPP-4 inhibitors among short peptides occurring in hydrolysates of β-lactoglobulin, the major whey protein found in the milk of ruminants, by *in silico* and *in vitro* analysis.

The peptide Ile-Pro-Ala (IPA), called β-lactosin A have been previously identified in β-lactoglobulin hydrolysates prepared using proteinase K. We tested IPA in vitro for DPP-4 inhibitory activity, due to its structure similarity to the well-known inhibitor Ile-Pro-Ile. Although the substitution at position 3 resulted in a weakening of the inhibitory effect vs the reference compound Ile-Pro-Ile ($IC_{50}$ value of 59 vs 7 μM), our results showed that the β-lactoglobulin-derived peptide IPA can be regarded as a moderate DPP-4 inhibitor.

Two more peptides (Leu-Leu and Leu-Ala) proved to act as DPP-4 inhibitors but their potency was tenfold lower as compared with IPA.

Based on *in silico* analysis of published β-lactoglobulin amino acid sequences, the peptide IPA is conserved across bovine, ovine and caprine species and within bovine variants but it is absent in β-lactoglobulin from donkey. More interestingly, our results suggest that the differential level of expression of β-lactoglobulin across and within species could significantly affect DPP-4 inhibitor content of whey.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P584

**Decreased sucrose preference in humans and rodents with diabetes**

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Objective

Craving of sweet foods and sucrose may be a big obstacle for glycemic control of diabetic patients. It is generally believed that diabetic patients may prefer sweet taste. However, few studies have examined sucrose preference in diabetic patients. Therefore, we investigated changes in sucrose preference in diabetic condition.

Research design and methods
We performed two-alternative staircase methods and forced-choice tracking procedures to assess sucrose thresholds and preferences, respectively in 172 type 2 diabetic patients and 104 age and sex-matched controls. To examine if glycemic control state may affect sucrose preference and sensitivity, 88 diabetic patients underwent follow-up test in 3 months later. To test the emotional effect of sweet taste in diabetic patients, we also performed sucrose preference test in streptozocin-induced diabetic rats.
Results
Unexpectedly, diabetic patients liked a lower concentration for sucrose compared to non-diabetic subjects (most palatable sucrose concentration: diabetic $0.32 \pm 0.02$ M vs non-diabetic $0.40 \pm 0.02$ M, $P=0.001$). Similarly, streptozocin-induced diabetic rats had reduced preference for sucrose compared to normal rats, suggesting that diabetes itself may reduce sucrose preference. Meanwhile, sucrose detection threshold was elevated in diabetic patients (sucrose concentrations detectible sweet taste: diabetic $21.2 \pm 1.0$ vs non-diabetic $16.2 \pm 1.0$, $P<0.001$), indicating that diabetic patients taste sweet less effectively. Both the preference and sensitivity for sucrose were not affected by glycemic control status in follow-up tests.
Conclusions
Diabetic patients and rodents showed lower sucrose preference. The preference and sensitivity for sucrose were independent of changes in blood glucose levels.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P585

**Regulatory T cells number and function in patients with LADA**

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CD4+CD25+lymphocytes (Tregs) play a crucial role in self-tolerance and autoimmunity. LADA is associated with the loss of immune tolerance to β-cell autoantigens similar to classic type 1 diabetes mellitus. The aim of the study was to evaluate changes in terms of Tregs number and function (FOXP3 expression) in LADA patients.
Material and methods
We examined 74 patients with LADA at different stages of the disease. Fifty-six healthy subjects (control group) were included. The HLA-DR and DQ alleles were detected by using PCR method. CD4+, CD8+, CD25+, CD4+25+, CD95+, CD95L+ cells were analyzed by flow cytometry. FOXP3 expression was determined by real time PCR. All subjects were tested for ICA, GADA, IA-2A, C-peptide, HbA1c.
Results
In recent-onset LADA FOXP3 expression was similar to that in control subjects. However, the percentage of Tregs was significantly higher: 2.1 (0.3; 2.3) vs 0.8 (0.6; 0.8). During the period from 6 to 12 months upon the onset of the disease a reduced FOXP3 expression 0.43 rq (0.11; 0.75) was observed in LADA patients if compared with control subjects with 1.11 rq (0.66; 2.26; $P<0.05$). There was no significant difference in the percentage of Tregs in this period ($P>0.05$). FOXP3 expression became normal again after the first and until the 5th year of the disease. Correlation between FOXP3 and Tregs amount was found only in control group. No correlation was established between C-peptide level and autoantibodies titers in LADA patients. In all LADA patients expression of apoptosis markers (CD95 and CD95L) was comparable with the control group ($P>0.05$). Correlation analysis showed strong positive association between GADA and CD95L expression ($r=0.94$, $P=0.05$).
Conclusions
Tregs functional deficiency is delayed in LADA patients. It appears to be compensated by a rise of Tregs number in the first 6 months. This may contribute to slowing β-cells autoimmune destruction.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P586

**Depression as a predictive factor for glycemic control and family functioning in Japanese patients Swith type 2 diabetes: a 6-month follow-up study**

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Purpose
To investigate the impact of depression for diabetes-related quality of life and glycemic control among Japanese patients with type 2 diabetes and their family members.
Methods
Ambulatory patients with type 2 diabetes were drawn consecutively from the inpatient population participated in a 2-week educational intervention program at two general hospitals affiliated with Hiroshima University. Written informed consent for the study was obtained from 123 patients and 75 family members. Before and after the intervention program, the subjects and their family members completed the Zung self-rating depression scale, the Zung self-rating anxiety scale (SAS), and the subjects also completed the diabetes quality of life (DQOL) and the problem areas in diabetes scale. Family functioning was assessed by the family assessment device (FAD) before the program. This study was approved by the Institutional Review Boards and the Ethics Committees of those two hospitals and Hiroshima University.
Results
'Depressed patients' ($n=69$; SDS at baseline $\geq 40$) perceived significantly worse diabetes-related quality of life including family functioning than 'non-depressed patients' ($n=54$; SDS at baseline $<40$) before the intervention. At the 6-month follow-up after the intervention. Depressed patients apparently improved in their mood, but still reported significantly worse diabetes-related quality of life including family functioning and tended to show higher HbA1c values than non-depressed patients. Furthermore, at the 6-month follow-up, family members of depressed patients perceived significantly higher level of conflict in their families than those of non-depressed patients.
Conclusion
The findings of the study suggested that, as for patients with diabetes, depression at baseline might predict worse quality of life, less effective family functioning, and worse glycemic control afterward. Consequently, diabetes care professionals should devote attention to taking care of mood status of patients, and intervene to promote appropriate affective interaction among patients and their family members.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P587

**What arises from the decrease of visceral adiposity in Japanese diabetic patients with visceral fat accumulation?**

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Aim
To clarify what arises from the decease of visceral adiposity in Japanese diabetic patients with visceral fat accumulation.
Patients
Diabetic patients ($n=30$) whose visceral fat areas (V), determined with abdominal CT, were larger than 100 $cm^2$.
Methods
$V$ was followed at every 6 month, and clinical profiles and course were compared of eight patients (group D), whose changes of $V(\Delta V=(V$ at the second exam or

more) − (*V* at the first exam)) were under the (mean - S.D.) of Δ*V*, with of the other patients (group C).

Results

In group D, at the beginning *V* was larger, frequency of fatty liver and serum concentrations of TG and LDL-C were higher, insulin therapy was fewer, and fibrates were used more than in group C, during the following BMI was decreased, frequency of more than 5000 steps/day was increased, A1C (NGSP %) was decreased, and use of ACEI/ARB was increased, though diastolic pressure was decreased in group C, and at the ending serum TG was yet higher than in group C.

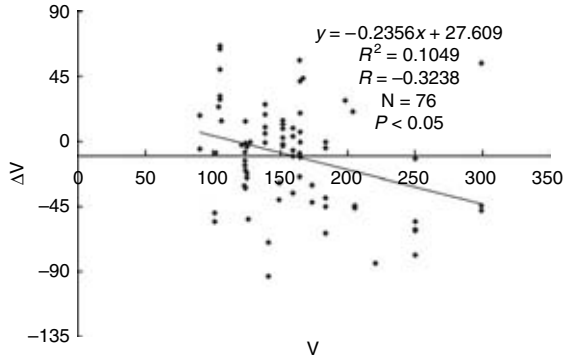
Discussion

As *V* was decreased, body weight was decreased and the metabolism of glucose and lipids were improved. Increase of ADL was important for decrease of visceral adiposity.

**Table 1** Comparison of clinical profiles and course between the groups

| Clinical parameter | Group | At the begining | At the ending | *P* 1 |
|---|---|---|---|---|
| *V* ($cm^2$) | D (*n*=8) | 191±66 * | 128±70 | 0.000 |
| | C (*n*=22) | 140±29 | 134±39 | 0.277 |
| BMI | D | 27.3±4.2 | 26.2±4.2 | 0.015 |
| | C | 27.9±3.4 | 27.8±3.5 | 0.696 |
| A1C (NGSP%) | D | 8.2±2.2 | 6.6±0.6 | 0.039 |
| | C | 7.2±1.3 | 7.2±1.4 | 0.818 |
| Fatty liver | D | 6/8 * | 3/8 | 0.131 |
| | C | 7/22 | 7/22 | 1.000 |
| TG (mg/dl) | D | 427±456(8) * | 227±186(7) * | 0.140 |
| | C | 146±107(20) | 128±64(22) | 0.416 |
| LDL-C (mg/dl) | D | 144±15(8) * | 135±38(7) | 0.514 |
| | C | 108±42(17) | 106±41(22) | 0.231 |
| Times of counseling | D | 0.6±1.1 | 1.8±1.3 | 0.174 |
| by dietitian 2) | C | 0.9±0.9 | 2.4±3.6 | 0.056 |
| More than 5000 | D | 3/8 | 7/8 | 0.039 |
| steps/day | C | 11/18 | 13/20 | 0.804 |

Mean±S.D.(*n*)/ratio 1) (at the beginning) vs (at the ending), statistically analysed with paired *t*-test/$\chi^2$ test 2) compared at the ending(during the following) with at the beginning(during the same length of period before following) *$P<0.05$ (D vs C), analysed with unpaired *t*-test/$\chi^2$.




Relationship between visceral fat area (V) and the change of *V* (Δ*V*). In *y* axis, the crossing level with *x* axis shows the mean of Δ*V*, and the level of −45 is the (mean-S.D.) of Δ*V*.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P588

### Outcome of a young adolescent diabetes transition clinic in an inner city ethnic population in Birmingham: an audit

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Introduction

Management of adolescent diabetes is challenging due to a complex interplay of physiological and psychological factors.

Aims

To audit the outcome of our local hospital adolescent diabetes clinic.

Method

Adolescents aged 16–24 years are seen by our multi-disciplinary team (adult and paediatric diabetes consultant, diabetes specialist nurses and a dietician) every 4 months after transfer from paediatric service. We looked at the clinic records and electronic data of 56 patients over 5 years (2006–2010).

Results

Forty-seven had TIDM, eight T2DM and one possible MODY (31 males, 25 females). Mean age 20 (range 17–26) years and average diabetes duration 9.5 years (Asian – 28, Afro-Caribbean – 9, Caucasian – 16, African – 3).

Seventeen patients had undergone structured education (DAFNE) and four are on waiting list. Four had 1:1 carbohydrate counting sessions.

Two patients had coeliac disease, two used recreational drugs and eight took alcohol. Four patients developed retinopathy and five microalbuminuria over these 5 years. Mean HbA1c in 2006 was 9.2% and in 2010 was 9.6%. HbA1c improved in 15/56 (27%) patients and was static in eight patients. There were 40 patients in 2006 on multi-dose insulin injection, increasing to 52 by 2010. There was a 44% (nine in 2008, five in 2010) and 35% (35 in 2007, 23 in 2010) reduction in DKA and hypoglycemia related hospital admissions respectively. Clinic attendance rates improved from 68 to 79%. User questionnaire results show significant patient satisfaction.

Conclusions

Our ethnically mixed adolescent diabetes clinic remains a big challenge. We have started to convert more patients to multi-dose insulin injections and enrol into structured education (DAFNE). Empowerment and clinic attendance is getting better, DKA and hypoglycaemia admissions have fallen through more frequent diabetes specialist nurse contact and support (by e-mails, mobile text messaging, drop-in clinics) – hopefully, we shall see concomitant improvements in HbA1c in future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P589

### Microcirculatory disorders in adolescents with type 1 diabetes mellitus

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Aim

To study microcirculatory disorders in adolescents with type 1 diabetes mellitus (T1DM).

Patients and methods

Eighty-five adolescents 12–17 years with T1DM were examined. The duration of T1DM was: <1 year – in 29 (HbAc1 7.1±0.4%) – group 1, from 1 to 5 years – in 26 (HbAc1 9.2±1.1%) – group 2, more then 5 years – in 30 patients (HbAc1 10.7±1.1%) – group 3. The indexes of tromboresistance of vessel wall measured by manjetic method. The indexes of trombocyte aggregation by laser method and intravascular aggregation were evaluated.

Results

Tromboresistance of vessel wall was normal in group 1 ($P<0.05$). Levels of adrenalin-stimulating and intravascular aggregation of thrombocytes were increased ($P<0.05$). Tromboresistance of vessel wall was decreased in group 2 ($P<0.05$). Levels of ADP-, kollagen-stimulating and intravascular aggregation ($P<0.05$) were increased in group 2. Decrease of tromboresistance of vessel wall ($P<0.001$), increase of activity of thrombocytes ($P<0.05$) and intravascular aggregation ($P<0.001$) were found in patients of group 3.

Conclusions

Microcirculatory disorders in adolescents with T1DM correlated with duration of the disease and may demand treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P590

**Screening for unknown diabetes mellitus or IFG in a population of South Italy**

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The importance of early detection of diabetes mellitus helps to prevent the development of related chronic complications.
Screening, which lasted a week, has been implemented in 31 pharmacies in the territory of the Province of Brindisi (Apulia–Italy) on a random sample of 1048 people. Blood glucose was measured by glucometer; a questionnaire was administered with anthropometric data and medical history and evaluation of the lifestyle of exercise and different dietary habits.
In total, 964 people did not know they have diabetes mellitus or impaired glucose. In 44 of these (equivalent to 4.6%; 23 males M and 21 females F) there was a fasting glucose greater than 125 mg/dl (mean blood glucose 149 mg/dl with S.D. 30.3), and 178 (18.5%; 89 M and 89 F) had blood glucose between 101 and 125 mg/dl.
About 77% of the 44 newly diagnosed with diabetes mellitus and 83% of subjects with fasting glucose between 101 and 125 mg/dl did not exercise more than two times a week. Of the 44 subjects with fasting blood glucose greater than 125 mg/dl, 27% had a normal BMI, 34% overweight, 30% obesity class I, 7% obesity class II and 2% class III. Of the subjects with fasting blood glucose levels between 101 and 125 mg/dl, 1% were underweight, 32% normal weight, 42% overweight, 18% obesity class I and 7% class II. Finally, there was a significant positive correlation between the presence of a known pre-existing hypertryglyceridemia and/or hypertension and the detection of fasting hyperglycemia.
This study demonstrates the importance of screening for the blood glucose determination in the population, especially in the presence of one or more components of metabolic syndrome, to allow, as early as possible, to investigate the diagnosis of an unknown overt or preclinical diabetes mellitus.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P591

**Adiponectin among markers of low grade inflammation in gestational diabetes mellitus**

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Background and aims
The subclinical inflammation connected with hypoadiponectinaemia and inflammatory pattern may favor the development of gestational diabetes mellitus (GDM).
Methods
In total, 66 pregnant women were enrolled in the study during systematic testing for GDM between 24 and 28 weeks of gestation. GDM was diagnosed in 42 cases, 24 were normal glucose tolerant control group (NGT). In blood serum concentration of glucose, insulin, adiponectin (AdipoQ), hyaluronic acid (HA), CRP were assessed during pregnancy and 12 weeks after delivery.
Results
Fasting and 2 h glucose, insulin concentration, HOMA IR, HOMA B were significantly higher, Quicki lower in women with GDM than in NGT. AdipoQ (12.5 vs 20.6 ng/ml; $P<0.001$) and HA (7.3 vs 24.1 μg/ml; $P<0.001$) concentrations were significantly lower in women with GDM than in NGT. CRP levels did not differ between groups (3.3 vs 2.7 mg/l; $P=0.54$). AdipoQ significantly correlated with basal insulin ($r=-0.477$), HOMA IR ($r=-0.394$), HOMA B ($r=-0.428$), Quicki ($r=0.394$), CRP ($r=-0.399$). HA concentrations correlated with glucose at baseline ($r=0.448$) and 2 h ($r=0.509$). Postpartum levels of AdipoQ negatively correlated with CRP ($r=-0.526$) and glucose at baseline ($r=-0.546$). HA was considerably higher (8.8 μg/ml vs 13.3 μg/ml; $P=0.031$), and CRP lower (3.8 vs 2.2 mg/l; $P=0.012$) at postpartum than in pregnancy.
Conclusions
The course of gestational diabetes is connected with low levels of AdipoQ and HA. After delivery most of women with GDM returned to NGT, which was linked with rise in HA and AdipoQ concentrations. The way of delivery itself had no effect on serum HA levels whatsoever.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P592

**Comparative analysis of adiponectin isoforms distribution in pregnant women with gestational diabetes mellitus and after delivery**

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Objective
The aim of the study was to evaluate distribution of circulating adiponectin (AdipoQ) isoforms at pregnant women with gestational diabetes mellitus (GDM) and comparison analysis of isoform distribution changes 12 months after delivery.
Design
The study was conducted on 64 pregnant women who underwent routine pre-natal tests for GDM between 24 and 28 week of gestation and 30 of them 12 months after delivery. The study group included 36 women diagnosed with GDM based on the results of 75 g OGTT and the control group of 28 healthy pregnant women with a normal screening test and no risk factors for GDM.
Methods
Quantitative determination of total AdipoQ and its isoforms in serum was performed with the multimeric adiponectin ELISA (Alpco Diagnostics, Salem, NH, USA). The assays were run according to the manufacturer recommendations.
Results
Total AdipoQ concentration in women with GDM was significantly lower than in women without GDM. Among adiponectin isoforms significantly lower concentrations of HMW multimers and LMW trimers in women with GDM were found in comparison to the control group during pregnancy, while the concentration of MMW hexamers did not differ between the groups. After delivery a significant increase in total AdipoQ as well as in the concentration of HMW and LMW was found in women with the history of GDM. While searching for correlations between different forms of AdipoQ we found that HMW and MMW negatively correlated with BMI before and during gestation as well as after delivery.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P593

**Three novel mutations in maturity-onset diabetes of the young genes in Spain**

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Introduction
Maturity-onset diabetes of the young (MODY) is a genetically, metabolically and clinically heterogeneous subtype of diabetes mellitus characterized by an early onset, an autosomal dominant inheritance, and a primary defect in insulin secretion. They are caused by mutations in a reduced number of β-cell genes. Mutations in glucokinase gen (MODY 2) and hepatic nuclear factor 1α (HNF 1α) (MODY 3) are the most common.
Materials and methods
We present the cases of monogenic diabetes diagnosed in our environment between 2006 and 2010. This study involves 13 families with 23 patients (13 index cases and 10 relatives). The clinical features are listened in Table 1.
Mutations in the glucokinase gene were detected in 82.5% of the families studied, two of them have not been previously described (c984delA and C380C > A). Only one patient presented mutation in HNF 1α, neather previosly described (T156M). In the other three families no mutation where detected.
Conclusions
– Most of our patients were MODY2, since its clinical presentation makes it suspect the probability of monogenic diabetes.

– It is important to improve in the genetic study of MODY patient's relatives to enlarge de map of mutations involved.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1** Clinical features of MODY patients

| | MODY 2 | MODY3 | MODY X |
|---|---|---|---|
| Number of patients | 19 | 1 | 3 |
| Gender | 11/8 | 1/0 | 1/2 |
| Age at diagnosis | 21 (2–59) | 10 | 1/27 (5–9) |
| BMI | 20.4 | 19.1 | 20 |
| Fasting glucose (mg/dl) | 117.8 (103–134) | 94 | 105 (102–112) |
| HbA1c | 6.3 (5–6.4) | 5.2 | 5.7 (4.9–7.2) |
| Treatment (diet/insuline/oral hypoglucemic agent) | 15/3/1 | 1/0/0 | 2/1/0 |

## P594

### Proteomic identification of plasma biomarkers in type 1 diabetes mellitus: an implication of hemopexin overexpression in diabetes mellitus

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Type 1 diabetes mellitus (T1DM) not only is congenital disease that known as insulin-dependent diabetes, often occurs in children and adolescents. Recent advances in quantitative proteomics including fluorescence two-dimensional differential gel electrophoresis (2D-DIGE) and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) have offered opportunities to discover plasma proteins as biomarkers for tracking the progression and for understanding the molecular mechanisms of diabetes. Proteomic analysis of the plasma proteome in T1DM and healthy donors indicated that 41 proteins representing 36 unique proteins are T1DM associated biomarkers including heme-associated protein, hemopexin. Further investigation of glucose mediated hemopexin induction was determined *in vitro*. Our data indicated that hemopexin can be induced in numerous cell lines (ARPE19, chang's liver cells, HT29 and Hela) by increasing the glucose concentration in the medium. Interestingly, the glucose-induced hemopexin overexpression can be reduced by ROS scanvenger such as glutathion implying hemopexin expression is linked to glucose-induced oxidative stress. To sum up, the current work has identified numerous T1DM specific biomarkers by proteomic strategy. In addition, to our knowledge, this is the first report showing the expression of hemopexin can be modulated by glucose concentration which is mediated by ROS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P595

### Comparative incidence of type 1 diabetes mellitus in school-aged children between immigrated families and the background population living in Piedmont or in Morocco, 2005–2009: a role for vitamin D?

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Introduction

The aim was to compare the incidence of type 1 diabetes between immigrant Moroccan and Romanian children and background population in Piedmont, Italy, and between Moroccan children living in Italy and their counterpart in Morocco.

Patients and methods

All new cases of type 1 diabetes aged 6–13 years in Piedmont and Morocco in 2005–2009 were retrieved by regional registries. Numbers of children regularly registered to primary and middle school were chosen as reference population to bypass the demographic impasse of irregular immigration. Skin phenotype and vitamin D levels were evaluated in diabetic and healthy, Moroccan and Italian children, living in Piedmont or in Morocco. Incidence rates and their ratios were calculated.

Results

Increased incidence rates in brown skinned Moroccan children living in Piedmont (44.8, 95% CI 25.6–71.6) were recorded respect to white skinned Italian and Romanian children in Piedmont (16.8, 95% CI 14.7–19.1 and 9.1, 95% CI 2.4–22.5) and Moroccan children in Morocco (10.2, 95% CI 9.0–11.6). Moroccan children living in Piedmont had a higher risk to develop type 1 diabetes respect to the Italian population (incidence rate ratios, 2.663, 95% CI 1.858–4.897). Lower vitamin D levels were associated with type 1 diabetes in children living in Piedmont, but not in those living in Morocco.

Conclusions

The Moroccan origin is a strong determinant of type 1 diabetes in immigrant children living in Piedmont, with brown skin as one of the major risk factors for the disease. Vitamin D status could play a role in type 1 diabetes onset in immigrant brown skinned population living in the Northern part of the Mediterranean area.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P596

### HbA1c as a tool for the diagnosis of type 2 diabetes in 208 premenopausal women with polycystic ovary syndrome

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Objective

To study HbA1c as a tool for diagnosing diabetes and to study HbA1c as a cardiovascular risk marker in patients with polycystic ovary syndrome (PCOS).

Methods

A retrospective observational study at an academic tertiary-care medical center. In total, 208 premenopausal women with PCOS participated. Patients underwent clinical evaluation (Ferriman–Gallwey score, body mass index, waist, blood pressure), hormone analyses (testosterone, sex hormone-binding globulin, fasting lipids, insulin, glucose, HbA1c), transvaginal ultrasound, and 2-h oral glucose tolerance tests (OGTT) measuring capillary blood glucose (BG) at 0 (BG 0) and 120 (BG 120) min, insulin, and C-peptide.

Results

In total, 20 patients were diagnosed with type 2 diabetes during OGTT. The sensitivity and specificity of HbA1c $\geq$ 6.5% for the diagnosis of diabetes were 35 and 99%, respectively, compared with the diagnosis established by OGTT. HbA1c showed closer correlation with waist, body mass index, and lipid profile than BG 120, suggesting that HbA1c could be a cardiovascular risk marker.

Conclusion

The clinical utility of HbA1c for diagnosing impaired glucose tolerance and type 2 diabetes in PCOS in daily practice is low. Long-term prospective studies are needed to determine whether HbA1c is superior to glucose levels as a cardiovascular risk marker in patients with PCOS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P597

**Non-HDL and LDL cholesterol in type 2 diabetes mellitus patients at tertiary care hospital of Pakistan**

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Objective
The objective of this study was to determine level of non-HDL and LDL cholesterol in type 2 diabetic patients with or without coronary artery disease irrespective on lipid lowering therapy and the common factors associated with elevated non-HDL cholesterol.
Study design: cross-sectional study
Place and duration of study: diabetic clinic, Aga Khan University Hospital, Karachi. Data of Type 2 diabetes mellitus patients attended clinics during 2007 and 2011 were reviewed.
Patients and methods
All type 2 diabetic patients of either gender with fasting lipid profile irrespective of on lipid lowering therapy were included. T1DM, gestational diabetes, type 2 diabetes patients with pregnancy and those T2DM patients with incomplete data were excluded. Multivariable regression was done to identify common factors associated with elevated non-HDL cholesterol.
Results
A total of 5000 patients visited diabetes clinics during 2007–2011. Out of these 1352 patients fulfill the eligibility criteria. Among them 169 (13%) had coronary artery disease. Mean age of patients was $48 \pm 6.3$ years, 57% were males, more than 80% were obese with equal proportion had HBA $1C > 6.5$. Mean non-HDL cholesterol was 132 mg/dl S.D. $\pm 43$. Mean LDL cholesterol was103 mg/dl S.D. $\pm$ 37. Both LDL $\leq 100$ and non-HDL $\leq 130$ was achieved in 645(48%) patients. In total, 728 (61.5%) patients achieved target LDL of $\leq 100$ mg/dl among them 83 (11.4%) have non-HDL cholesterol $> 130$ mg/dl ($P < 0.05$). In patients with CAD, combined goal achievement of LDL $\leq 70$ and non-HDL $\leq 100$ was seen in 59 (35%). Among patients with LDL $\leq 100$ mg/dl, 8 (11.9%) patients have non-HDL $> 100$ mg/dl ($P < 0.05$). Multivariable analysis shows that age $\leq 60$ years and BMI $> 25$ are independently associated with NHDL $> 130$ (Adj. OR 1.5, 95% CI 1–2.2) and (Adj. OR 3.8, 95% CI 1.6–8.6), respectively. Similarly, HBA1C $>$ 6.5 was 40% more associated with NHDL $> 130$ (Adj. OR 1.4, 95% CI 0.9–2.3).
Conclusion
T2DM patients need aggressive lipid lowering therapy to achieve non-HDL cholesterol for residual risk reduction.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P598

**Prevalence of diabetes-associated antibodies and impaired insulin response to glucose in first degree relatives of diabetic patients Ebtissam M. Salah*, Hesham El-Hafnawy**, Mona Anwar*, Samar M. E. Salem*, Mai M. Youssef*, Atef Bassyoni** and Marry Aziz****

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Type 1 diabetes is a chronic autoimmune disease with a subclinical prodromal period characterized by the presence of circulating antibodies to various islet cell proteins. Our main objective is to estimate the prevalence of diabetes-associated autoantibodies in a group of 1st degree relatives, compared to healthy control subjects. Also, we tried to assess the insulin secretory capacity in subjects having multiple antibodies using first phase insulin response (FPIR) to intravenous glucose.
Eighty children and adolescents of the 1st degree relatives of diabetic patients attending the out patient clinic in the diabetic institute participated in our study. They were (34 boys and 46 girls, 50 siblings and 30 offspring of diabetic parents, aged 8–20 years with mean age $13.23 + 3.6$). 20 age and sex matched control subjects with negative family history of diabetes were enrolled from the child health clinic in the NRC. Sera of all subjects and controls were monitored for: islet cell antibodies (ICA), anti-insulin autoantibodies (IAA) and glutamic acid decarboxylase antibodies (GAD) using ELIZA technique, and (IA-2) antibodies using radioligand binding assay. It was found that: according to the considered cut off point for positivity, 23 out of the 80 relatives (28.75%) showed positive ICA. 21 out of 80 relatives (26.25%) showed positive IAA. 17 out of 80 relatives (21.25%) showed positive GAD antibodies. 5/80 relatives (6.25%) showed positive IA-2 antibodies. As regard the control group only one subject tested positive for ICA and another one tested positive for IAA. None of the control group tested positive for GAD or IA-2 antibodies. On studying different combinations of positive antibodies, it was found that only two subjects of the study group (2.5%) had three positive antibodies, 10% had positive ICA and IAA, 3.75% had positive ICA and anti-GAD. The same percent of the study group had positive IAA and anti-GAD. Those subjects showing more than one positive antibody underwent IVGTT to determine FPIR to predict subjects at high risk for developing type 1 diabetes. One subject was found to be at risk and four subjects were found to be at high risk for developing type 1 diabetes.
Declaration of interest
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## P599

**Is there a seasonal variation in the incidence of gestational diabetes mellitus?**

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Introduction
GDM seasonability has been rarely addressed, with conflicting results. The aim of the study is to evaluate monthly GDM incidence in a large group of Greek women.
Methods
7618 pregnant women underwent 100g OGTT during the third trimester (GDM: ADA 2000 criteria). Seasonal, monthly GDM incidences and mean seasonal glucose levels during OGTT were calculated. Mean month temperature data were obtained from the Greek Meteorological Service.
Results
GDM incidence, relative prevalence (RP), odds ratio (OR–95% CI) and mean temperature per month as in Table 1.
Seasonal GDM incidence was significantly different: Winter (W) = 28.1%, Summer (S) = 39.2%, Spring (Spr) = 32.4% and Autumn (A) = 32.4% ($\times 2 =$ 51.0, $P < 0.0001$). Glucose levels (Glu) during OGTT were calculated. There was no statistical difference in fasting glucose blood levels. On the contrary, significantly increased blood glucose values were observed at 60, 120 and 180 in S vs W, while Spr and A values were intermediate (ANOVA: $P < 0.0001$). Glu60 (mg/dl): $W = 163 \pm 42$, $Spr = 166 \pm 42$, $A = 167 \pm 39$, $S = 173 \pm 40$, Glu120: $W = 137 \pm 41$, $Spr = 138 \pm 41$, $A = 139 \pm 38$, $S = 146 \pm 40$, Glu180:$W = 109 \pm 33$, $Spr = 110 \pm 33$, $A = 111 \pm 32$ $S = 116 \pm 34$.
The effect of season on postload glucose levels remained an independent significant factor after adjustment for age, gestational age, BMI, weight gain and blood pressure (MANOVA model, $P < 0.0001$).
Conclusions
GDM incidence in Greece presents seasonal variation. GDM risk diagnosis in summer is significantly increased ($\sim 70\%$) compared to winter. Diifferences in seasonal incidence are due to post glucose load levels. Whether the observed variations could be attributed to differences in ambient temperature or other environmental factors, remains to be clarified.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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**Table 1**

| Month | GDM % | RP | OR (95%CI) | Mean temp C |
|---|---|---|---|---|
| Jan | 32.7 | 1 | Rel to Jan | 9.5 |
| Feb | 36.4 | 1.1 | 1.2(0.9–1.6) | 10.5 |
| Mar | 36.9 | 1.1 | 1.2(1.0–1.6) | 12 |
| Apr | 41.2 | 1.3 | 1.4(1.1–1.8) | 16 |
| May | 38.3 | 1.2 | 1.3(1.0–1.6) | 21 |
| Jun | 44.7 | 1.4 | 1.6(1.3–2.1) | 25 |
| Jul | 49.2 | 1.5 | 1.9(1.5–2.4) | 28 |
| Aug | 49.9 | 1.5 | 1.8(1.4–2.4) | 28 |
| Sep | 39.5 | 1.2 | 1.4(1.1–1.8) | 24 |
| Oct | 38.9 | 1.2 | 1.3(1.0–1.6) | 20 |
| Nov | 37.4 | 1.1 | 1.2(1.0–1.6) | 16 |
| Dec | 32.8 | 1.0 | 1.0(0.8–1.3) | 11.5 |

## P600

**We can do better: activity report of ambulatory educative sessions for the patients treated with external pumps**

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Since the end of 2006 in France we have the obligation to evaluate yearly the educational level in diabetic patients treated with pumps. In 2007 we created bimonthly ambulatory group sessions for these patients (six persons per group). After receiving an explanation letter, the patients have to register by calling the service. The aim of the study was to evaluate quality and quantity of the sessions we have been performed.
Methods
We conducted analyses between March 2007 and October 2010. The program of evaluation was conformed to French medical diabetologic recommendations for knowledge in patients treated with pumps. During this period, several members of the team passed an official exam (education to diabetic patients) to improve efficacy of the sessions. For the quantity evaluation we counted the number of patients who attended the session and for the quality evaluation we used satisfaction surveys.
Results
During 43 months we evaluated 179 patients through 40 ambulatory group sessions. For these patients, the pump was first initiated between 2006 and July 2009. Some of them have participated twice or more. The theoretical number of patients should have been about 240 if we consider the number of new patients treated by pump during year 2006 and until the end of 2009. This total amount represents 25% of non-evaluated subjects. We analyzed 173 satisfactory questionnaires. On one hand, 98% of the patients were satisfied from the cordial welcome, 95% appreciated the technique sessions (in particular 'catheter replacement') and the talks between each other or the exchanges with the team. On another hand 31% disagreed with the session called 'check your material' (new devices for glycemic control, replacement insulin pens) and 16% did not like the session called 'real life clinical situations'.
Conclusion
We need to increase the number of the patient's registration to this educative program and we need to improve the methods for practical session, although we previously demonstrated the feasibility of this specific ambulatory training for the evaluation of patients treated with pumps. We have to create a tool to evaluate the number of 'lost of follow-up', patients who never call the service to register.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P601

**Diagnostic value of cytogram analysis of different types of oral cavity mucous epithelium in type 2 diabetes mellitus elderly patients**

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Objective
To define diagnostic value of cytogram analysis of different types of oral cavity mucous epithelium in type 2 diabetes mellitus patients using cytological methods.
Material and methods
Material for cytological analysis was oral cavity mucous swab in 22 healthy people and 41 sick ones with type 2 diabetes mellitus at subcompensational stage. The group under study was homogenous in age, sex and biorhythmic type. Oral swabs were fixed in spirit–acetone and were May-Grunvald's and Romanovsky-Himsa's dyed. In swabs at the rate of 1000 cells the epithelical cells at various stages of differentiation were determined. Differentiation and cornification indexes of epithelial cells (DI and CI) were calculated in the swabs according to cytograms. Moreover, blood glucose level was determined. Correlation analysis and student's criteria were used.
Results
Research in lining type mucous (lips and cheeks) showed that in type 2 diabetes mellitus patients on the second day in hospital the DI and CI significantly increased ($P<0.01$). In specialized type mucous epithelium cytogram (tongue) DI and CI increased but in a less degree ($P<0.05$). According to the data obtained from the cytogram analysis of chewing type mucous epithelium (gingivia and hard palate) figures of DI and CI did not differ from the norm. Strong correlation between high DI and CI in the lip and cheek cytogram and increased blood glucose level in diabetes mellitus were observed.
Conclusion
The present study shows that cytological indexes of lining type mucous are the most informative for type 2 diabetes mellitus diagnostic. Obvious increase of DI and CI of lining type mucous cytogram against increased content of blood plasma glucose level indicate hyperglycemia during two previous weeks.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P602

**Socio-demographic factors associated with pediatric diabetic ketoacidosis admissions in Southern West Virginia**

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Background
Diabetic ketoacidosis (DKA) is a well-known complication in children with type 1 diabetes (T1DM) with a mortality rate estimated at 2%. Sparse data are available from the literature describing socio-demographic factors associated with DKA admissions in children. A previous study identified that children of non-Caucasian race and Medicaid, with T1DM, had increased incidence of DKA admissions.
Aims
To identify the socio-demographic factors associated with DKA admissions including type of insurance coverage, income by county, race, gender and HbA1c in West Virginia, a rural part of Appalachia.
Methods
A retrospective chart review of patients with known type 1 diabetes ages 1–18 years admitted to the Pediatric ICU with DKA in Charleston WV from January 2007 to December 2010 in comparison to our general type 1 diabetes population. The data collection tool included multiple socio-demographic factors and HbA1c.
Results
We reviewed a total of 167 patients with an admitting diagnosis of DKA; 63 charts were excluded because they did not meet either DKA criteria, age criteria, had new onset diabetes or lived outside of WV. About 57% were female, 43% male. Average age was 13.6 years (S.D. $\pm$2.81) 56% were covered by Medicaid or Chips insurance and 44% by commercial payers. About 11.5% were African American and 88.5% were Caucasian. The average HbA1c was 10.85% (S.D. $\pm$ 2.364).
Conclusions
Salient findings include higher HbA1c, higher rates in African American patients and in those covered by Medicaid.
Clinical implications
This study identifies socio-demographic factors associated with children admitted for DKA in WV. Patients identified at higher risk for DKA include those with elevated HbA1c, African American race and those covered by Medicaid/CHIPS. Findings can be utilized to identify patients at higher risks for DKA and implementation of prevention strategies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P603

**Hypersomatotropism and glucose metabolism disorders**

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Introduction
Glucose metabolism disorder (GMD) is a classic complication of acromegaly, but its frequency varies from study to study.
Aim
We aimed to analyze GMD frequency in our population, and predictive factors such as: family history of diabetes mellitus (DM), age, gender, and GH rates.
Subjects and methods
It is a prospective study where 75 hypersomatotropic subjects were analyzed. They all had fasting and postprandial blood glucose evaluation. When the last

ones were normal glucose tolerance test was done and analyzed according to the WHO recommendations.
Results
In our group GMD were observed in 41 patients (22M/19F) = 55%. Diabetes mellitus was found in 27 subjects (65%), and impaired glucose tolerance (IGT) in 14 (35%). The diagnosis of DM was made before the hypersomatotropism's one in 37%, the delay was 5 years. Mean age at diagnosis for diabetic patients was $40 \pm 11$years, and $38 \pm 13$ years for non-diabetic subjects. Glucose metabolism disorders were more frequent in women: 54 vs 46% for men, but the difference was not statistically significant. Mean plasma GH was = 75.25 ng/ml in DM subjects vs 46.1 ng/ml for non-diabetic patients ($P < 0.05$). For family history of diabetes mellitus there was no difference between both groups.
Conclusion
In this study 55% hypersomatotopic subjects suffer from GMD. DM is more frequent than IGT (65 vs 35%). On the statistical side, there is a positive correlation between GMD and GH concentrations, but not with gender, age and family history of DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P604

**Questionnaire survey about a hospital adolescent transition diabetic clinic**

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City Hospital, Birmingham, UK.

Introduction
Our adolescent transition diabetes clinic (16–24 years) is run by a multi-disciplinary team (adult and paediatric diabetes consultant, diabetes specialist nurses and a dietician) every 4 months.
Aim
Questionnaire survey of patients understanding of their diabetes and satisfaction about the running and set-up of this unique transition clinic.
Method
A two-page questionnaire given to patients attending clinic or posted out.
Results
In total, 34 out of 56 questionnaires were returned. About 70% were aware of their most recent HbA1c. About 77% felt that their diabetes knowledge was good to excellent. About 23 and 21% patients experienced between 1–2 and 2–5 minor hypoglycaemia per week respectively. About 42% had attended the DAFNE course.
With regards to clinic environment, 88% rated clinic staff to be friendly and helpful, 84% thought that privacy level was good to excellent and 86% felt that enough time was devoted to appointments with minimal waiting times.
Timely notification of appointment letter was appreciated by 93%. About 73% felt adequate information was provided in the clinical correspondence. About 86% of patients were very satisfied with the accessibility and on-going support from diabetes specialist nurse (drop-in clinics, e-mail and mobile contact).
About 91% felt that the quality of care received in this clinic was good to excellent. About 94% wanted this transition clinic in the hospital setting rather than in the community. About 89 and 97% felt very satisfied with the consultations received from the doctors and nurses respectively. About 97% believed that this transition clinic helped them deal more effectively with their diabetes.
Conclusion
Although majority of our adolescents were satisfied with clinic environment and overall care received in the transition clinic, we need to focus more on specific aspects to further improve patient empowerment, mainly, patients own knowledge of their HbA1c, uptake of DAFNE and written clinical information given to our patients. We plan to re-audit this in future.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P605

**Celiac disease, autoimmune thyroiditis, vitamin B12 deficiency and adrenal failure among adult Turkish patients with type 1 diabetes mellitus**

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Introduction
Autoimmune polyglandular syndromes are characterized by the coexistence of at least two autoimmune endocrinopathies. Organ-specific autoimmunity is frequent in patients with type 1 diabetes mellitus (T1DM). The aim of this study was to estimate the prevalence of celiac disease and other autoimmune diseases like thyroiditis, pernicious anemia and adrenal failure among adult patients with T1DM.
Methods/design
Sixty-nine, asymptomatic patients with T1DM (32 females, 37 males), aged between 16 and 51 years were screened for celiac disease using antigliadin Ig A-G (AGA), endomisyal antibodies (EMA), anti-tissue transglutaminase (tTG). Endoscopic biopsies were performed for patients with positive antibody results. HLA-DQA1, HLA-DQB1 and HLA-DRB1 were available for 37 patients. Thyroid function tests, antithyroglobulin, antiperoxidase antibodies, level of vitamin B12, basal cortisol were measured for each patient.
Results
AGA was positive for 15 patients, EMA for two patients and tTG was positive for three patients; EMA and tTG positive patients were also positive for AGA. Endoscopic biopsies were performed in 10 patients, five patient did not accept to go undergo endoscopy. Two cases (2.9%) took a diagnosis of celiac disease with positive endoscopic biopsies revealing total mucosal atrophy. Increased intraepithelial lymphocyte count was present in four patients and gastritis was detected for remaining three patients. All patients were screened for autoimmune thyroid disease, adrenal disease and deficiency of vitamin B12. There were four (5.8%) cases of subclinical hypothyroid and three (4,3%) cases of overt hypothyroid patients. All were antibody positive. Thyroid antibodies were detected in 16 (23%) patients with normal thyroid function. Insulin tolerance test was performed for two patients whom basal cortisol levels were low. One took a diagnosis of Addison (1.4%). There were seven (10.1%) patients with vitamin B12 deficiency. Of 37 patients, HLA-DQA1 was positive for 25 patients, HLA-DQB1 for 24 patients and HLA-DRB1 for 18 patients. Although six patients were negative for HLA, one had high levels of thyroid antibodies and one had deficiency of vitamin B12.
Conclusion
Clinicians should be aware of possible autoimmune disease among patients with T1DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P606

**Correlation of fasting blood glucose with menopausal status in middle aged women with their obesity status**

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Introduction
The association between type 2 diabetes mellitus and obesity is very close. Obesity is common in women aged between 45 and 49 years. Prevalence of prediabetes, i.e. impaired fasting glucose also seems to be higher in women than men in the Indian population. The present work is planned to study the prediabetic status in preobese and obese in pre- and post-menopausal women with the help of estimation of fasting blood glucose (FBG) levels.
Materials and method
Fasting blood glucose levels and BMI were estimated in 300 asymptomatic women with no family history of type 2 diabetes mellitus (DM) using GOD–POD

method. Pre- and post-menopausal subjects are divided in control (I), preobese (IIa) and obese group (IIb). The results is analyzed statistically ANOVA test.
Result and conclusion
Mean FBG levels in preobese and obese group are higher than control group in both pre- and post-menopausal women. There is significant difference between BMI and fasting blood glucose levels in pre- and post-menopausal women. In a nutshell obesity with & without menopause both act as a important predictor for type 2 DM. Thus in premenopausal women lifestyle modifications may prove as important means for prevention of obesity and type 2 DM to minimize the complication in post-menopausal status.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P607

**Insulin resistance and C reactive protein level**

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C reactive protein is a sensitive marker of subclinical inflammation. C-reactive protein is associated with obesity, diabetes mellitus and increased cardiovascular risk. The mechanism of elevated CRP levels could have connections with insulin resistance. Inflammation seems to have significant role in pathogenesis of diabetes and cardiovascular disease. The aim of this study is to show that the subjects with greater insulin resistance, measured by HOMA-IR have higher levels of CRP.
Material and method
We included in the study 154 subjects with BMI below 25 kg/m$^2$, with no history of diabetes mellitus, conditions and medications that influence glucose control. We excluded patients with diabetes mellitus and CRP higher than 10 mg/l. In all subjects we measure body weight and height, BMI, CRP, FBG and insulin and calculated HOMA-IR.
Results
Regarding HOMA-IR values we divided all subjects in Group A with HOMA-IR less than 2 and Group B HOMA-IR higher than 2. In Group A CRP level was $1.76 \pm 0.72$ mg/l. In Group B CRP level was $3.84 \pm 1.45$ mg/l. There is statistically significant higher value of CRP in group B comparing to Group A, $P < 0.001$.
Conclusion
Our results show that greater HOMA-IR is associated with higher levels of CRP and that low-grade inflammation is associated with insulin resistance. Sublicinal inflammation leads to endothelial dysfunction and increased peripheral resistance that promotes insulin resistance further. Levels of CRP higher than 3 mg/l seems too be associated with increased risk for cardiovascular disease and diabetes mellitus. Our observation maybe can be used like adjunctive method for early detection of type 2 diabetes mellitus.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P608

**The antropometric characteristic of type 1 diabetic patients in Kragujevac**

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Background
Diabetes mellitus is a condition of chronic hyperglicemia (as well as metabolic disorders of the other carbohydrates, lipids and proteins), as a result of absolute and/or relative lack of insulin or lack of insulin effects. The aim of the research presented in this study is examination of antropometric characteristics of patients suffering from type 1 diabetes in Kragujevac depending on gender.
Material and method
We examined 206 patients with type 1 diabetes in Kragujevac, and compare according to gender (107 men and 99 women). All these patients were treated with insulin therapy.
Results
The average duration of DM1 was $12.1 \pm 5.2$ years. The prevalence of overweight was 11.3%. The average height was $173.14 \pm 9.4$ vs $164.16 \pm 7.27$ cm (man vs women, $P < 0.001$), the average weight was $74.22 \pm 14.04$ vs $67.58 \pm 11.28$ kg, $P < 0.001$), the average value of BMI was $26.01 \pm 8.19$ vs $28.55 \pm 5.25$ kg/m$^2$, man vs women, $P < 0.001$), the average value of waist $95.6 \pm 12.01$ vs $79.5 \pm 10.35$ cm, man vs women, $P < 0.001$), the average value of hip $97.89 \pm 7.7$ vs $104.61 \pm 8.67$ cm, man vs women, $P < 0.001$).
Conclusion
There is significant difference in height, weight, BMI, waist and hip between man and woman in type 1 diabetic patients.
Keywords: diabetes mellitus type 1, antropometric characteristic.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P609

**Resolution of type 2 diabetes following IFN-α therapy for chronic hepatitis C**

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We report on a patient whose type 2 diabetes resolved following interferon-α (IFN-α) therapy for hepatitis C virus (HCV). A 57-years-old man, presented with fatigue, polyuria and polydipsia. He was newly diagnosed with type 2 diabetes. During the hospital day, he was also diagnosed with chronic hepatitis. Serological studies for chronic hepatitis demonstrated presence of hepatitis C, type 2a/2c genotype. He was started on subcutaneous insulin and IFN-α. After 24 weeks of treatment of IFN-α, HCV polymerase chain reaction was negative. And diabetes resolved despite an increase in his body weight. A number of papers have described associations between IFN-α therapy and the precipitation of diabetic ketoacidosis as the presenting feature of new-onset diabetes. Our case shows a resolution of diabetes after IFN-α therapy for chronic hepatitis C. HCV mainly affects the liver, but also several tissues outside the liver have been reported to be involved, resulting in a wide spectrum of extrahepatic manifestations. It has been hypothesized that diabetes also could be one of these extrahepatic conditions attributable to HCV infection. Previous HCV infection markedly increased the prevalence of type 2 diabetes, regardless of the presence of liver cirrhosis. Non-diabetic HCV patients have insulin resistance and specific defects in the insulin-signaling pathway. The specific mechanisms by which HCV lead to type 2 diabetes are not fully understood, but it seems that an increase of insulin resistance associated with both steatosis and the overproduction of pro-inflammatory cytokine could play a crucial role. Although it is unclear whether the resolution of diabetes in this case occurred as an effect of IFN-α or as a result of becoming HCV RNA negative, it has suggested a great deal about the role of IFN-α and HCV infection in the pathogenesis of diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P610

**Art and its role in therapeutic patient education**

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Art-painting, poetry, music, is an inexhaustible source of inspiration and motivation. Patient education (TPE) in chronic diseases is a long and tedious process. To achieve high motivation one must use all existing possibilities. Art is the most potent tool to influence the conscious and subconscious of a person to motivate him/her to adhere to treatment and healthy lifestyle. Literature, and mainly W. Shakespeare, could be used for TPE, as deep understanding of existing health problems are reflected in his works. Shakespeare's worlds are the mirror of the various aspects of medicine during the renaissance. Most of the problems, such as obesity/overweight, excessive appetite, and alcohol intake, hereditary factors, depression, influence of every day's life on person's wellbeing are described in Othello, Hamlet, Richard III, Henry VI, Love's Labor's Lost, Comedy of Errors and others. Longville's words (Love's Labor's Lost) 'fat paunches have lean pates; and dainty bits make rich the ribs, but bankers out the wits', may be used as strong motivational element when eating habits and weight control are aimed at. We are widely using painting, literature, music in patient education process. The questionnaires used to assess the effect of art showed that almost all patients give positive evaluation to such approach. Patients we are working with express their impression of the art as follows 'it penetrates deeply into the subconscious and helps to change the habitual world'. Case reports, presented by W. Shakespeare can be widely used to stimulate educational process.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P611

**The antropometric characteristic of type 2 diabetic patients in Kragujevac**

J. Petrovic, V. Mladenovic, A. Djukic & S. Sipetic
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Introduction

Diabetes mellitus is a condition of chronic hyperglicemia (as well as metabolic disorders of the other carbohydrates, lipids and proteins), as a result of absolute and/or relative lack of insulin or lack of insulin effects.

The aim of the research presented in this study is examination of antropometric characteristics of people suffering from type 2 diabetes in Kragujevac.

Material and method

We examined 3108 patients with type 2 diabetes in Kragujevac, and divide patients in two groups: 2470 patients (73.8%) with oral antidiabetic therapy (DM2) and 638 patients (19.1%) with insulin therapy (DM2I).

Results

The average duration of DM2 was $6.37 \pm 5.8$ years, and for DM2I $12.87 \pm 7.39$ years. The average height was $165.8 \pm 9.68$ vs $167.2 \pm 9.69$ cm (DM2 vs DM2I, $P = 0.041$), the average weight was $79.37 \pm 14.19$ vs $74.19 \pm 13.73$ (DM2 vs DM2I, $P < 0.001$), the average value of BMI was $28.82 \pm 4.72$ vs $26.56 \pm 4.59$ kg/m$^2$ (DM2 vs DM2I $P < 0.001$), the average value of waist $98.09 \pm 11.13$ vs $92.59 \pm 11.72$ cm (DM2 vs DM2I $P < 0.001$), the average value of hip $108.67 \pm 9.88$ vs $104.61 \pm 8.67$ cm (DM2 vs DM2I $P < 0.001$). We also compared patients according to sex: the average height was $174.14 \pm 9.4$ vs $161.16 \pm 7.27$ cm (man vs women, $P < 0.001$), $81.99 \pm 15.11$ vs $74.22 \pm 14.04$ (man vs women, $P < 0.001$), the average value of BMI was $26.86 \pm 4.43$ vs $28.55 \pm 5.25$ kg/m$^2$ (man vs women, $P < 0.001$), the average value of waist $98.92 \pm 10.75$ vs $95.6 \pm 12.01$ cm (man vs women, $P < 0.001$), and the average value of hip $107.7 \pm 7.49$ vs $109.39 \pm 10.6$ cm (man vs women, $P < 0.001$). There is significant difference in height, weight, BMI, waist and hip between man and woman.

Keywords: diabetes mellitus type 2, antropometric characteristic.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P612

**Prevalence of microvascular complications in youth with type 2 diabetes: systematic review and meta-analysis**

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Introduction

Type 2 diabetes (T2D) has increased in youth and complications rates may be higher than type 1 diabetes (T1D).

Aims

To estimate the prevalence of complications and hypertension in youth with type 2 diabetes and compare complication rates between T2D and T1D.

Design and methods

Systematic review and meta-analysis of observational studies, analysed with random effects models.

Data sources

Medline and Embase (until Oct-2011), article bibliographies and contact with experts.

Eligibility criteria

Longitudinal or cross-sectional observational studies measuring incidence or prevalence of nephropathy, hypertension, retinopathy, peripheral neuropathy and/or autonomic neuropathy in youth or young adults ($\leq$28 years) with T2D. Studies of youth with both T2D and T1D were included; where possible meta-analysis was conducted to compare complication rates. Evidence levels were assigned using the Oxford CEMB and quality assessed using the Newcastle–Ottawa scale.

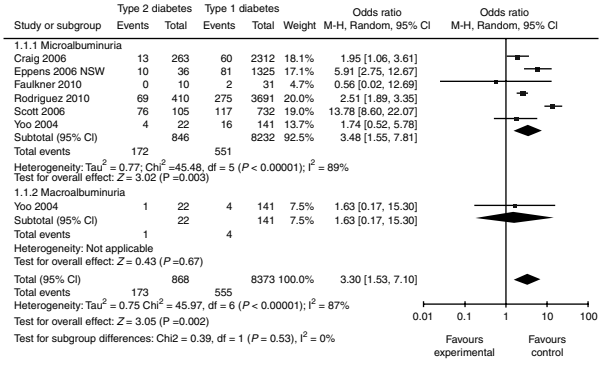
Results

The final search yielded 25 studies involving 3321 youth. Overall, methodological quality was poor. Among those with T2D, median age was 14.5 years, duration 1.7 years and HbA1c 7.7%. Pooled prevalence (95% CI) for T2D was: microalbuminuria 18% (17–20%); macroalbuminuria 5% (3–7%); hypertension 28% (26–29%); retinopathy 2% (1–3%); peripheral neuropathy 2% (1–4%); and autonomic neuropathy 43% (25–63%). Compared with T1D, microalbuminuria was more common in T2D: odds ratio (OR) 3.5, 95% CI 1.6–7.8, and hypertension: OR 3.4, 95% CI 2.4–4.8. There was significant heterogeneity across studies for microalbuminuria, hypertension and nephropathy ($P < 0.01$).

Conclusions

This is the first systematic review of complications in youth with T2D; they have higher rates of microalbuminuria and hypertension compared with T1D, despite shorter diabetes duration. There is a paucity of quality data on complications in youth with T2D. Large-scale prospective cohort studies are required to better understand their specific risk factors for microvascular complications, and to guide complications screening and treatment.

Figure 1 – Odds ratio for nephropathy prevalence in youth with type 2 vs type 1 diabetes.




Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P613

**The intake of fiber suppresses the high fat high carbohydrate meal induced endotoxemia, oxidative stress and inflammation**

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Our previous work has shown that the intake of a high fat high carbohydrate (HFHC) meal results in an increase in endotoxin (LPS) and LPS binding protein (LBP) concentrations concomitant with an increase in the expression of TLR-4, the receptor for LPS, and CD-14, which facilitates the binding of LPS to its receptor. In addition, there is an increase in ROS generation, NFkB binding and the expression of TNF-$\alpha$ and IL-1$\beta$. Our work has also demonstrated that an equicaloric meal rich in fiber and fruit (American Heart Association (AHA) recommended meal) does not induce these changes. We hypothesized, therefore, that the addition of fiber to a HFHC meal will prevent the changes induced by HFHC meal. Eight fasting normal subjects (BMI $<25$ kg/m$^2$) were given 900 Calorie meals of either HFHC or AHA or HFHC with additional fiber (30 g) (Fiber One Original) on three sequential visits one week apart in a randomized crossover design. As expected, the HFHC meal induced an increase in LPS concentrations (by $69\pm14\%$) with an increase in ROS generation (by $120\pm24\%$) and the expression of TLR-4, CD14 and IL-1$\beta$ (by $71\pm14$, $86\pm14$ and $127\pm14\%$, respectively; $P<0.05$ for all) in MNC. The AHA meal did not induce any of these changes. The addition of fiber to HFHC meal significantly reduced the increase in LPS concentrations (by $58\pm10\%$; $P<0.05$), ROS generation (by $47\pm14\%$; $P<0.05$) and the expression of TLR-4, CD14 and IL-1$\beta$ (by $37\pm11$, $46\pm12$ and $52\pm15\%$, respectively; $P<0.05$ for all) observed with the HFHC meal alone. Additionally, the intake of fiber with the HFHC meal reduced postprandial glucose excursion and increased insulin concentrations compared to HFHC meal alone. We conclude that the addition of fiber to a pro-inflammatory HFHC meal has a beneficial anti-inflammatory and metabolic effect. Thus, the fiber content of the AHA meal may account for its non-inflammatory nature. Since ROS, TLR-4 and IL-1 $\beta$ potentially interfere with insulin signal transduction and are known to be pro-inflammatory in the arterial wall, this action of dietary fiber may contribute to its benefits in the prevention of insulin resistance, type 2 diabetes and atherogenesis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P614

**Blocking Notch pathway improves wound healing in diabetic mice**

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Aim

We have proposed to study the modulation of the Notch pathway in diabetes having in mind the essential role played by the Notch system in the regulation of cell differentiation and angiogenesis.

Methods

The effect of hyperglycemia on Notch system was studied *in vitro* and *in vivo* using western blot, reporter gene assay or evaluation of target genes (qRT-PCR). The functional consequences of the Notch system modulation were studied by the assessment of the migration and angiogenesis potential. Notch pathway inhibition was induced either chemically with gamma secretase inhibitors (DAPT, L-685,458). The effect of the Notch inhibition on wound healing was evaluated in db/db mice.

Results

Hyperglycemia activates Notch pathway at several levels as shown by increased NICD level, increased reporter gene activity and enhanced expression of several essential target genes as evaluated both *in vitro* and *in vivo*. The inhibitory effect of high glucose on migration of HDF and angiogenesis was cancelled by blocking the Notch signaling with different gamma-secretase inhibitors (DAPT or L-685,458). Specific inhibition of different Notch receptors (1–4) by siRNA pointed out to a crucial role of Notch1 in migration and angiogenesis. Local treatment with gamma-secretase inhibitors (DAPT or L-685,458) improved the wound healing in db/db mice (percentage of wound closure on day 12 was $60\pm2\%$ (DAPT), $70\pm4\%$ (L-685,458) and $43\pm5\%$ (placebo) respectively ($P<0.01$). Blocking Notch pathway in diabetic wounds was followed by increase in granulation and epidermal formation, increase in blood vessel formation and increase in expression of chemokine (SDF-1 alpha) responsible for better recruitment of EPCs.

Conclusions

Hyperglycemia activated Notch signaling with deleterious effects on cell migration and angiogenesis. Blocking the overactive Notch pathway by local treatment with gamma-secretase inhibitors improves wound healing rate in diabetic animal model.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P615

**The role of AMP-activated protein kinase in myocardial tolerance to ischemia in rats with diabetes mellitus type 2 and its relation to metformin therapy**

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Introduction

The aim of our experimental study was to investigate the effect of both T2DM and metformin treatment on myocardial tolerance to ischemia-reperfusion injury and cardiac AMP-activated protein kinase (AMPK) activity.

Methods

The protocol included four groups: i) controls ($n=13$) – non-diabetic rats treated with i.p. vehicle injections for 3 days prior to the heart perfusion, ii) controls + metformin (CM; $n=12$) – non-diabetic rats treated with metformin (200 mg/kg) i.p. for 3 days prior to the perfusion, iii) T2DM ($n=12$) – diabetic animals receiving vehicle, iv) T2DM + metformin (T2DMM; $n=7$) – diabetic rats treated with metformin. In all groups, the hearts were subjected to 30-min global ischemia and 120-min reperfusion according to Langendorff followed by histochemical determination of infarct size. Phosphorylated $\alpha$-AMPK was evaluated with western blot analysis.

Results

Infarct size and postischemic recovery of left ventricular function were not different between controls and CM-group. Infarct size in T2DM was significantly lower than in controls ($24.4\pm7.6$ vs $45.0\pm10.4\%$, respectively, $P<0.01$), which is indicative of the phenomenon of metabolic preconditioning in T2DM. At the same time in T2DMM group there was no appreciable effect on infarct size ($37.7\pm8.3\%$). In comparison to controls, $\alpha$-AMPK phosphorylation was significantly increased in T2DM and, to a lesser extent, in CM-animals. However, AMPK activity was not different between T2DM and T2DMM groups.

Conclusion

AMPK phosphorylation was increased in both T2DM and CM, but it did not provide a significant cardioprotection in CM group. Moreover, combination of T2DM and metformin treatment had not potentiated infarct-limiting effect of T2DM, meaning that AMPK activation by metabolic preconditioning is not additive to that seen with metformin treatment. As the diabetes may act through many different pathways in the cell the effect of diabetes was more manifested in ischemia tolerance improvement.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P616

**A novel case of diabetes mellitus and aniridia in a Caucasian family**

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Introduction

We describe a novel case of familial congenital aniridia in a Caucasian woman with associated insulin requiring diabetes mellitus. This human combination has been described in Asian families in association with a heterozygous Paired Box 6 (PAX6) mutation (1) with subsequent evidence supporting that the mutation in

this transcription factor causes decreased Proconvertase 1/3 function in the pancreas and a high proinsulin to insulin ratio (2). Our case does not have a PAX6 mutation and study ratios of proinsulin to insulin were not consistent with impaired conversion.

Clinical case:

A 54-years-old woman of Caucasian descent was diagnosed with diabetes mellitus aged 28. She commenced a basal-bolus regimen soon after diagnosis, currently taking $<20$ units insulin per day. She has a HbA1C of 7.5% without microvascular complications. Her body mass index is 21 kg/m$^2$. Recent glutamic acid antibody and islet cell antibody tests were negative.

The patient has congenital bilateral aniridia with subsequent bilateral cataracts. She has an intraocular lens implant in her left, and a contact lens in her right eye. There is a significant family history with her father having aniridia without diabetes, her sister having aniridia with poorly controlled diabetes, renal failure, and severe retinopathy, her nephew having aniridia alone.

Conclusions

Up to 10% of congenital aniridia cases do not have identifiable PAX6 mutation (3). This could be due to genomic alterations in regulatory elements of PAX6 or a mutation in another gene known to cause congenital iris abnormality (4). An ocular developmental gene forkhead box C1 (FOXC1) heterozygous mutation (W152G) has been suggested to produce aniridia in man by resulting in failed autophagy of poorly folded proteins (5).

We postulate that this mechanism could offer another means by which the beta cell may be vulnerable in man and should be sought in PAX6 negative aniridia cases with diabetes.

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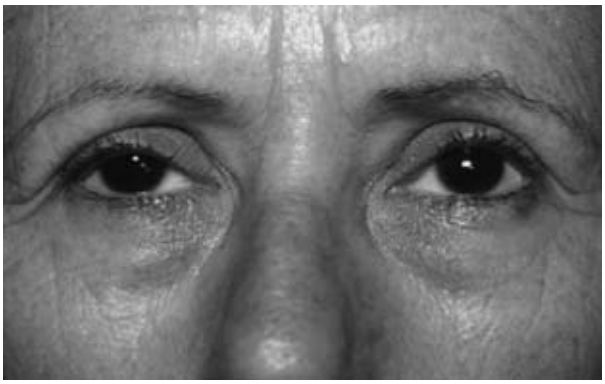

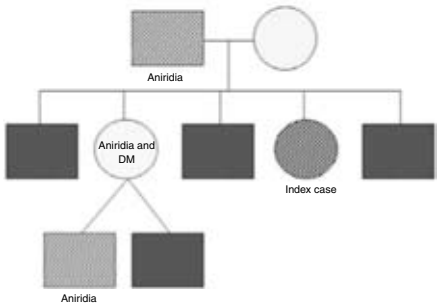


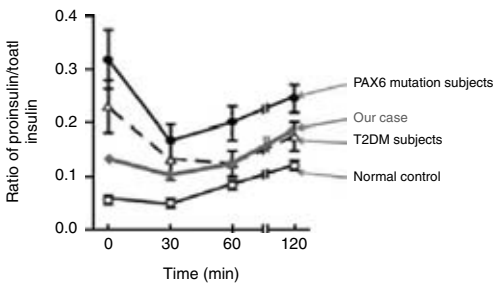


Top: Congenital bilateral aniridia Middle: Family tree Bottom: Meal study ratios of proinsulin to insulin were not consistent with impaired conversion

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P617

**Experience of diabetic dyslipidemia correction in patients with ischemic heart disease and type 2 diabetes mellitus using α-lipoic acid in combination therapy**

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Purposes

To investigate the effects of α-lipoic acid (ALA) on diabetic dyslipidemia, endothelial dysfunction, levels of adiponectin and proinflammatory mediators in combination therapy of patients with ischemic heart disease (IHD) and type 2 diabetes mellitus (T2DM).

Methods

We examined 40 patients with IHD and T2DM (19 males, age $60.5\pm4.7$ years). Baseline characteristics of patients included history of IHD ($7.2\pm2.3$ years), T2DM ($4.7\pm0.5$ years). The level of HbA1c was $<7.5\%$. All patients were divided into two groups: the 1st ($n=20$) – received the standard therapy, the 2nd ($n=20$) in the standard therapy received ALA 600 mg once daily. In all patients were determined the levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL), triglycerides (TG), high-density lipoprotein cholesterol (HDL) by enzymatic colorymetric method, proinflammatory mediators (TNF-α, hsCRP), vascular endothelial growth factor (VEGF) and adiponectin by ELISA method at baseline and in 6 months.

Results

Using in combination therapy ALA increased plasma levels of HDL on 5% (0.4 mmol/l), decreased TC, LDL and TG levels on 4, 5.2 and 6.3% respectively (all $P<0.001$), substantially lowered plasma levels of TNF-α by $6\pm1.5\%$ ($P<0.05$) and hsCRP from $1.53\pm0.13$ to $0.98\pm0.11$ pg/ml ($P<0.05$), increased plasma levels of adiponectin by $18\pm2\%$ ($P=0.001$) compared with the 1st group. The serum VEGF concentrations in patients who received in the standard therapy ALA were significantly reduced from $320\pm26$ pg/ml at baseline to $212\pm22$ pg/ml in 6 months ($P=0.022$). There were correlations between changes in adiponectin levels and the VEGF concentrations ($r=-0.31$, $P=0.043$).

Conclusions

Combination therapy with ALA significantly reduced TC, LDL, TG and proinflammatory mediators, VEGF, increased HDL in patients with IHD and T2DM.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P618

**Changes of NO concentration in the cause of streptozotocin diabetes mellitus and under action of different NOS inhibitors in streptozotocin rats**

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Background

Diabetes mellitus (DM) and its complications cause numerous health and social problems throughout the world. Pathogenic action of nitric oxide (NO) is responsible to a large extent for development of complications of the disease. Thus modification of NO production in DM patients appears to be an option for treatment of DM complications.

Objective

To study changes in NO concentration in diabetic rats after administration of different compounds with potential influence of NO production: iNOS inhibitors

aminoguanidine and 1400 W, specific inhibitor of Kupffer cells GdCl3 and xantine oxidase inhibitor allopurinole.
Methods
Severe diabetes mellitus in rats was induced by single injection of streptozotocin (STZ) 50 mg/kg, i.v., after 1 week of diabetic state compounds of interest were administered. Production of NO was monitored by means of ESP spectroscopy of Fe–DETC–NO complex in brain cortex, cerebellum, liver, kidneys, whole blood, skeletal muscle, subcutaneous fat and aorta.
Results
Development of STZ DM was followed by increase of NO production in the liver from 45.39±5.93 to 101.81±12.12 ng/g tissue, kidneys (from 2.64±0.97 to 15.04±2.04 ng/g tissue), blood (from 29.36±2.15 to 41.62±3.93 ng/g tissue), muscles (from 7.82±1.87 to 12.65±0.87 ng/g tissue), aorta (from 14.4±0.2 to 42.2±4.2 ng/g). In adipose tissue NO production decreased from 34.00±3.68 to 18.56±1.81 ng/g tissue. In diabetic rats, 1400 W decreased NO concentration in liver, kidney, muscle, whole blood, fat and aorta, aminoguanidine – in all studies organs except brain cortex, allopurinol – in kidneys, muscle, aorta and fat.
Conclusions
Diabetic state provokes increase of NO production in rats. iNOS inhibitors aminoguanidine and 1400 W, as well as allopurinole attenuated increase of NO concentration in most organs studied. Modification of NO production could be useful in prevention of some diabetic complications.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P619

**Cardiac dyssynchrony as a pathophysiological mechanism of heart failure development and progression in type 2 diabetic patients**

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Objectives
Diabetes mellitus (DM) is an independent predictor of new onset heart failure (HF) and adverse outcomes among patients with already existing HF. However mechanisms leading to impaired cardiac performance due to DM are still unclear. The purpose of investigation was to assess the influence of cardiac dyssynchrony (DYS) on systolic and diastolic function in patient with type 2 diabetes mellitus (T2DM) with concomitant stable coronary artery disease (CAD) and without arterial hypertension (AH).
Methods
In total, 42 patients (67±12 years, 20 males) with stable CAD, without AH in HF II–III NYHA class were divided into two age-matched groups: 1st group ($n=22$) – patients with T2DM (average duration 5.6±2.1 years) and 2nd group ($n=20$) – patients without DM. In all patients were determined body mass index (BMI), HbA1c level, HOMA-IR and specific echocardiographic tissue Doppler imaging (TDI) parameters as myocardial mitral annular (Sa) and basal segmental (Sm) systolic and early diastolic (Ea and Em) velocities, transmitral to TDI Ea ratio (E/Ea) and time to peak and onset of Sm from four basal and four mid left ventricular (LV) segments. Maximal difference in time between Sm-Ts-diff > 90 ms and standard deviation-Ts-SD > 37 ms were considered DYS.
Results
In the 1st group DYS and diastolic dysfunction were significantly more frequent than in the 2nd one (45.5 vs 35.0% and 68.2 vs 60.0% respectively, both $P<0.05$). Subanalysis of 1st group has shown correlation between Ts-SD and HbA1c level ($r=+0.48$, $P<0.05$), BMI ($r=+0.51$, $P<0.05$) and HOMA-IR ($r=+0.57$, $P=0.048$). The highest values of Ts-SD and Ts-diff were obtained in patients with Ea = 2.2 m/s and E/Ea = 42.
Conclusions
It was found that in patients with T2DM DYS is one of the factors which have negative impact on cardiac performance and thus the prognosis. The degree of mentioned disturbances correlates with glycemic control and insulin resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P620

**Possible risk factors of type 2 diabetes and role of long term oral hypoglycemic agent, metformin**

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Introduction
Over the past decades diabetes emerged as epidemic dimension due to person's genetic pre disposition and environmental condition. Despite significant advancement in the development of oral hypoglycemic agents, an ideal drug for treating T2DM and its complication is still a distant reality. Hyperglycemia has a variety of toxic effects, including elevated generation of ROS nitrosative stress and inflammation. This work attempts to explore the role of oxidative and nitrosative stress for the electron leakage in the mitochondria initiating cardiovascular disease pathogenesis. Apart from classical risk factors, elevated serum concentration of Hcy is closely associated with the increased risk of atherosclerosis. Here we report, how Hcy upregulation may be controlled in diabetic patients administered with hypoglycemic agent, metformin.
Methods
Metformin administered and placebo T2DM subjects participated in the study.
Results
Metformin treatment diminished ROS production, MMP, inflammation and restore nitric oxide production in diabetic subjects. Homocysteine level is higher in metformin administered patients (16.2+3.5 vs 14.5+2.9 μmol/l). Vitamin B12 level is greatly reduced in metformin treated patients compared to placebo ($P=0.001$). The mean values of Hcy level for genotypes (677C > T MTHFR)11, 12 and 22 are 14.76, 23.37 and 29.12 μmol/l respectively.
Conclusion
Greater reduction of ROS, AOPP, CRP and pentosidine level and restoration of NO from baseline were achieved in metformin administered patients, elucidating that metformin extends added protection to combat the disease pathogenesis. Metformin treatment balance mitochondrial homeostasis by normalizing the ion transport. Enhanced Hcy production is not related with metformin treatment rather it is a drug independent phenomena and depend greatly on polymorphism in MTHFR 677 C > T phenotype. These information will be beneficial for the emergence of new pharmacogenomic initiatives.
ROS generation (1a,1c-baseline,1b-metformin administered,1d-placebo) and hyperpolarisation of mitochondrial membrane (3a,3b,3e,3f-baseline,3c,3d-metformin administered 3g,3h-placebo) reduced in metformin administered subjects in comparison to placebo. Nitric oxide production (2a,2c-baseline,2b-metformin administered,2d-placebo) is restored in metformin treated subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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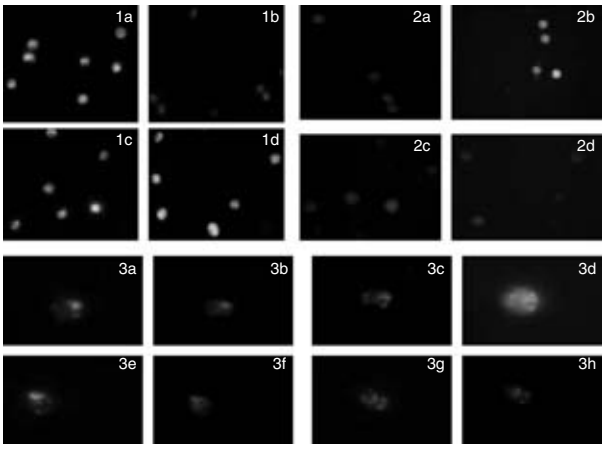

## P621

**Complex ventricular arrhythmias with early repolarization in type 2 diabetes mellitus**

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Background and aims
Mortality rate from cardiovascular disease (CV) including sudden death (SD) is pronounced in type 2 diabetic (T2DM) patients with cardiac autonomic neuropathy (CAN) and/or complex ventricular arrhythmias (CVA). Early repolarization (ER) is also considered to be a significant risk factor for SD. The aim of the study was to evaluation the relation between CAN, CVA and ER in T2DM patients.
Materials and methods
Participants were 44 T2DM patients (24m/20f, mean age 56.9 $\pm$ 7.3 years) with ER pattern (Gr.1) at the surface ECG defined as a J-point elevation of at least 0.1 mV in at least two leads I,II,III,avF,avL,V4,V5 or V6, when slurring of the terminal part of QRS was associated with an elevation of ST segment. As controls (Gr.2) 35 sex, age and diabetes duration matched patients were selected. In all patients 24 h Holter ECG was performed.
Results
CVA, such as paired ventricular extrasystoles, episodes of ventricular tachycardia (VT) were more frequently registered in Gr.1 (43.2 vs 34.3%), while frequent supraventricular extrasystoles were more often observed in Gr. 2 (37.1 vs 34%), though this difference was not significant. Heart rate variability (HRV) parameters SDNN (0.091 $\pm$ 0.023 vs 0.118 $\pm$ 0.029, $P = 0.000$) and triangular index (25.4 $\pm$ 6.3 vs 33.6 $\pm$ 8.1 $P = 0.000$) were significantly lower in Gr 1.
Conclusion
CVA were more frequently observed in T2DM patients with ER. HRV parameters were lower in patients with ER. Increased risk of sudden cardiac death in T2DM patients may be associated with repolarization abnormalities due to cardiac autonomy neuropathy that predisposes to fatal ventricular arrhythmias. Further studies are required in order to assess the role of cardiac autonomy neuropathy in the development of repolarization abnormalities in T2DM patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P622

**Diabetic nephropathy in pregnant women with type 1 diabetes mellitus**

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Background
Diabetic nephropathy represents one of the most common causes of end-stage renal disease. During pregnancy it is associated with an increased perinatal morbidity as a striking result of the consistently high rate of preterm deliveries, mainly due to the frequent development of pre-eclampsia.
Aim
To describe pregnancy outcome in type 1 diabetic women with normoalbuminuria, microalbuminuria, or diabetic nephropathy after implementation of an intensified antihypertensive therapeutic strategy.
Design and methods
In our prospective study were enrolled 80 pregnant women with type 1 diabetes. Antihypertensive therapy, mainly methyldopa, was given to obtain blood pressure $<$135/85 mmHg and urinary albumin excretion $<$300 mg/24 h. Blood pressure and HbA1C were recorded during pregnancy. The pregnancy outcome was compared with recently published studies of pregnant women with microalbuminuria or diabetic nephropathy.
Results
Antihypertensive therapy was given in 9 of 54 women with normoalbuminuria, 8 of 18 women with microalbuminuria, and all eight women with diabetic nephropathy. Mean systolic blood pressure during pregnancy was 124 mmHg (range 105–151), 125 mmHg (119–138), and 138 mmHg (113–147) in women with normoalbuminuria, microalbuminuria, and diabetic nephropathy, respectively ($P = 0.0095$). No differences in mean diastolic blood pressure or HbA1C were detected between the groups. No women with microalbuminuria developed preeclampsia. The frequency of preterm delivery was 22% in women with normoalbuminuria and microalbuminuria, in contrast to 75% in women with diabetic nephropathy ($P < 0.01$) where the median gestational age was 254 days.
Conclusions
With intensified antihypertensive therapy and strict metabolic control, comparable pregnancy outcome was seen in type 1 diabetic women with microalbuminuria and normoalbuminuria. Diabetic nephropathy was associated with more adverse pregnancy outcome, compared to other studies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P623

**Effects of chronic administration of Vardenafil on endothelial function in patients with type 2 diabetes mellitus. A longitudinal, randomized, placebo-controlled, double blind, non-sponsored clinical trial**

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Introduction
The endothelium produces molecules regulating vascular function, such as nitric oxide (NO). NO protects blood vessels from endogenous injury and causes vasodilation through activation of second messengers. In diabetes mellitus (DM), hyperglycemia provokes oxidative stress, reduction of NO and increased synthesis of vasoconstrictors (e.g. endothelin). Cyclic nucleotide phosphodiesterases (PDEs) are enzymes, controlling the rate of degradation of second messengers. In short-term studies PDE-5 inhibitors showed promising results on endothelial function parameters. No data about chronic treatment in diabetic subjects are available.
Aim
To investigate if chronic treatment with Vardenafil for six months can prevent or delay the deterioration of systemic endothelial function in patients with type 2 DM.
Methods
Longitudinal, randomized, placebo-controlled, double-blind clinical trial involving men with type 2 DM. Study duration: 52 weeks including enrollment (4 weeks), treatment (24 weeks), follow-up (24 weeks). Treatment: 2 $\times$ 10 mg/day of Vardenafil/placebo. Parameters: andrological and instrumental examination and blood sampling performed at each visit. Primary end-point: serum endothelin-1. Sample size: 53 patients per arm.
Results
Interim analysis of blinded data: of 35 patients enrolled so far, 18 (51.43%) completed the treatment phase, 12 (34.29%) ended the study. Five patients dropped-out (14.29%). All patients, had a good adherence to treatment (mean of tablets taken: 92.2%). Glycated hemoglobin, considering 18 patients who ended the treatment phase, improved in seven (38.9%), worsened in 2 (11.1%) and remained elevated in five (27.8%) patients. Flow-mediated dilation (FMD, 13 patients) improved in 1 (7.7%), worsened in three (23.1%) and persisted elevated in three (23.1%) patients. Intima-media thickness (IMT) and IIEF-15 improved in 1 patient. Endothelin-1 and other serum parameters will be measured at the end of the study.
Conclusion
Intervention per se results in variations of glycated hemoglobin and FMD values (improvement/impairment) in 50% and 30.8% of patients, respectively but does not seem to influence IMT and IIEF-15 values.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P624

**Pattern of chronic complications among Nigerian patients with DM: the need for focused prevention initiatives**

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Introduction
The prevalence of diabetes mellitus among Nigerian adults has been increasing over time. A considerable number of chronic complications of DM can be

adequately prevented, however, the proportion of patients presenting with chronic complications of DM in our setting is still relatively high.
Objectives
This study was carried out to assess the pattern of chronic diabetic complications among patients attending the diabetic clinic of Lagos University Teaching Hospital and to identify the factors associated with these complications.
It is hoped that this data will provide information to physicians and public health workers for focused prevention initiatives for these patients.
Methodology
A cross-sectional descriptive survey was carried out among patients attending the diabetic clinic from January to March 2011 using a systematic random sampling method. In total, 419 patients were selected and interviewed using a pre tested interviewer administered questionnaire after written informed consent.
Results
The age of the patients ranged from 25 to 84 years with a mean of $55.2 \pm 12.5$ years. There were more females (62%) than males (38%). Most of them were married (65%) with a mean duration of DM of $8.4 \pm 6.5$ years. Up to 16.9% of the patients had at least one complication of DM at the time of the survey. Eye problems were by far the commonest type accounting for 65% of these. This was followed by neurological complications (16.7%). Cardiovascular problems accounted for 11.6% while foot complications and renal problems were 3.3 and 1.7%, respectively. A bi-variate analysis revealed that increasing age, lower educational status, increasing duration of DM, the presence of co-existing hypertension and a higher waist circumference were significantly associated with the presence of chronic complications among these patients. A logistic regression revealed that increasing age and co-existing hypertension were the strongest predictors of chronic complications among these patients.
Conclusion
There is therefore a need to focus on routine preventive eye screening especially among the elderly diabetics attending this clinic. The prevention and appropriate management of hypertension among known diabetics will also play an important role in the prevention of chronic complications among these diabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P625

### The correlation between metabolic syndrome and albuminuria in type 2 diabetes mellitus

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Background
Diabetic nephropathy (DN) represents the leading cause of end-stage renal disease (ESRD). It is known that patients with ESRD have the highest risk of cardiac morbidity and mortality. Thus, identifying and treating risk factors associated with cardio-vascular disease in diabetes may offer the best approach for preventing and delaying adverse renal and cardiovascular outcomes.
Aim
To determine the relationships between metabolic syndrome (MS), diabetic nephropathy (DN) and renal function in type 2 diabetes mellitus (DM).
Methods
In our clinic-based cohort study were enrolled 374 type 2 diabetic patients, from which 52% males and 48% females, with a mean age $58 \pm 10$ years. We analysed MS, detected DN and estimated glomerular filtration rate (eGFR) during a 4 years period.
Results
Prevalence of both microalbuminuria and macroalbuminuria were higher in subjects with MS, increasing proportionally with the number of MS components. eGFR was lower in subjects with MS than in those without ($89 \pm 18$ vs $91 \pm 20$ ml/min per 1.73 $m^2$; $P < 0.001$). The lowest eGFR values were found in those with four or more components of MS. Prevalence of low eGFR increased with the stage of DN and was affected by MS only in normoalbuminuric patients. The most important result was that MS was independently associated with DN, but not with low eGFR, after adjustment for all of the individual components of the MS.
Conclusions
In this study we have noted an independent association between MS and DN. This is a strong argument for treating the MS by an intensive multifactorial therapy, in order to prevent the progression of DN to the renal failure.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P626

### Diabetic nephropathy and risk factors associated with DM in newly diagnosed type 2 diabetics

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Objective
Nephropathy is a common complication in diabetes mellitus (DM), with significant morbidity and mortality. Diabetic nephropathy (DN) is more likely to develop in the presence of some risk factors associated with type 2 diabetes mellitus, even in newly diagnosed diabetics.
Aim
To study the incidence of diabetic nephropathy in newly diagnosed type 2 diabetics and to study the relationship of development of nephropathy with various risk factors associated with DM, like age, sex, blood pressure, blood sugar and body mass index (BMI).
Material and methods
We analysed 100 newly diagnosed type 2 diabetics (diagnosed within 2 years), between September 2008 to October 2010. Presence of urinary microalbuminuria in two samples in a period of 6 months was taken as criteria for detecting nephropathy.
Results
Incidence of DN in newly diagnosed type 2 diabetics was 15% (15/100). This incidence had significant correlation with age, increasing significantly with increase in age (33% in age group $>60$ years). Also, DN resulted more frequent in male sex and high blood pressure, with incidence of nephropathy being as high as 8% at BP $>160/100$ mmHg ($P = 0.001$). The incidence of DN also increased with increase in BMI as well as HbA1c. Dyslipidemia also significant effect, especially hypertrigliceridemy ($P = 0.01$). The familly history of DM didn't result significantly important in development of DN.
Conclusion
Incidence of DN in newly diagnosed type 2 diabetics is as high as 15%. Hypertension is the most important associated factor contributing to development of nephropathy in these patients. Age over 60 years old, male sex, poor glycemic control, high BMI and dyslipidemia play significant role.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P627

### Clinical experience of gel testosterone in men with diabetes type 2 and late-onset hypogonadism

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Aim
To study the effect of testosterone replacement treatment in the aging male with T2DM.
Material and methods
Twenty-six hypogonadal men with T2DM and late-onset hypogonadism between the age 50–65 years (55.00 (50.00; 58.00)) were treated with a 1% testosterone gel within 12 months.
Results
Testosterone levels increased from 4.65 (2.70; 6.10) nmol/l at baseline to 13.65 (12.60; 15.20) nmol/l after 48 weeks of treatment ($P < 0.001$). Free testosterone increased from 0.106 (0.062; 0.152) nmol/l to 0.426 (0.345; 0.553) nmol/l ($P < 0.001$). SHBG decreased from 43.75 (31.83; 48.48) nmol/l to 30.40 (26.28; 32.03) nmol/l ($P < 0.001$). We showed reduction of levels of TG from 2.09 (1.37; 3.42) mmol/l to 1.33 (1.12; 1.62) mmol/l ($P = 0.002$), HbA1C from 8 (6.93; 10.68) to 6.8 (5.90; 8.15)% ($P < 0.05$), leptin from 10.0 (4.0; 22.7) μg/l to

5.25 (4.03; 8.23) μg/l, HOMA-IR from 4.37 (1.98; 5.76) to 2.46 (1.98; 3.04). PSA levels fluctuated minimally within in the normal range. All patients reported improved mood, sexual function and QoL.
Conclusions
Treatment with the 1% testosterone gel was associated with significant improvements in the testosterone, triglyceride, HbA1C, leptin, HOMA-IR levels in men with late-onset hypogonadism and T2DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P628

**Influence of planning pregnancy in pregestational diabetes**

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Introduction and objective
The pregnancies in women with pregestational diabetes are associated with increased risk of abortion, birth defects, complications of pregnancy and perinatal morbidity and mortality. Pregnancy planning would be associated with better metabolic control, and therefore better obstetric and perinatal outcomes. The objetive of this study has been to evaluate the obstetric results of women with pregestacional type 1 diabetes and compare planned with not planned pregnancies.
Material and methods
Retrospective cross-sectional study including pregnancies followed in our Endocrinology and Pregnancy Unit (1996–2010). We compare the results between planned and unplanned pregnancies and present descriptive data (frequencies, means and S.D.). The frequencies are compared with the $\chi^2$ test, the means by parametric tests (Student' *t*-test) or nonparametric (Mann–Whitney). It was considered significant a $P<0.05$.
The results in planned (122 pregnancies) vs non planned (136 pregnancies) were: we found differences in: age (years): 30.7 vs 28.8, *P* 0.007; evolution DM (years): 16.8 vs 14.1, *P* 0.014; first visit (week): 6.8 vs 10, *P* 0.000; CSII treated: 34.7 vs 5.9%, *P* 0.000; HbA1c pregestational: 6.6 vs 8.2, *P* 0.000; HbA1c first trimester: 6.4 vs 7.5%, *P* 0.000; HbA1c second trimester: 6.07 vs 6.48, *P* 0.000; respiratory distress: 3.2 vs 12.2%, *P* 0.021; birth trauma: 0 vs 10%, *P* 0.002 and Jaundice (phototherapy): 14.4 vs 29.5%, *P* 0.015. There were no differences in HbA1c third trimester: 6.1 vs 6.2; retinopathy worsening: 6.4 vs 7.5%; nephropathy worsening: 2.9 vs 1%; abortion: 14.4 vs 16.5%; preterm (<37w): 20.8 vs 27.4%; cesarean: 56.8 vs 56.6% and other perinatal complications.
Conclusions
Planning pregnancy is associated with better glycemic control before conception, in first and second trimesters and better perinatal outcomes (reduced presence of respiratory distress, birth trauma and jaundice requiring phototherapy).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P629

**Diabetic nephropathy and retinopathy: complications in pregnancy and labor**

Y. Themeli[1,5], V. Bajrami[1], M. Barbullushi[2,5], A. Idrizi[2], K. Zaimi[1], K. Mustafaraj[1,5], J. Lulo[1], A. Shtylla[3], J. Gjoshe[4] & E. Peci[6]
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Background
Diabetes microangiopathy is an important negative factor, that affects directly the maternal and neonatal morbidity.
The objective
To determine the risk factors for the morbidity of the mothers and their fetus in patients with diabetic retinopathy and/or nephropathy.
Methods and design
We followed the course of 50 pregnancies in diabetic woman with nephropathy and/or retinopathy and 50 other pregnancies in diabetic woman without severe microangiopathy. We compered the two groups for the diabetic complications and the maternal and neonatal morbidity.
Results
We found a correlation between retinopathy progression and hyperglycaemia during the first trimester ($P<0.05$). There was an increase in the deterioration of visual acuity up to blindness due to the progression of microangiopathy in cases of proliferative retinopathy. There was a significant increase of the mean diastolic blood pressure and preeclamptic symptoms occurred in of 67% of the cases with severe microangiopathy ($P<0.05$). Deterioration of the diabetic nephropathy with excessive proteinuria (>10 g/day) and unmanageable hypertension or a progression of the retinopathy led to an earlier delivery in 83% of the patients ($P<0.05$). A high rate of preterm deliveries (39%) and a frequent occurrence of intrauterine growth retardation's (13%) characterised the fetal outcome.
Conclusions
All womans diagnosed with diabetes mellitus during childhood or if the course of the disease is between 10 and 15 years, should have periodic ophthalmological evaluations, control of the renal function, contraceptive advice and an improvement of the metabolic situation. In case of a diabetic nephropathy in combination with hypertension the patients shoud be warned against pregnancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P630

**Quality of life of people with diabetes living in rural area**

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Introduction
Diabetes with a high prevalence in Iran could affect all aspects of the individual life. Iranian people with diabetes living in rural area have access to health care system and family physician through the health care homes which offer health related care and education to the population. The services are provided by family physician and assistant health provider for remote rural area by weekly visiting tour to provide the opportunity to use health care system's services. All the activities aim to improve QOL of people living in rural area special those living with diabetes. The information about the QOL in the population can be considered as baseline information.
Objective
The aim of the study was to access the QOL in the remote rural population of Malayer (a small town in the west of Iran) who live with diabetes.
Methods
In this descriptive study 39 people with type 2 diabetes participated who were visited by the family physician during weekly tour. The physician filled out the Iranian version of WHOQOL-BREEF for all.
Results
The participants were both male (46.2%) and female (53.8%) with the rage of 39–80 years old. Mean (S.D.) of domain scores of the aforementioned questionnaire in the population under study were 10.76 (S.D. = 1.38) for physical health, 10.43 (S.D. = 1.19) for psychological health, 10.28 (S.D. = 1.64) for social relationships, and 10.51(S.D. = 1.19) for environment. In analysis of different sex bands, scores of the environmental and physical health for female were significantly lower than male. In addition, people with higher education level had higher scores in the environmental and physical health. Moreover, there was no difference between marriage status and job of the population.
Conclusion
However the QOL of Iranian people with diabetes living in remote rural area were lower than the population studied by WHO in overall; the score of QOL in domains such as psychological and environmental was very close to the result in Argentina, India and USA.
Keywords: Quality of life, WHOQOL-BREEF, remote rural area, people with diabetes, Iran.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P631

**Insulin resistance: policy plan to address burning issue by NGO from rural India**

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Issues
Newer anti-diabetic therapies out of reach of >84% populations of Asia. Pharmaceuticals donot provide price discounts in resource-constrained-countries. incidence of insulin resistance neglected issue in resource-poor nations. Diabetes forums failed to address this concern. Hence we formed liaison study group of four clinics to analyse this issue and make policy paper. Resource poor nations have little technical expertise/manpower to study insulin resistance. Lack of resources for such research had demotivates young researchers.
Description
In developing countries cost-economics of diabetes treatment in rural/tribal India leads to poor-therapeutic-compliance. National/international programs donot offer discounted drugs, or diagnostics/technical assistance in diabetes-care. Insulin-resistance must be evaluated and corrective steps to minimise it. Health-care NGO's pivotal to facilitate development of sound/sustainable diabetes-care-programs. Its need of hour to organise specialised sessions on Insulin-resistance at ICE/ECE-congress for participants like us from Asian-countries.
Results/observations
There is need to provide technical/research skills from Industrialised-nations researchers to filter it to lesser privileged from developing nations like India. Currently no facility to learn/train on insulin-resistance. Major Lacunae is absence of system linking diabetes-caregivers from rural/tribal zones. Forums needed to implement/expand this suggestion/policy. Most of conferences address issues of cost/availability/ADR's BUT issues of drug-resistance neglected.
Recommendations
Development of tolls to tackle insulin-resistance is in infantile stage in developing-nations. Dialogue with western/European-researchers will accelerate policy implementation, increases resource and access to technical expertise. We need to break north-south barriers to counter resistance-issues. patient-advocates at ICE/ECE-2012 need to collaborate in future research-projects on this issue. We NGO-representatives from developing-nations need exposure to research technicalities/methodologies used by European/American experts. This is possible by our participation at Trondheim-meeting.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P632

**Seeking a new identity: the empowerment process in Iranian people with diabetes**

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Facilitating empowerment among people with diabetes is the responsibility of all health care providers. However, research suggests that the health care providers do not have the knowledge and skills to facilitate empowerment. As a result, many Iranian people with diabetes experience low levels of empowerment. The aim of the study was to explore and understand how empowerment occurs in people with diabetes in Iran. Grounded theory method using in-depth interviews, field notes, and memos, were used to collect data from 25 people with diabetes, their families, and health care providers. Constant comparative analysis was used to analyze the data. Findings showed that empowerment is a transitional, perceptual and continuous process in people with diabetes and includes threatened identity, reconstructing pre-diabetes identity and gaining a new identity, which is influenced by a combination of knowledge, social support, values and beliefs, psychosocial issues, and the nature of diabetes. Facilitating empowerment can help people with diabetes integrate diabetes into their self-identity; however, achieving empowerment is complex and occurs over time.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P633

**Prevalence of Charchot arthropathy after the introduction of electronic foot ulcer patient record**

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Introduction
Loss of protective sensation, inflammation and trauma may lead in predisposed diabetics with peripheral neuropathy to Charcot foot.
Methods
Regular foot screening enables identification of those individuals who are at highest risk for foot ulceration. Special attention must be paid to infection. Differentiation between osteomyelitis and Charcot arthropathy is mandatory, the two conditions may coexist. Basic conservative treatment for Charcot arthropathy is offloading with bed rest or total contact cast with healing shoe. The active acute form should be detected promptly in order to apply bisphosphonate or calcitonin therapy which may improve healing and stabilisation of the disease and postpone deformities. Few data on this treatment are available. In our clinic we keep an electronic diabetic foot patient record since 2009. Among 6000 diabetics, 185 have electronic record, 89 an active foot ulcer. Seven with Charcot arthropathy were detected, all having peripheral neuropathy, one due to alcohol, two women and five men. One patient died in 2009. One has chronic bilateral form of the disease, other presented with an acute form. All have been referred to orthopaedic surgeon with a native X-ray, one with scintigram, one MRI was done. All were complicated with infection and healed in 8–24 months. One had transmetatarsal amputation. Two have ischemia, others mediocalcinosis. In the observational period no one received drug treatment, one received bisphosphonates in 2006 when the disease was active on his left foot.
Results and conclusions
We conclude that in this short period the incidence of the acute form of Charcot arthropaty was higher. The acute active form of Charcot arthropathy can be found if it is searched for. Early detection of patients with acute Charcot foot and appropriate treatment are cornerstones of foot ulcer and deformity prevention.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P634

**Development of a high-sensitive urinary albumin immunosensor based on MCM-41-PVA nanocomposite and AuNPs**

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Accurate and sensitive detection of the biomarkers is important both for preclinical research fields such as biochemistry, biomedicine, and clinical diagnostics. Immunosensors are the miniaturized analytical devices that combine high specificity of immunological reaction with sensitivity of detection techniques.
In this study, we developed a new competitive immunosensor with employing antibody (Ab) labeled AuNP (Ab-AuNP) and mobile crystalline material-41 (MCM-41)-polyvinyl alcohol (PVA) mesoporous nanocomposite modified screen-printed carbon electrode (SPCE) surface to detect the urine albumin. Field emission scanning electron microscopy (FE-SEM) of modified electrode showed a suitable and stable attachment between HSA antigen – mAb and AuNP. Cyclic voltammetric (CV) method demonstrated that modification process was well performed.
Differential pulse voltammetry (DPV) was employed for quantitative determination of antigen in biological samples. The electrochemical measurements performed with other proteins mixed with samples demonstrated a high specificity and selectivity for this biosensor in detecting the HSA. In optimal conditions, this immunosensor could detect HSA in a high linear range (0.5–200 μg/ml) with a low detection limit of 1 ng/ml. The proposed method showed acceptable reproducibility, stability, and reliability and could also be applied to detect the other antigens.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P635

### Metformin-associated lactic acidosis: prevention is the real challenge!

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Introduction
More than 50 years after its introducing in 1957 (1996 in USA), metformin is used today in more than 90 countries. It is the most efficient, safe and costless anti-hyperglycemic agent in the treatment of type 2 diabetes mellitus. Its principal risk remains metformin-associated lactic acidosis (MALA), subject of controversies as reported in a recent Cochrane database review in 2010.
Patients and methods
We present personal series of all cases of MALA diagnosed in an 8 year-period in an Emergency department admitting 16 000 patients yearly.
Serum lactic acid over than 5 mmol/l at admission (normal value $<2$ mmol/l) in a context of metabolic acidosis is the key of the diagnosis.
Results
Four cases of MALA are diagnosed in this 8 year-period. The incidence is three for 100 000 patients admitted in emergency.
The indication of the metformin was not relevant in 1 case (mitochondrial disease) and subject of discussion in two others (with chronic alcoholism in on case and chronic use of anti-inflammatory drugs in the other). The case 4 treated with 3000 mg of metformin, presents also a mild asthma.
The clinical presentation is misleading in the four cases. The evolution was favorable in 50% of the cases, fatal in two cases (severe impaired renal function, hepatic insufficiency).
Discussions and conclusions
MALA incidence is probably underestimated. The frequency of co-morbidity makes its diagnosis difficult. Checking serum lactic acid for all diabetic patients treated with metformin when admitted for any acute problem in emergency is the main 'take home message' of this report.
Diagnosed and treated without delay, the evolution of MALA can be quickly favorable.
In UKPDS, Metformin given not more than 2550 mg daily has shown to reduce total mortality but no clinical evidence for the superiority of higher doses in studies.
We suggest, in addition to the strict respect of contraindications, the integration of the benefit-risk balance when prescribing higher doses.
Finally, the expertise of Diabetologists in Emergency rooms could improve the quality of care for diabetic patients. Helpful in the management and prevention of complications, it is enhancing educational messages of safety for patients and physicians.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P636

### Oxidative stress and histopathological differences on the skin of diabetes mellitus compromised rats

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Background
Several studies on diabetes mellitus and cutaneous wounds have been reported. However, only few studies show the influence of diabetes on the skin regarding its oxidative stress, antioxidants mechanisms and histopathological aspects.
Objectives
The aim of this study was to evaluate these parameters by comparing them to non-diabetic rats skin.
Methods
Ten Wistar rats were assigned to groups: diabetic rats (induced by streptozotocin 45 mg/kg; DM; $n=5$) and non-diabetic rats (N-DM; $n=5$). After verifying the diabetes status (hyperglycemia), animals were euthanized and biopsies were taken from the dorsal surface using a skin biopsy punch (1.5 mm diameter). Biochemical analysis was carried out to measure markers of oxidative stress (MDA, malondyaldeide; NO, nitric oxide; FOX, $H_2O_2$) and antioxidant activity (GSH, glutathione). Histological analysis (HE and Gomori's trichrome staining) was performed to assess inflammatory cells, blood vessels, fibroblasts and the percentage of collagen area.
Results
Hyperglycemia was confirmed for DM group, reaching statistical difference compared to N-DM group ($P=0.0001$). Diabetes pathology increased the number of inflammatory cells ($P=0.0001$) and NO production ($P=0.0473$) on the skin, however, it decreased MDA ($P=0.0001$), FOX ($P=0.0001$) and GSH ($P=0.0035$). The number of blood vessels was lower for the DM group compared to the N-DM group ($P=0.0001$), suggesting that there is an influence of diabetes pathology on the quantity of skin blood vessels. The number of fibroblasts an collagen content were similar for both groups ($P>0.05$).
Conclusion
The pathophysiology of diabetes changed the skin by increasing the influx of inflammatory cells and NO production, decreasing the antioxidant defense and the number of blood cells.
Glycaemia (mg/dl) of diabetic (DM) and non-diabetic (N-DM) rats. Distribution of variables inflammatory cells, NO, MDA, FOX, GSH, blood vessels, fibroblast and collagen of dorsal skin in diabetic (DM) and non-diabetic (N-DM) rats.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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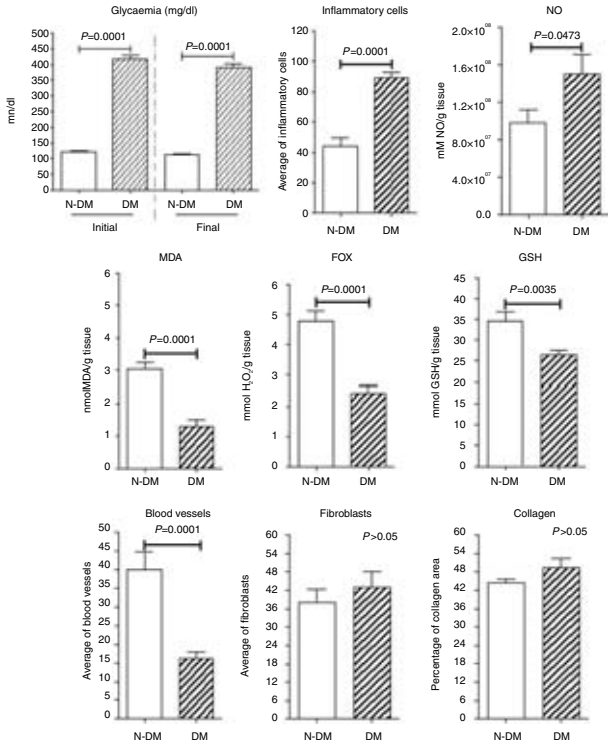


## P637

### Relationships between hyperglycemia and the oxidative stress in type 2 diabetes

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A continuous glucose monitoring (CGM) provides a good tool to investigate the influence of glucose fluctuation on oxidative stress regarding as the pathogenesis of arteriosclerosis in type 2 diabetes (T2DM). The CGM can detect min-to-min glucose fluctuation under the daily activity and eating habit. We measured various indexes for oxidative stress and compared with the glycemic factors detected by the CGM.

Methods
T2DM ($n=25$) and healthy controls ($n=14$) were subjected to the CGM for 72 h and they were simultaneously measured blood levels of HbA1c, LDL, HDL, triglyceride, small dense (sd)- and oxidative (ox)- LDL, remnant-like particle lipoprotein (RLP), high-sensitivity (hs)-CRP, leptin, adiponectin, total free radical derived from reactive oxygen species (ROS), urinary output of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 15-isoprostane F2t (isoprostane).
Results
The mean and 2s.d. of the glucose in the control group were ranged from 73.2 to 148.3 mg/dl. Therefore, the hyperglycemic excursion was defined as the AUC above 140 mg/dl of glucose. The total ROS and urinary output of 8-OHdG and isoprostane were not different between the T2DM and control groups. However, the mean glucose and hyperglycemic excursion measured by CGM were correlated with total ROS ($r^2=0.191$, $r^2=0.230$, $P<0.05$) and urinary isoprostane ($r^2=0.187$, $r^2=0.200$, $P<0.05$). The glycemic indexes were not correlated with LDL, HDL, TG, RLP, sd-LDL, or ox-LDL. The glucose instability expressed as s.d. of the mean glucose and the combined percentages of hyper-plus hypoglycemia were not correlated with the oxidative stress indexes.
Discussion and conclusion
Hyperglycemia *per se* increased the production of oxidative stress detected by total ROS and urinary output of isoprostane and may accelerate the arteriosclerosis. The markers for oxidative stress might depend on its half-life and the exposed time for the hyperglycemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P638

**Somatostatin eye drops prevent retinal neurodegeneration in experimental diabetes**

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Introduction/aim
There is growing evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy (DR). Somatostatin (SST) has a neuroprotective action and we previously found a downregulation of SST associated with the hallmarks of retinal neurodegeneration (glial activation and apoptosis) in human retinas. The aim of the study was to test the hypothesis that SST is useful in preventing retinal neurodegeneration.
Methods
Male Sprague–Dawley rats in which diabetes was induced by streptozotocin were treated with either eye-drops of SST (10 mg/ml; 20 µl/eye; $n=8$) or vehicle ($n=8$) for 15 days. Non-diabetic Sprague–Dawley rats ($n=8$) treated with vehicle served as control group. Electroretinography (ERG) studies were performed before starting treatment and one day prior death. Glial activation was evaluated by measuring glial fibrillar acidic protein (GFAP) by immunofluorescence. Apoptosis was assessed by TUNEL assay.
Results
Treatment with SST eye drops prevented ERG abnormalities (reduction in b-wave amplitude and increase in b-wave implicit time), as well as the characteristic features of neurodegeneration (glial activation and apoptosis) caused by diabetes. In fact, glial activation and apoptosis in diabetic rats treated with SST were similar than in non-diabetic rats.
Conclusion
SST eye drops prevented both functional and morphologic abnormalities in the diabetic retina. Based on these promising results in rats we have designed and initiated a multicentric clinical trial (EUROCONDOR. FP7-Health-2011-278040) in order to demonstrate the potential beneficial effects of SST topical administration in preventing or arresting DR development.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P639

**Serum high sensitivity c-reactive protein as predictor of metabolic syndrome and its relationship with albuminuria in type 2 diabetes mellitus**

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The high sensitivity C-reactive protein (hs-CRP), mediator of atherosclerotic disease, is known to be a sensitive predictor of coronary heart disease in type 2 diabetes mellitus (T2DM).
Aims
To evaluate the relationship of hs-CRP levels to metabolic syndrome (MS) and albuminuria in T2DM.
Methods
Four hundred and eighty-eight T2DM subjects were recruited. Patients with hs-CRP $\geq 10$ mg/l, creatinine $\geq 1.4$ mg/dl, chronic inflammatory and cardiovascular diseases, smokers or on antiplatelet therapy were excluded. In all the 178 subjects ($65.2 \pm 10.7$ years, 71 men and 107 women) enrolled, we measured hs-CRP, waist circumference (WC), fasting plasma glucose (FPG), systolic and diastolic blood pressure, high density lipoprotein-cholesterol (HDL-Ch), triglycerides (TGs), and 24 h. Albumin excretion (ALB-e). The MS was defined according to the IDF criteria. Student's *t*-test was used to compare the means, and the Mann–Whitney *U* test to analyze the relationship between hs-CRP levels and the presence of MS. Spearman partial correlation coefficients were calculated to assess the correlations of log hs-CRP with all variables. A *P* value $<0.05$ was considered significant (SPSS).
Results
The hs-CRP level was higher in those subjects with WC $>94$ cm in men and $>80$ cm in women ($P=0.000$), TGs $>150$ mg/dl ($P=0.01$), HDL-Ch $<40$ mg/dl for men and $<50$ mg/dl for women ($P=0.012$), in subjects with MS ($P=0.025$), and with macroalbuminuria ($P=0.045$). After adjusting for age and sex, the log hs-CRP correlated significantly with WC ($r=0.368$), BMI ($r=0.297$), log TGs ($r=0.382$), FPG ($r=0.172$), HbA1c ($r=0.162$), and log ALB-e ($r=0.233$).
Conclusions
The serum hs-CRP level may be used to predict MS in T2DM patients. The correlation between hs-CRP level and albuminuria, suggests that the inflammatory process plays a role in nephropathy in T2DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P640

**Relationship between hyponatremia and diabetes mellitus**

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Aims
Our aim is to retrospectively analyze the diagnosis of hyponatremia in the discharge sheets of a tertiary hospital during 5 years and its relationship with type 1 and 2 diabetes mellitus.
Methods
Data from the MBDS (Minimum Basic Data Set) of the discharged patients from the Hospital Clínico San Carlos (Madrid) between the years 2005 to 2009 was analysed. The 5th Edition of the ICD-9-CM was used for the codification of the diagnoses and procedures. The system of patient classification of Diagnosis Related Groups – AP-DRG (version 21.0) was used for the grouping of discharge processes. The Charlson index was use as a measure of comorbidity. Patients with a main or secondary diagnosis of hyponatremia suffering also from type 1 or type 2 diabetes were selected and analyzed. Age, sex, length of stay, comorbidity, procedures and mortality were taken into account.
Results
Reported hyponatremia was 1.7% in type 1 diabetes and 2.3% in type 2. Mean age was 33.6 years for type 1 and 68.3 years for type 2. In type 1 diabetes, 48.7% were males vs 43.1% in type 2 diabetes. Mean stay was 7.2 days for type 1 vs 9.9 days for type 2. A Charlson index $>2$ was present in 17% of type 1 diabetic patients and in 22% of those with type 2. Mortality reached 14% in type 1 and 7% in type 2.
Discussion
The low prevalence of the notification of hyponatremia, very far from the real prevalence of this disturbance is our first observation. In our series, two

populations are distinguished: patients with type 1 diabetes are younger, with a shorter stay, less comorbidity and higher mortality. On the other side, type diabetics are older, predominantly female, with higher comorbidity and longer hospitalizations but lower mortality.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P641

### The relationship between daytime, nighttime and total heart rate with albumin and protein excretion in newly diagnosed type 2 diabetic patients

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Introduction
Autonomic nervous system dysfunction has been widely observed in patients with type 2 diabetes. Twenty-four hour ambulatory heart rate measurements (AHRM) have been found to associate with autonomic dysfunction in patient with type 2 diabetes. Since albumin excretion is also related with autonomic dysfunction; in the current study, we analyzed whether 24-h AHRM were related with 24 h urinary albumin excretion (UAE) and urinary protein excretion (UPE) in newly diagnosed patients with type 2 diabetes.
Methods
All patients underwent following procedures: history taking, physical examination, blood pressure measurement, 12 lead electocardiographic evaluation, routine urine analysis, biochemical analysis, 24-h urine collection to measure urinary sodium and protein excretion and creatinine clearance calculation. Twenty-four hour ambulatory blood pressure and heart rate monitoring were performed for all patients. Diagnosis of diabetes was based on at least two fasting blood glucose.
Results
In total 80 patients (34 male, 46 female aged $50.4 \pm 9.2$ years) with newly diagnosed type 2 diabetic patients were included. Stepwise linear regression of factors including age, gender, body mass index, smoking status, presence of coronary artery disease, dipping status, averaged fasting blood glucose, 24-h creatinine clearance, 24-h average ambulatory systolic blood pressure (AASBP), 24-h average ambulatory diastolic blood pressure (AADBP), daytime AHRM, nighttime AHRM and 24-h AHRM revealed that logarithmically converted 24-h UAE were independently related with 24-h AASBP (*b*: 0.02, CI: 0.09–0.031, *P*: 0.001) and nighttime AHRM (*b*: 0.05, CI: 0.03–0.069, $P<0.0001$). Using the same independent variables logarithmically converted 24-h UPE were independently related with age (*b*: $-0.014$, CI: $-0.027$ (to) $-0.001$, *P*: 0.032), with averaged fasting blood glucose (*b*: 0.011, CI: 0.002–0.021, *P* 0.023), 24-h AASBP (*b*: 0.013, CI: 0.005–0.020, *P* 0.002), nighttime AHRM (*b* 0.021, CI: 0.009–0.033, *P* 0.001).
Conclusion
Nighttime AHRM but not daytime and 24-h AHRM were related with both 24-h UAE and UPE in patients with type 2 diabetes. Whether augmentation of autonomic dysfunction specifically during nighttime should be evaluated in patients with type 2 diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P642

### Cystatin C and TGF-β as a biomarker of diabetic nephropathy in normo and microalbuminuric patients

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Background and aims
This study was done to predict renal impairment in type 2 diabetic patients with normo or microalbuminuria by detection of serum cystatin C, serum and urinary TGF-β levels.
Material and methods
Seventy-eight patients were categorized into four groups depending on urinary albumin excretion and eGFR: eGFR $<60$ ml/min per 1.73 $m^2$ with normoalbuminuria in group 1; eGFR was $<60$ or $>60$ ml/min per 1.73 $m^2$ with microalbuminuria in group 2; normoalbuminuria in group 3 and 4 patients with eGFR $>120$ and eGFR between 90–120 ml/min per 1.73 $m^2$ respectively.
Results
Baseline characteristics of subjects are shown in Table 1. Although blood glucose regulation was best, blood urea, serum creatinine were higher and eGFR was significantly lower in group 1. While serum cystatin C level was significantly high in group 1, serum and urinary TGF-β levels were high in group 2.
Conclusion
Although urinary albumin excretion is suggested for detection of type 2 diabetic nephropathy, there is subgroup of patients with impaired renal function without increased urinary albumin excretion. This study showed that serum cystatin C level could be used as an early biomarker of diabetic nephropathy in normoalbuminuric patients.
M, male; F, female; BUN, blood urea nitrogen; Cr, Creatinin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; TGF, transforming growth factor.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Baseline demagrophic, metabolic and laboratory parameters of patients.

| | Group 1 | Group 2 | Group 3 | Group 4 | *P* value |
|---|---|---|---|---|---|
| Sex (M/F) | 10/10 | 10/11 | 15/2 | 10/10 | 0.039 |
| Age (years) | 62.9±5.8 | 59.7±6.9 | 49.0±8.0 | 53.7±8.3 | 0.001 |
| BMI (kg/$m^2$) | 29.6±4.2 | 32.0±5.3 | 32.2±6.6 | 31.6±5.4 | 0.43 |
| Diabetic duration (months) | 116.4±100.1 | 127.4±96.7 | 96.0±71.8 | 78.0±45.1 | 0.243 |
| Glukoz (mg/dl) | 118.8±26.6 | 152.1±62.7 | 143.9±42.6 | 121.1±32.7 | 0.043 |
| HbA1c (%) | 6.1±0.7 | 6.9±1.2 | 7.3±1.1 | 6.7±1.3 | 0.028 |
| BUN (mg/dl) | 21.5±6.7 | 17.8±7.2 | 13.5±2.8 | 13.7±3.4 | 0.001 |
| Serum Cr (mg/dl) | 1.2±0.2 | 0.9±0.3 | 0.6±0.0 | 0.6±0.1 | 0.001 |
| hsCRP (mg/dl) | 5.1±4.5 | 6.0±7.7 | 5.5±5.5 | 5.6±5.2 | 0.969 |
| Total kolesterol (mg/dl) | 171.3±46.5 | 182.8±35.9 | 203.8±32.9 | 186.2±35.4 | 0.109 |
| HDL (mg/dl) | 41.2±10.1 | 38.4±7.8 | 39.8±10.9 | 40.5±5.7 | 0.774 |
| LDL (mg/dl) | 102.6±35.5 | 115.3±34.3 | 129.6±30.3 | 105.9±29.6 | 0.079 |
| TG (mg/dl) | 148.3±91.1 | 174.8±79.9 | 175.9±50.9 | 184.6±68.1 | 0.074 |
| Serum TGF-β (ng/ml) | 16.7±6.9 | 18.9±3.01 | 16.1±6.1 | 13.2±6.4 | 0.022 |
| Urinary TGF-β (pg/mg cr) | 558.6±230.0 | 633.2±100.6 | 539.3±204.3 | 442.2±214.3 | 0.022 |
| Cystatin C (mg/l) | 1.4±0.6 | 1.1±0.4 | 0.6±0.1 | 0.7±0.1 | 0.001 |
| Urinary micro-albumin (mg/day) | 18.0±5.9 | 89.8±37.7 | 22.2±8.9 | 16.3±5.1 | 0.001 |
| eGFR (ml/min per 1.73 $m^2$) | 54.7±7.6 | 78.2±23.5 | 125.7±6.9 | 106.1±29.8 | 0.001 |

## P643

### The impact of hyperinsulinemia on the serum IL12p40 subunit concentration

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Introduction
Numerous studies indicate an association between low-grade chronic inflammation and predisposition to type 2 diabetes and atherosclerosis. IL12 is a proinflammatory cytokine with proatherogenic properties. IL12 is a disulfide-linked, 70 kDa (p70) heterodimeric glycoprotein composed of a 40 kDa (p40) subunit and a 35 kDa (p35) subunit. Many data reported higher levels of p40 subunit than total IL12. The aim of the present study was to investigate the influence of hyperinsulinemia on serum p40 subunit.
Methods
Our study involved 35 young (age: $24.31 \pm 2.81$ years), apparently healthy men with normal glucose tolerance. Anthropometric measurements, blood

biochemical analysis and euglycemic hyperinsulinemic clamp were performed in the studied group.
Results
The serum concentrations of p40 was significantly lower after the clamp than the baseline state ($P<0.05$). The change in IL12p40 during the clamp was already to the steady-state insulin (SSI) concentrations ($r=0.35$, $P=0.037$) – the higher SSI the greater decrease in serum IL12/p40. We found inverse correlations between post-clamp serum p40 and total cholesterol and LDL-cholesterol ($r=-0.34$, $P=0.049$ and $r=-0.46$, $P=0.006$ respectively). A significant association between basal and post-clamp p40 subunit and lymphocyte cell count ($r=0.35$, $P=0.037$ and $r=0.45$, $P=0.006$ respectively) and significant negative correlations with neutrophile cell count ($r=-0.41$, $P=0.014$ and $r=-0.51$, $P=0.002$ respectively) was observed in the studied group.
Conclusion
Our data indicated that hyperinsulinemia decreased serum IL12/p40 concentration.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P644

**Association of cardio-ankle vascular index with diabetes mellitus-related peripheral arterial disease in chronic hemodialysis patients**

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Introduction
Peripheral arterial disease (PAD) is increased associated with an accumulation of patients with end stage kidney disease and diabetes. Recently, the cardio-ankle vascular index (CAVI) has been developed as an index reflecting the stiffness of the aorta independently of blood pressure. The aim of this study was to assess the prevalence of PAD in hemodialysis patients with diabetes.
Methods
To examine the usefulness of CAVI to screen for the presence of PAD, cross-sectional studies were performed on 94 patients undergoing chronic hemodialysis. Patients were referred to echocardiography or analyzed HbA1c and a blood inflammatory marker, high-sensitivity C-reactive protein (hs CRP). We also analyzed the ankle brachial index (ABI) and values of skin perfusion pressure (SPP) in all patients.
Results
CAVI significantly correlated to age but not blood pressure. CAVI in diabetics was significantly higher than that in non-diabetics. In contrast, ABI or SPP was no differences between diabetics and non-diabetics. Furthermore, CAVI was markedly elevated in patients with a history of PAD or cardiovascular disease. When diabetic patients were classified on the basis of CAVI quartiles, the odds ratio for the prevalance of PAD was increased. Increased CAVI was also associated with other risk factors for PAD (HbA1c, hs CRP, body mass index or history of PAD). However, the association with HbA1c was not observed in ABI.
Conclusion
The prevalence of PAD is high in diabetic elderly patients with hemodialysis. The present findings suggest that CAVI can be useful index that predicts the occurrence of microvascular complications in dialysis patients with diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P645

**Pituitary function in patients with autoimmune type 1 diabetes evaluated by CRH test**

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This work aimed at acquiring data on pituitary response to CRH test in autoimmune type 1 diabetics with adult onset with/without thyroid autoimmunity. Our previous study showed 25% of such diabetic patients without clinical and immunological signs of adrenal insufficiency had lower response to the Synacthen test with normal basal levels of ACTH and cortisol.
Twenty-seven diabetics were investigated; age $44\pm10$ years (mean $\pm$ S.D.), age at diagnosis $28.5\pm10$ years, disease duration $15\pm8$ years, BMI $24.5\pm2.7$ kg/m$^2$, HbA1c $7.2\pm1.2$%. The group of patients consisted of 13 diabetics without and 14 diabetics with thyroid autoimmunity. The study was approved by the ethical committee.
The pituitary function was tested by CRH test. We evaluated serum ACTH and cortisol, salivary cortisol, thyroid function and metabolic paramerts of diabetics. The group of patients with subnormal cortisol response to Synacthen showed a significantly lower cortisol response but a significantly higher ACTH response to CRH test. We divided this group to diabetics with and without thyroid autoimmunity. We found significantly lower cortisol response to CRH in diabetics without thyroid autoimmunity but significantly higher ACTH response to CRH, in comparison with diabetics with thyroid autoimmunity.
The results indicate that both groups of patients with subnormal response to Synacthen required the higher levels of ACTH to achieve normal cortisol response to CRH in contrast to group of patients with normal cortisol response to Synacthen. This higher activity on the pituitary level may be the first step of latent adrenocortical hypofunction to offset the stressful event. These results obtained may contribute to better understanding latent adrenal insuficiency adaptation in type 1 diabetics. The study was supported by grant no. NT 11 277 and NT 12340-5 of the IGA MZCR.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P646

**Microvascular complications in type 1 diabetes mellitus: prevalence and associated risk factors in DIACAM 1 study**

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Background and aims
DIACAM1 study was designed to investigate the clinical characteristics of a representative group of type 1 diabetic (T1D) population in Castilla-La Mancha (Spain). The aim of this report is to describe the prevalence of microvascular complications and the factors associated with such complications.
Materials and methods
This is an observational, cross-sectional, prospective and multicentre study of 1465 patients who received assistance during 2010. All reported patients were aged $>16$ years at the time of the study and duration of diabetes was $>5$ years. There were 48.5% women, mean age $39.4\pm13.5$ years, and mean diabetes duration $19.4\pm10.6$ years. Patients underwent clinical and laboratory evaluation. A multivariate logistic regression analysis was used to assess variables independently associated with microvascular complications.
Results
The prevalence of retinopathy was 32.5%. Non proliferative retinopathy was found in 20% and severe retinopathy in 12.5% (proliferative 8.9%). The prevalence of nephropathy was 18%. Without renal function impairment in 12.9% and with evident renal injury in 5.1% (NFK stages 3–4 in 3.5% and ESRD in 1.6%). There is significative increase ($P<0.001$) of microvascular complications with time evolution (Table 1). Independent variables for the presence of retinopathy were duration of diabetes ($\geq10$ years. OR 19.9 and $\geq30$ years. OR 6.8, $P<0.001$), hypertension (OR 3.1, $P<0.001$) and current HbA1c $>8$% (OR 1.4, $P<0.05$). Independent factors associated with nephropathy were: hypertension (OR 7.04, $P<0.001$), diabetes duration $\geq30$ years (OR 2.8, $P<0.001$) and current HbA1c $>8$% (OR 1.84, $P<0.05$).
Conclusions
Retinopathy and nephropathy are less prevalent than expected. Microvascular complications are strongly influenced by the time of evolution of the disease, higher glycosilated hemoglobin and the presence of hypertension. These results are consistent with the trends of reduction of diabetes complications in other studies, due to intervention therapies and improvement of diabetes care.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1** Tabele 1 Cumulative incidence of microvascular complications (%) by diabetes duration.

| Time | Retinopathy (all) | Proliferative *R* | Nephropathy (all) | MAU/protei-nuria (NFK stages 1–2) | ESRD |
|---|---|---|---|---|---|
| <10 years | 2.4 | 0.3 | 3.9 | 3.2 | 0 |
| 10–19 years | 16.2 | 1.4 | 10.2 | 9.2 | 0.2 |
| 20–29 years | 51.8 | 13 | 25.5 | 18.6 | 2.0 |
| >30 years | 79.5 | 31.4 | 43.2 | 25.6 | 6.3 |

## P647

### Urinary pentosidine concentration in patients with type 2 diabetes and risk of vascular complications

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Aims
We investigated the association between urinary pentosidine concentration (UPC) and vascular complications.
Methods
UPC was measured in 66 patients (31 men and 35 women) with type 2 diabetes. We compared UPC between patients with and without diabetic microangiopathy, and between patients with and without macroangiopathy (cerebral infarction (CI), coronary artery disease, and peripheral arterial disease). Stepwise regression analysis was performed to examine the effects of various factors on UPC, and the following factors were considered as independent variables: age, duration of diabetes, microangiopathy, CI, and systolic blood pressure (SBP). Logistic regression analysis was performed to examine the effects of various factors on vascular complications, and the following factors were considered as independent variables: UPC, eGFR, age, duration of diabetes, HbA1c, SBP, and smoking habit.
Results
UPC was significantly higher in patients with retinopathy ($71.3 \pm 32.7$ vs $54.5 \pm 25.5$ pmol/mgCr, $P=0.026$), nephropathy ($72.9 \pm 36.9$ vs $54.2 \pm 19.9$ pmol/mgCr, $P=0.022$), and neuropathy ($68.7 \pm 32.5$ vs $53.5 \pm 24.2$ pmol/mgCr, $P=0.045$) compared with that in patients without complications. UPC in patients with CI ($87.3 \pm 36.5$ vs $59.1 \pm 28.0$ pmol/mgCr, $P=0.026$) was significantly higher than that in patients without CI. Stepwise regression analysis demonstrated that retinopathy ($\beta=0.31$, $F=4.15$, $P=0.049$) and CI ($\beta=0.37$, $F=7.25$, $P=0.011$) were independent factors for UPC. Logistic regression analysis demonstrated that UPC (OR 1.03, 95% CI 1.00–1.06, $P=0.048$) and duration of diabetes (OR 1.17, 95% CI 1.07–1.28, $P=0.0007$) were independent factors for retinopathy.
Conclusions
High UPC could be an important factor for the incidence of vascular complications, especially retinopathy, in type 2 diabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P648

### The mechanisms of anemia development in patients with early stages of diabetic nephropathy

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Anemia occurs early and predicts high risk of cardiovascular events and death in patients with diabetic nephropathy (DN). It may result from various factors including erythropoietin (EPO) deficiency, iron and vitamin deficiencies, systemic inflammation, adverse effects of some drugs and others. However, the clinical significance of each factor is not fully known. The aim of this study was to assess the relative contribution of EPO deficiency, iron deficiency and systemic inflammation as pathogenetic factors of anemia development in patients with early stages of DN.
We investigated 72 anemic patients with type 2 diabetes mellitus and chronic kidney disease (CKD) stages 1–3. GFR was calculated by Cockcroft-Gault formula. Anemia was defined according to World Health Organization criteria (2008). Serum levels of EPO, ferritin, vitamin B12, interleukin 1β (IL1β), IL6 and tumor necrosis factor-α (TNF-α) were measured by immunoassay (Labsystems MR 600 analyser).
EPO deficiency was in found in 48.6%, low serum ferritin levels – in 11.1%, low vitamin B12 levels – in 2.8% of patients. Most individuals had elevated levels of at least one of the proinflammatory cytokines (IL1β, 73.6%; IL6, 54.2%; TNF-α, 33.3%). The contribution of these factors varied depending on the degree of renal impairment. EPO deficiency was found in 20.8, 45.8 and 79.2% patients with CKD stages 1, 2 and 3 respectively. The prevalence of iron deficiency was 16.6, 12.5 and 4.2% in patients with CKD stages 1, 2 and 3 respectively.
The results of the study suggest that pathogenesis of anemia in patients with early stages of DN is multifactorial. The most prevalent pathogenetic factors are EPO deficiency and systemic inflammation. The role of EPO deficiency increases and the role of iron deficiency decreases with progression of DN.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P649

### Non-alcoholic fatty liver disease and cardiovascular disease in type 2 diabetic patients with metabolic syndrome

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Background
Type 2 diabetes is often associated with non-alcoholic fatty liver disease (NAFLD). Patients with NAFLD may be at greater risk for CVD than those without. The relationship between NAFLD and metabolic syndrome (MS) is very well recognized. The aim is to determine the association of NAFLD and the CVD between type 2 diabetic patients with and without MS.
Materials and methods
One hundred and thirty type 2 diabetic patients (M: 71, F: 59, mean age 59, 32 +11, 94), were studied. All subjects were assessed for diabetes duration, the degree of obesity, CV risk factors, HbA1c, C reactive protein and lipid profile. NAFLD was assessed by patient history and ultrasound. MS was defined based on NCEP-ATP 3 criteria. The previous and current CVD (myocardial infarction, angina or revascularization) was assessed. The patients were categorized as four groups: MS (−) and NAFLD (+), MS (−) and NAFLD (−), MS (+) and NAFLD (+), MS (+) and NAFLD (−).
Results
The prevalence of CVD in type 2 diabetic patients with NAFLD was higher than in those without NAFLD (44.2 vs 38.1%). The prevalence of CVD in type 2 diabetic patients with MS was higher than in those without (40.1 vs 35.8%). The risk of CVD in patients with MS was significantly increased by the presence of NAFLD (53.4 vs 32.3%). In type 2 diabetic patients with MS (+) and NAFLD (+) the number of components of MS, BMI, and systolic BP were positively associated with CVD.
Conclusions
What this study suggests to us is that the presence of NAFLD increases the risk of CVD in type 2 diabetic patients with MS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P650

**Microalbuminuria and blood pressure in normotensive subjects with type 2 diabetes mellitus**

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Objective
Albuminuria has been shown to predict cardiovascular disease in populations with diabetes mellitus. The aim of this study was to assess the mean pressure values and the circadian rhythm of blood pressure during its ambulatory monitoring in normotensive diabetic patients, dividing them according to the presence of microalbuminuria.
Methods
The study group comprised of 77 type 2 diabetic patients. Their mean age was 56.5±6.7 years, and the mean duration of their disease was 8 years. For microalbuminuria, spot urine samples were collected in the early morning and microalbuminuria was defined as, a urinary albumin excretion between 30 and 300 mg/g. These patients, also underwent determination of ambulatory blood pressure monitoring.
Results
9 (24.6%) patients were microalbuminuric. Ambulatory blood pressure monitoring in the microalbuminuric patients had higher mean pressure values, mainly the systolic pressure, during sleep as compared with that in the normoalbuminuric patients (120.1±8.3 vs 110.8±7.1 mmHg; $P=0.007$). The pressure load was higher in the microalbuminuric individuals, mainly the systolic pressure load during wakefulness (6.3 (2.9–45.9) vs 1.6 (0–16%); $P=0.001$). This was the variable that better correlated with the urinary excretion of albumin ($r_S=0.61$; $P<0.001$). Systolic pressure load >50% and diastolic pressure load >30% during sleep was associated with microalbuminuria ($P=0.008$). The pressure drop during sleep did not differ between the groups.
Conclusion
Microalbuminuric normotensive type 2 diabetic patients show greater mean pressure value and pressure load during ambulatory blood pressure monitoring, and these variables correlate with urinary excretion of albumin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P651

**The relationship Between Self-Reported Nocturnal Sleep Duration, Daytime Sleepiness and 24-hour urinary Albumin and Protein Excretion in Newly Diagnosed Type 2 Diabetic Patients**

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Aim
Few studies demonstrated that self-reported nocturnal sleep duration (NSD) were related with urinary protein excretion. However no previous study has specifically addressed the relationship between 24-hour urinary albumin excretion (UAE) and 24-hour urinary protein excretion (UPE) in newly diagnosed type 2 diabetic patients.
Methods
All patients underwent history taking, physical examination, blood pressure (BP) measurement, 12 lead electrocardiographic evaluation, routine urine analysis, biochemical analysis, 24-hour urine collection to measure UAE, UPE and creatinine clearance. Self reported NSD and daytime sleepiness (using Epworth Sleepiness Scale (ESS)) were recorded for all patients.
Results
In total 110 patients (56 male, 54 female) were included. The average 24-hour UPE, and UAE were 370.4±571.6 mg/day and 162.8±250.5 mg/day respectively. The self reported sleep duration was 7.17±1.07 hours. The mean ESS score was 5.59±2.48. Stepwise linear regression of independent factors including age, gender, smoking status, body mass index, clinical SB and diastolic BP fasting blood glucose, Hba1c, creatinine clearance, insulin resistance as evaluated by HOMA-Index, self reported NSD and ESS score revealed that logarithmically converted 24-hour UAE was related with clinical systolic BP (b:0.012, CI:0.004–0.021, *P*: 0.005), Hba1c (b:0.097, CI:0.011–0.183, *P*: 0.027), self reported NSD (b:-0.114, CI:-0.0253 (-)-0.036, *P*:0.009) and ESS score (b:0.049, CI:0.001–0.096, *P*: 0.044). Using the same independent parameters 24-hour UPE was related with clinical systolic BP (b:0.01, CI:0.002–0.018, *P*: 0.011) and self reported nocturnal sleep duration (b:-0.167, CI:-0.0267 (-)-0.067, *P*: 0.001).
Conclusion
Self reported NSD and daytime sleepiness were related with both UAE and UPE in newly diagnosed type 2 diabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P652

**Office hypertension and masked hypertension and their association with left ventricular hypertrophy and diastolic dysfunction in patients with type 2 diabetes and hypertension**

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Background
The association between isolated office hypertension (IOH) and masked hypertension (MH) with left ventricular hypertrophy (LVH) and diastolic dysfunction cannot be the same, determining different increased cardiovascular risk.
Aims
To evaluate the prevalence of MH and IOH in treated hypertensive patients with type 2 diabetes and their association with LVH and diastolic dysfunction.
Methods
A cross-sectional study was conducted in 193 consecutively selected hypertensive patients with type 2 diabetes, at the outpatient clinic of a hospital in Southern Brazil. Patients performed an evaluation with office blood pressure (BP), 24-h ambulatory BP monitoring (ABPM) and echocardiography. They were classified according to their BP control (office: <140/90 or ≥140/90 mmHg; daytime ABPM: ≤130/85 or >130/85 mmHg) in controlled hypertension (CH: low office BP, low ABPM), IOH (high office BP, low ABPM), MH (low office BP, high ABPM), and truly uncontrolled hypertension (TUH: high office BP, high ABPM).
Results
Seventy patients were men, mean age 56.8±6 years, HbA1c 8.1±1.9%, diabetes duration 11.9±8.8 years, of which 19.7%, 15.5%, 24.4%, and 40.4% were identified as having CH, IOH, MH and TUH, respectively. Interventricular septum was 0.98±0.21, 0.95±0.15, 1.04±0.17 and 1.03±0.16 mm in CH, IOH, MH and TUH, respectively, $P=0.05$. The posterior wall thickness was 0.91±0.14, 0.90±0.13, 0.98±0.13 and 0.99±0.15 mm in CH, IOH, MH and TUH, respectively, $P=0.004$. In a logistic regression model using body mass index and age as covariates, LVH (septum>1 mm) was not associated with IOH (OR 0.53, CI 0.19–1.51, $P=0.241$) or MH (OR 1.70, CI 0.683–4.23, $P=0.254$). Diastolic dysfunction did not differ among groups.
Conclusions
The prevalence of MH and IOH was lower than estimated in the literature in diabetic patients. Masked hypertension and TUH seem to be similar entities with respect to their effects on hypertrophy parameters, while IOH would be a more benign condition.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P653

**Dynamics of insulin-like growth factor-1 concentrations during diabetic ketoacidosis**

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Background and Aim
Clinical and experimental studies demonstrated that total insulin-like growth factor 1 (IGF-1) levels are reduced in the patients with poorly controlled diabetes. The aim of the present study was to investigate the levels of IGF-1 in patients with acute metabolic acidosis.
Materials and Methods
17 patients with thype 1 diabetes and moderate or severe metabolic acidosis were recruited for this study. Serum levels of IGF-1 were determined by radioimmunoassay. Results were compared with 12 diabetic controls without metabolic acidosis.

Results. The subjects of study group: 8 women (47.05%) and 9 men (52.94%) were 26 to 38 years (30.20±3.22 years). 13 patients (76.47%) exhibit moderate metabolic acidosis (according to American Diabetes Association, moderate acidosis is characterized by pH 7.00–7.25, bicarbonate 10–15 mmol/l), and 4 patients (23.52%) severe ketoacidosis (according American Diabetes Association severe acidosis is characterized by pH below 7.00, bicarbonate below 10 mmol/l) Diabetic ketoacidosis occurs due to intercurrent illness and poor compliance with insulin therapy. The levels of IGF-1 were significantly decreased in patients with thype 1 diabetes with metabolic acidosis compared to those without metabolic acidosis (122 ng/dl vs 286 ng/dl, $p<0.005$). The normal IGF1range for age 25–39 years 114 to 492 ng/dl

Conclusion. In this study we found significant differences for the IGF-1 levels between patients with diabetic ketoacidosis and patients with thype 1 diabetes without ketoacidosis. Our results are consistent with several studies that have reported that in diabetic ketoacidosis IGF-1 is impaired.

metabolic acidosis, insulin-like growth factor 1 levels, thype 1 diabetes

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P654

### Diabetic peripheral arterial occlusive disease is associated with microvascular complications but coronary atherosclerosis might not be associated with them

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Background and aims

The distribution of arterial occlusive disease is not fully explained in diabetes. The aim of this study was to examine the prevalence of CHD and PAD in diabetic patients complicated by abnormal test of distal autonomic function (Neuropad®) and large fibre neuropathy.

Materials and Methods

237 patients were analysed, M/F 111/126, age (mean±SD) 61.3±9.5years (ys), diabetes duration 16.4±10.6ys., type 1 n=22 (9.3%), HbA1c 9±1.98%, 66.5% on insulin therapy, BMI 25.5±4.8 kg/m2, W 97±12.4 cm, high BMI 28.8 ±5.96. Sudomotor neuropathy was determined on the sole of the foot using the Neuropad® response. 32 patients (pts) with small fibre neuropathy (DN), (group 1; G1); 42pts with small and large fibre DN (group 2; G2); 60pts with large fibre neuropathy, VPT<6, AR>3 (group 3; G3); and 103pts without DN (G4).

Results

ANOVA: age (G1 58.3±11.3, G2 64.5±7.8yrs; p 0.01), DM duration (G1 13.5±8.6, G2 20.5±9.6, G3 19.3±11.9, G4 14±9.8yrs; $p<0.01$), proteinuria (G1 147.2±131.8, G2 350.9±485.2, G3 615.4±1174.2, G4 446 ±1197 mg/dU; $P<0.05$), W (G1 92.1±14.8, G2 99.7±10.8, G3 99.3±10.6, G4 96.1±12.7; $P$ 0.02;). The contingency tables (X2) were used: maculopathy (G1 15.6%, G2 46.6%, G3 40%, G4 17.5%; $p<0.01$), prolipherative retinopathy (G1 0%, G2 14.3%, G3 16.6, G4 2.9%; p 0.01), CHD (G1 15.6%, G2 40.5%, G3 33.3%, G4 29.1%; $P$ 0.02), PAD (G1 14.8%, 30.95%, 26.7%, 8.3%; $P$ 0.05), major amputation (G1 0%, G2 11.9%, G3 0%, G4 0%; $P$ 0.04).

Conclusion

Small fibre neuropathy on feet together with large fibre neuropathy is strongly associated with both micro- and macro- vascular diabetic complications. In patients without neuropathy (G4) there is still increase in prevalence of coronary artery disease but not peripheral artery disease. It is possible that endogenous insulin may exert an adverse effect on the incidence of coronary atherosclerosis and protective on microvascular in this group of patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P655

### Predictors of Cardiovascular Risk: Metabolic Syndrome vs. Diabetes Mellitus and Hypertension

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Introduction

Metabolic syndrome (MS) is an independent risk factor of coronary artery disease (CAD). We investigated how influenced this risk is by the definition of MS, and whether the risk is greater than that conferred by its components.

Methods

We analyzed 200 consecutive patients (35–79 years, mean 59.23±8.6, men 132, women 68) who underwent coronary angiography due to suspected myocardial ischemia. MS was obtained by several definitions: ATPIII, IDF, and JIS from 2009. CAD was defined by the presence of stenosis of any severity. CAD severity was determined by Gensini, and extent of disease scores. The effects of predictors on the presence of CAD and its severity were analyzed using binary logistic regression, and multiple linear regression analysis.

Results

MS was present in 151(75.5%), 167(83.5%), and 172(86%) patients, using ATPIII, IDF, and JIS criteria, respectively. Regardless of used criteria, MS was associated with increased risk of CAD (OR=3.30, CI95%: 1.64–6.63, OR=4.48, CI95%: 2.05–9.82, OR=5.62, CI95%: 2.43–13.0, for ATPIII, IDF and JIS criteria, respectively). Overall risk was significantly higher in women with MS than in men with MS, using all three criteria. Patients with ≥3 clustered components were in greatest risk of CAD (OR=4.19, CI95%: 2.03–8.67). However, diabetes and hypertension, each or together, had similar strength in predicting CAD. Waist circumference did not have effect on CAD risk. The higher the number of components of MS included, the higher the value of Gensini and extent of disease score was obtained ($r=0.36$, $P=0.0001$, and $\tau=0.4$, $P=0.001$, respectively). However, the strongest independent risk factor of severity of disease defined by Gensini score was diabetes, while hypertension was the strongest predictor of extent of disease score.

Conclusion

Irrespectively of diagnostic criteria, MS has similar clinical significance as diabetes and hypertension individually.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P656

### Risk Factors and Therapeutic Management of type 2 Diabetes in Georgia

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Background and aims

Cardiovascular disease (CVD) is a leading causes of death in type 2 diabetes mellitus (T2DM), thus prevention of CVD is of utmost importance. Our aim was to determine whether the ESC/EASD guidelines on CVD prevention in T2DM patients are followed in clinical practice in Georgia.

Materials and methods

In total, 824 T2DM patients (age <80 yrs) with no history of coronary or other atherosclerotic disease, who entered Georgian Diabetes Center in 2009–2010 were included in a retrospective study. Data collection was based on a review of Patients' Medical Notes, and interviews and examinations during at least six months post drug therapy initiation.

Results

Only 46.9% of patients had HbA1c <6.5%; 67.3% had average blood pressure (BP) ≥ 130/80 mmHg. Only 27.8% of patients on antihypertensive medications achieved BP goals; 74.7% had total cholesterol (TC) ≧4.5 mmol/l; out of all patients on lipid lowering therapy 67.7% achieved TC, 38.8% - triglycerides and 37.9% HDL-C goals. Following cardioprotective medications were used: aspirin or other anti-platelets - in 37.3% of patients, ACE inhibitors/ARB - in 61.5%, beta-blockers - in 27.1%, calcium channel blockers - in 33.5% and statins in 37.4% of patients.

Conclusion

Our study shows that glucose, blood pressure and lipid control are completely inadequate in a large majority of T2DM patients in Georgia; targets defined in the prevention ESC/EASD guidelines are not achieved. To some extent it is due to the

fact that part of the patients quits or changes prescribed antihypertensive and/or lipid-lowering therapy. The fact that in patients on statin therapy triglycerides and HDL-C levels, when compared to TC ones, were mostly poorly controlled indicates to the necessity of administering additional agents to achieve target levels of lipids. Primary prevention needs systematic approach to therapeutic management that addresses various risk factors, and health care system that invests in prevention.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P657

**Effects of Hyperglycemia on Oxidative Stress and Antioxidant Potential in Patients with Type 2 Diabetes**

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Background
Many *in vitro* studies have suggested that oxidative stress induced by hyperglycemic condition has pivotal role for pathogenesis of vascular complications in diabetics. This study intended to evaluate oxidative stress and antioxidant potential in patients with type 2 diabetes mellitus (DM), and to clarify the relation between oxidative stress and metabolic derangements including chronic hyperglycemia.
Materials and methods
We measured the levels of derivatives of reactive oxidative metabolites (ROM) and biological antioxidant potential (BAP) in 59 patients with type 2 DM and 10 healthy controls. Values for ROM and BAP were assessed using Free Radical Analytical System 4 (FRAS4; H&D srl, Parma, Italy). The adjusted BAP/ROM ratio was calculated by dividing the BAP/ROM ratio by 7.510, which was the average value obtained from healthy Japanese adults, and thus the data were standardized to a level 1.00.
Results
ROM levels in patients with type 2 DM increased significantly compared with those in controls ($370 \pm 69$ vs. $299 \pm 23$ CARR U, respectively; $p < 0.01$). There were no differences in BAP levels between the two groups. On the other hand, adjusted BAP/ROM ratios were significantly decreased in patients with type 2 DM compared with those in controls ($0.84 \pm 0.20$ vs. $0.98 \pm 0.17$ μM/CARR U, $p < 0.05$). It was found that ROM levels positively correlated with HbA1c, plasma glucose, and waist size, and adjusted BAP/ROM ratios negatively correlated with HbA1c, plasma glucose, waist size, and serum triglycerides. Stepwise multiple regression analysis indicated that HbA1c and waist size were independent factors contributing to the elevated ROM levels. The ROM level in type 2 DM patients complicated with metabolic syndrome was significantly 10% higher than that in DM patients without metabolic syndrome ($p < 0.05$). Treatment with either glimepiride($n = 10$), miglitol ($n = 5$)or vildagliptin($n = 13$) for 3 months improved HbA1c level from 11.1% to 7.4%, 6.8% to 6.4%, 7.6% to 6.9% and reduced ROM levels significantly by 13%*, 4%*, 7%*(from $330 \pm 46$ to $290 \pm 45$, from $337 \pm 66$ to $322 \pm 61$, from $289 \pm 40$ to $269 \pm 49$ CARR U, $*P < 0.05$), respectively.
Conclusions
These results clearly demonstrate that glycemic control and visceral obesity are independently associated with an increasing oxidative stress in patients with type 2 DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P658

**Serum vitamin D and parathormone (PTH) concentrations as predictors of the development and severity of diabetic retinopathy.**

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Introduction
Vitamin D is suggested to be an inhibitor of angiogenesis. The degree of severity of Diabetic retinopathy (DR) may be related to serum Vitamin D concentration so this study aims at investigating vitamin D and parathormone (PTH) concentrations as predictors of the development and severity of diabetic retinopathy.
Material and methods
Levels of vitamin D [25(OH) D3 and Calcitriol] and PTH were measured in 200 diabetic patients presenting with suspected diabetic retinopathy. Diabetic retinopathy was assessed using 7-field stereoscopic Fundus photography.
Results
Mean serum concentration of 1, 25 dihydroxy vitamin D 3 (1, 25(OH)2 D3) was significantly lower in diabetic subjects with retinopathy than in diabetic subjects with no retinopathy and There is a significant negative correlation between the mean level of 1, 25 (OH) 2 D3 and the degree of severity of retinopathy. Level of PTH was significantly higher in severe NPDR and PDR compared to patients with no retinopathy.
Conclusions
low levels of vitamin D might be a risk marker of development or progression of diabetic retinopathy. It might be advisable that detailed ophthalmologic examination is needed for diabetics whose serum 1, 25(OH) 2D3 concentrations are gradually decreases. The measurement of serum 1, 25(OH) 2D3 concentrations could become a useful biochemical means to predict the severity of DR in patients with diabetes mellitus.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P659

**Epidemiology of Diabetic Ketoacidosis in National Institute of Diabetes and Endocrinology (NIDE) by:Bassyouni, A.; El Ebrashy, I.; El Hefnawy, H**

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Background
Diabetic ketoacidosis (DKA) is the most serious acute metabolic complications of diabetes. Unfortunately, there is a dearth of published data on this entity from Egypt. The aim of our work was to determine the clinical characteristics, precipitating causes and mortality rate of patients with diabetic ketoacidosis in National Institute of Diabetes and Endocrinology.
Methods
Our work is a retrospective study in which we reviewed and analyzed the data of all patients (1297 diabetics: 85.8% type1 and 14.2% type2 diabetics, 59.6% females and 40.4% males) who admitted to NIDE in Cairo, Egypt with diagnosis of DKA over the last three years. The mean age of the patients was $22.9 \pm 15.6$ year and the mean duration of DM was $5.5 \pm 5.7$ year.
Results
DKA among our study diabetics was attributed to non-compliance (45.5%), infection (27.2%),myocardial infraction (6.4%), and new-onset type 1 diabetes (20.9%).Respiratory infection (46.9%), urinary tract infection (36.3%), gastoentritis (9.7%),diabetic foot (2.5%) and abscess (4.6%) were the most frequent infection in the our patients. The patient's age,weight, blood pressure, pulse, serum creatinin, duration for recovery from DKA, type of DM and presence of coma were associated with mortality in these patients. Logestic regression revealed that duration for recovery from DKA was the only independent risk factor for mortality among diabeticswithDKA.
Conclusion
Most of diabetics with DKA are young, females and have type1 DM. Noncompliance and infection were the leading precipitating causes for DKA among type1 and type2 respectively. Recurrent admission for DKA and delayed diagnosis of new onset type1 DM are frequent and may indicate inadequate education. Respiratory and urinary tract infections accounted for the majority of infections in these patients. Mortality was infrequent finding among our diabetics with DKA. Although young age, underweight, tachycardia, hypotension, high serum creatinin, type 2 DM, rapid recovery from DKA and coma were associated with higher mortality rate among patients with DKA, rapid recovery from DKA was the only independent risk factor (predictor) for mortality in these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P660

**Evaluation of capillary Beta-hydroxybutyrate measurement In diabetic ketoacidosis By: Hesham El Hefnawy By:El Hefnawy, H.*; Bassyouni, A.** and Emara, I.*** From: *Pediatric, **Internal Medicine and ***Biochemistry departments, National Institute of Diabetes & Endocrinology (NIDE)**

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Background
Current criteria for the diagnosis of diabetic ketoacidosis (DKA) are limited by their nonspecificity (serum bicarbonate [HCO3] and pH) and qualitative nature (the presence of ketonemia/ketonuria). A new method is now available to measure capillary levels of beta-hydroxybutyrate (beta -0HB), It is a quantitative and enzymatic test that uses the same equipment as for home capillary blood glucose determination but with specific strips. Aim of the work: The aim of this study was to evaluate the use of measurement of capillary B-hydroxybutyrate (beta-OHB) during the diagnosis and follow up of type 1 diabetic patients with DKA.
Subjects and Methods
This study was conducted on 40 type 1 diabetic patients presented to (NIDE) with DKA and who were aged 4–20 years and half of them was males. 140 capillary blood samples were tested for beta-hydroxybutyrate using blood beta-OHB test strips. Measurement of serum bicarbonatre (HCO3), blood glucose and urine acetone and estimation of venous blood pH and anion gap had been done for all patients.
Results
Linear regression revealed highly significant correlation between capillary β-OHB levels and all indices of acidosis but no significant correlation between acetone in urine and serum bicarbonate. Using regression to predict values of HCO3, pH and anion gap from β-OHB levels revealed that levels of β-OHB of 1.4, 1.9, 2.7,3.5 and 4.0 mmol/l corresponded to HCO3 levels of $>18$, 15–18, 10-$<15$, 5-$<10$ mEq/l.
Conclusions
The measurement of capillary β-OHB levels is easy and give rapid and objective results. Capillary β-OHB levels are more predictable than urinary acetone for degrees of acidosis and severity of DKA and can be used at diagnose and follow up of type1 diabetic patients with DKA to improve their management.
Key words: Acetoacetic acid (ACAC) B-hydroxybutyrate (β-OHB) Diabetic Ketoacidosis (DKA) bicarbonate (HCO3) and National Institute of Diabetes & Endocrinology, (NIDE).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P661

**Clinical characteristics of diabetic peripheral neuropathy in past and present in Korea**

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Diabetic peripheral neuropathy (DPN) is a most common complication of diabetes mellitus. Patients with DPN have chronic, painful symptoms that disrupt sleep, depressed mood and can lead to diminish quality of life. We evaluated clinical characteristics of diabetic peripheral neuropathy in past and present in Korea
We performed the study to determine the prevalence and to understand the clinical characteristics of diabetic peripheral neuropathy in Koreans on 2005 and 2010.
A cross-sectional study was carried out on type 2 diabetic patients from the diabetic clinics of 33 medical centers throughout Korea on 2005. The study pool consisted of 875 randomly selected type 2 diabetic patients (45% men and 55% women, mean age: $59\pm10$ years). Of the 875 patients, 54% (51% men and 49% women) had DPN. There was significant differences in prevalence between the genders (40.7% men and 59.3% women, $P<0.002$) and the different age groups ($60.7\pm10.6\%$ vs $56.6\pm11.7\%$, $P<0.001$). The prevalence increased steadily with the duration of diabetes since the time of diagnosis. The DPN patients had higher HbA1c values than did the diabetic patients without DPN (8.0% vs 7.2% respectively $P<0.001$). The mean number of DPN symptoms was $3.2\pm1.7$. Among them, 'severe pain on the leg or feet at night', 'a sudden cramp on the leg or feet', and 'feeling a pin-prick on the leg or feet' were common, and the incidences of those symptoms were 31%, 29% and 29% respectively.
A cross-sectional study was carried out on 4,000 type 2 diabetic patients from the diabetic clinics of 40 medical centers throughout Korea on 2010. Of the 4000 patients, 33% (51% men and 49% women) had DPN. There was significant differences in prevalence between the genders (40.7% men and 59.3% women, $p<0.002$) and the different age groups ($60.7\pm10.6\%$ vs $56.6\pm11.7\%$, $P<0.001$). The prevalence increased steadily with the duration of diabetes since the time of diagnosis. The mean number of DPN symptoms for the patients was $3.1\pm1.7$. The mean number of DPN symptoms was $3.1\pm1.7$. Among them, 'a numbness on the legs or feet', 'feeling a pin-prick on the legs or feet' and 'severe pain on the legs or feet at night' were common, and the incidences of those symptoms were 65%, 46% and 36% respectively. DPN is a common complication of diabetes affecting 33–54% of patients with diabetes in Korea.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P662

**The effects of metoprolol,diltiazem and pilocarpine in Prolonged QTc interval caused by insulin induced hypoglycemia in rats**

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Introduction
Insulin induced Prolonged QTc interval cause severe ventricular arrhythmias and sudden cardiac death.
The purpose of this study is to reveal the effects of metoprolol,diltiazem and pilocarpine on insulin induced prolonged QTc interval(Bazett)
Material-Method
In this study 24 Sprague-Dawley adult male rats were used.Before application under anesthesia ECG were taken in derivation(D)I and normal QTc interval was determined in normoglycemic rats.
For the induction of hypoglycemia 40 U/kg crystalline insulin were injected to the 24 rats.Rats were divided into 4 groups ($n=6$). After 2.5 hours insulin injection, For first group saline(S), for second 1 mg/kg metoprolol, for the third group 0.8 mg/kg pilocarpine and fort he forth group 1 mg/kg diltiazem were applicated intraperitoneally.
3 hours after insulin injection, blood sugar was measured from all four groups rats tail with stick. 40 mg/dl defined as the limit of hypoglycemia. The all hypoglycemic rats below this value, under anesthesia,QTc was calculated and interpreted by taking ECG in DI
Results
In the first group hypoglycemic (HG) rats which is taken saline QTc interval ($0.143\pm0.01$s)is significantly ($P<0.05$)prolonged than normoglycemic rats QTc ($0.123\pm0.009$s)
In the second group HG rats which is taken metoprolol QTc ($0.121\pm0.004$s), in the third group HG rats which is taken pilocarpine QTc ($0.116\pm0.002$s), in the forth group HG rats which is taken diltiazem QTc ($0.129\pm0.001$s) were founded. QTc intervals in second,third, forth groups HG rats which is taken metoprolol, pilocarpine and diltiazem QTc intervals were significantly ($P<0.05$)shorten than HG rats which is taken saline QTc ($0.143\pm0.01$s).
Conclusion
Pilocarpine,metoprolol and diltiazem prevented elongation in QTc intervals in HG rats.The prophylactic use of this drugs may be an hope by reducing sudden cardiac death in patients taking insulin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P663

### Proximal upper and lower extremities severe pain, muscle weakness in a young diabetic male with multiple chronic complications. A rare and unusual clinical presentation

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A 49 years old male patient with 12 years history of poorly controlled type 2 diabetes mellitus on insulin therapy, complicated with neuropathy, nephropathy, retinopathy, peripheral arterial disease (right toe amputation), hypertension and dyslipidemia. He presented with upper extremities proximal muscle and upper back moderate pain that progressed to excruciating pain with restriction of motion, weakness, and stiffness of 3 days of evolution. He denied trauma, abnormal exercise, arthralgia, fever, nausea or vomiting. Physical examination was remarkable for bilateral upper extremities edema, local tenderness and indurations without palpable crepitus. Laboratory results showed normocytic normochromic anemia, hyperkalemia, creatinine of 1.9 mg/dl, high anion gap metabolic acidosis, erythrocyte sedimentation rate of 76 mm/hr and increased creatine kinase of 225 U/L. Electrocardiogram demonstrated a sinus tachycardia and peaked T waves. Blood cultures were negative. Upper extremities Venous Doppler was negative for DVT. Echocardiogram revealed moderate LVH, non thrombus or vegetation. MRI of the left arm showed diffused soft tissue swelling and T2 hyper intensity of the deep biceps, brachialis, and brachioradialis muscles in the anterior muscle compartment with heterogeneous patchy contrast enhancement consistent with diabetic myonecrosis. Similar findings noted in latissimus dorsi muscle. After two week he started with the same symptomatology in both thighs, with gradual relief of upper extremities affection, but with residual muscle wasting. A muscle biopsy with electronic microscopy allowed us to rule out other conditions. Currently he has symptomatic improvement.
Diabetic muscle infarction is a rare complication of longstanding diabetes referring to spontaneous ischemic necrosis of skeletal muscle, unrelated to atheroembolism or occlusion of major arteries. This condition cause acute or subacute pain, swelling and tenderness, typically in the thigh or calf. Rarely, the upper limb may be involved and only one case has been reported. Diabetic myonecrosis is an indicator of poor prognosis, tends to resolve spontaneously but with frequent relapses. A review of the literature, identified at total of 116 patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P664

### Risk factors for cardiovascular diseases in T1DM

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Rationality
T2DM is a component of the metabolic syndrome, the occurrence of overweight, arterial hypertension (AH), and dyslipidemia in T1DM is variable according to the literature.
Objective
Study of the occurrence of overweight, AH and dislipidemy in T1DM
Materials and methods
Total 726 patients with T1DM (348m, 378f), mean age ($M \pm \sigma$) $34.71 \pm 0.58$ yrs (1–78), age of T1DM manifestation $12.41 \pm 0.36$ yrs and special form including questions about T1DM. Levels of triglycerides (TRIG), total cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and HbA1C (%) were measured by biochemical analyzer 'ARCHITECT C8000' (Abbott, USA). Body mass index (BMI), systolic blood pressure (sBP), diastolic blood pressure (dBP) were analysed. Control includes 206 healthy volunteers of similar age and sex.
Results
The levels of sBP, dBP, HbA1C, TRIG and LDL were significantly higher in T1DM, than in the control (*$P<0.001$). Differences of rates are fixed in patients in their division into age categories: under 18, 18 and over (table -^$P<0.001$).
BMI of 25–29.9 kg/m2 was recorded in 188 patients (31%) and 41 subjects of control (28%), BMI of 30–34.9 kg/m2 in 89 patients (14%) and 16 subjects of control (11%), BMI of 35–39.9 kg/m2 in 25 patients (4%) and 5 subjects of control (4%), BMI of 40 kg/m2 and more (obesity of the third degree) in 5 patients (1%) and 6 subjects of control (4%). The levels of sBP, dBP, CHOL, TRIG and LDL ($P=0.001$) were higher and the level of HDL was lower ($P=0.015$) in the presence of T1DM and overweight, such differences occurred in men and women.
Conclusion
The patients over 18 with T1DM have often AH, dislipidemy, overweight and obesity. The patients with overweight and T1DM had high BP (>130/80 mm Hg) and metabolic changes in the form of atherogenic shifts in blood lipid spectrum. That indicates the risk increase of cardiovascular diseases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1** Differences of rates in patients in their division into age categories

| Indicator | T1DM total | T1DM under 18 | T1DM 18 and over | Control group |
|---|---|---|---|---|
| Chol,mmol/l | 4,7 [4,0/5,5] | 4,2 [3,7/4,7]^ | 4,8 [4,1/5,6] | 4,7 [3,9/5,5] |
| TRIG, mmol/l | 1,1 [0,7/1,5]* | 0,8 [0,6/1,2]^ | 1,1 [0,8/1,7] | 0,9 [0,6/1,3] |
| HDL, mmol/l | 1,5 [1,2/1,8] | 1,5 [1,2/1,7] | 1,4 [1,2/1,7] | 1,3 [1,1/1,5] |
| LDL, mmol/l | 3,1 [2,5/4,2]* | 2,7 [2,3/3,2]^ | 3,2 [2,6/4,3] | 2,9 [2,3/3,4] |
| sBP, mm Hg | 120,0[110,0/140,0]* | 100,0[100,0/110,0]^ | 120,0[120,0/140,0] | 110,0[100,0/130,0] |
| dBP, mm Hg | 80,0 [70,0/90,0]* | 70,0 [60,0/70,0]^ | 80,0 [75,0/90,0] | 70,0 [60,0/80,0] |
| HbA1c, % | 8,5 [7,3/10,4]* | 9,3 [7,6/11,3]^ | 8,4 [7,3/10,2] | 5,1 [4,9/5,3] |

*$P<0.001$ ^$P<0.001$

## P665

### Relationship Between HbA1c and Asymptomatic Coronary Artery Disease in Type 2 Diabetes Patients

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Background
Patients with type 2 diabetes mellitus (T2DM) have elevated risk of coronary artery disease (CAD) development. In this population CAD often proceedsa-symptomatically, especially if cardiac autonomic neuropathy (CAN) is present. Our aim was to assess relation between glycemia control and asymptomatic CAD inT2DM pts. Materials and methods: Participants were 160 T2DM patients without documented CAD divided into four groups (Gr.) according to HbA1c levels: Gr. 1 -HbA1c6.0–7.0%($n=42$; mean age (MA)- 48.3+9.1yrs; 27m/15f); Gr. 2 - HbA1c 6.0–8.0% ($n=46$; MA 51.4+6.7yrs; 26m/20f); Gr. 3 - HbA1c8.0–10.0% ($n=40$; MA 49.9+7.1yrs; 22m/18f); Gr. 4 - HbA1c>10.0% ($n=32$;MA 51.9+9.7yrs; 17m/15f). In Gr. 1 target HbA1c (6.0–7.0%) were selected individually. In all pts 12 - lead rest ECG,exercisestress-tests (veloergometry) or 24-hr. Holter ECG were performed. To put final CAD diagnosis coronary angiography was performed in subjects with positive stress-test or ischemic episodes on Holter ECG. Results: Abnormal ECG atrest was observed in 7.1% of Gr. 1, 6.5% - Gr. 2, 12.5% - Gr. 3 and 15.6% of Gr. 4 patients. Positive stress test or ischemic episodes on Holter ECG were found in 38 out of 160 (23.8%) patients, and were more frequently observed, when HbA1c levels were high - 11.9% - Gr. 1; 19.6%-Gr. 2; 30%-Gr. 3 and 37.5%ofGr. 4 patients. Among 38 pts with positive stress-test or ischemic episodes on Holter ECG, 23 subjects received CAG, CAD was diagnosed in 14(60.9%) patients. Conclusion: Hyperglycemia, reflected by significantly elevated HbA1c levels, is associated with increased risk of CAD development that often proceeds asymptomatic. This may be due to CAN, caused by elevated glycemia levels. In uncontrolled T2DM more attention should be paid to glycemia normalization and early CAD revealing. This will permit to timely take necessary measures to improve the outcomes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P666

### Treating depression in type 2 diabetic patients improves depressive symptoms and quality of life but not metabolic control

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Introduction:Type 2 Diabetes (T2DM) almost doubles the risk of comorbid depression, with lifetime prevalence up to 29%. It is important to recognize and treat depression in T2DM because its association with hyperglycemia, increased diabetic complications and poor quality of life (QoL) is well established. However, currently available medical therapy for depression is effective in reducing depressive symptoms, but does not consistently improves HbA1c levels. Aims:Determine the effects of antidepressant therapy on depressive symptoms, health-related QoL and metabolic control in T2DM. Material and methods:33 T2DM (47.8%♂, age 59.8±11.1, progression period of DM 9.5±6.5 years) who had a Beck Depression Inventory (BDI) test greater than 16 were prescribed citalopram 20 mg once daily; 10 out of 33 refused it. BDI score, BMI, HbA1c and the Spanish version of the SF-36 Health Survey were recorded baseline and after 6 months of treatment. Sociodemographic characteristics (marital status, educational level, labor situation), complications related to T2DM and comorbidities were also registered. Results:No differences in baseline characteristics were observed between the two groups. When compared with the untreated group ($n=10$), patients treated with citalopram ($n=23$) showed significant improvements in BDI score and in almost all areas of quality of life. No differences in HbA1c, waist circumference or BMI were seen (Table 1). Conclusions:Treating depressive symptoms with medical therapy in T2DM is associated with improvements in QoL and depression with lack of efficacy in metabolic control or weight.

**Table 1** BMI: body mass index. BDI: Beck Depression Inventory

| | Baseline | 6 month-treatment | p |
|---|---|---|---|
| BMI | 30.79±4.83 | 31±4.54 | $p=$ 0.56 |
| Waist circumference(cm) | 104±13 | 106±13 | $p=$ 0.091 |
| HbA1c (%) | 7.77±1.97 | 7.73±1.6 | $p=$ 0.923 |
| BDI | 22±6.9 | 14±7.9 | $p=$ 0.000 |
| General Health | 42.54±21.68 | 48±24.63 | $p=$ 0.337 |
| Physical Functioning | 59.1±20.33 | 67.27±21.47 | $p=$ 0.042 |
| Role-physical | 47.5±44.95 | 64.77±43.41 | $p=$ 0.023 |
| Role-emotional | 32.73±39.27 | 65.67±43 | $p=$ 0.001 |
| Social Functioning | 39.57±28.79 | 59.54±23.65 | $p=$ 0.002 |
| Bodily Pain | 60.68±27.8 | 59.5±27.61 | $p=$ 0.845 |
| Vitality | 38.86±17.45 | 50.22±23.37 | $p=$ 0.021 |
| Mental Health | 36.89±25.41 | 51.18±21.44 | $p=$ 0.012 |
| Health Evolution | 3.45±0.73 | 2.63±0.49 | $p=$ 0.000 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P667

### Cardiorespiratory fitness is decreased and not correlated with C-Reactive Protein levels in adults with Diabetes Mellitus

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Diabetes Mellitus (DM) has been associated with lung dysfunction and impairment of cardiorespiratory fitness and increasing evidences have suggested that comorbidities and systemic inflammation may be involved in these derangements. However, the results regarding cardiopulmonary dysfunction and its underlying mechanisms in DM are still controversial. In this study, we evaluated the relationship between metabolic variables and inflammation marker, including hemoglobin A1C (HbA1C), lipid profile and C-reactive protein (CRP) levels, and cardiorespiratory fitness in adult patients with DM. Nineteen men with diabetes (aged 51±6 years; mean ± SD) and nineteen age and sex-matched control subjects (aged 49±7 years) were studied. The average duration of diabetes was 11 years (range, 2–25 years) and the HbA1C mean levels were 8.4% (range, 6–12.9%). All individuals were subjected to incremental cardiopulmonary exercise test and spirometry. Blood pressure, triglycerides, HDL, LDL, total cholesterol and CRP were not different in control subjects and individuals with DM. Body mass index (28.7±1 vs 25.6±0.4 kg/m2, mean ± SD, $P<0.01$) and resting heart rate (78.3±2.2 vs 71.3±2.1 beats/min, mean ± SD, $P=0.03$) were significantly higher in individuals with DM than in control subjects, respectively. No significant difference was noted in spirometric variables, including forced vital capacity (FVC), forced expiratory volume in 1s (FEV1), forced expiratory flow midexpiratory phase (FEV25–75%) and peak expiratory flow (PEF). In the exercise test, maximal overload (W), peak heart rate (HRpeak), peak oxygen consumption (VO2peak) at anaerobic threshold (VO2AT) and respiratory exchange ratio (RER) were significantly lower in patients with diabetes than in control subjects. No correlations were observed between HbA1C and CRP plasma levels and cardiorespiratory variables. These data indicate that cardiorespiratory fitness is reduced and spirometric values are preserved in patients with diabetes. However, decreased cardiorespiratory fitness is not correlated with CRP levels or HbA1C.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P668

### Evaluation of Brainstem Auditory Evoked Potential in Diabetics

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Background
Brainstem auditory evoked potentials (BAEP) have been used for electrophysiological assessment of central neuropathy in diabetes. However, the role of this test in documenting the abnormality, the site of abnormality and relation of these abnormalities with metabolic control of diabetes are not clear as yet. The present study was done to explore the presence of abnormalities, if any, in the test parameters and relation of these with diabetic status.
Methods
It was a cross sectional study with controls. Thirty patients of diabetes mellitus (group 1) and thirty healthy controls (group 2) were included in the study. All the patients were subjected to laboratory examination including haemogram, fasting and postprandial plasma sugar (2 hours), HbA1c, urine R/E, 24 hour urine for proteins, ECG, RFT, LFT and lipid profile. BAEP was done in all the subjects
Results
Mean peak latency of waves I, III, V and interpeak latency of I-III, III-V, I-V were prolonged in group 1, but were not statistically significant. Abnormal BAEP response was found in 8 patients (27%) in group 1. Abnormal BAEP response reflex response was significantly correlating with postural drop in blood pressure ($P$-value 0.013 and 0.046). There was no significant relation between abnormal BAEP response with age, sex, type of diabetes, duration of diabetes since detection, fasting plasma sugar level, postprandial plasma sugar level, glycosylated haemoglobin, presence of retinopathy, nephropathy and peripheral neuropathy.
Conclusions
BAEP is a useful method for obtaining an early diagnosis of central and cranial nerve abnormalities in diabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P669

### The Loss of Work Productivity in Type 2 Diabetes Mellitus in Turkey

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Introduction: An update of health economics analysis of diabetes mellitus (DM) in Turkey was performed. The primary objective was to collect and calculate the healthcare resources utilized for the management of DM.
Methods: Thirty-one out of 40 randomly chosen centers completed the study. Medical files were reviewed for the data recorded for at least two years prior to the study. Collected data included laboratory tests, treatments, inpatient/outpatient follow-up visits, consultations and patient education. Data on work loss collected via validated "Work Productivity and Activity Impairment Questionnaire: General Health V2.0 (WPAI:GH)".

Results: A total of 942 patients' data were included in the analysis. Mean age was 57.7 ± 11.4 years; 58% were female, median DM duration was 8 years. Metabolic (28%), ocular (21%), cardiovascular complications (12%), neurologic (11%) and renal (4%) were the first five causes for seeking care. A total of 657 (69.7%) patients answered the WPAI:GH questionnaire. The percentage of patients, who had a job, at the time of the study conducted, was 14%. The proportion of the working patients who stated that health problems had caused 50% or more productivity loss while working in last 7 days was 21%. This figure for regular daily activities other than the ones at the job was 30% of all patients. Scores of work impairment and activity impairment were 23% and 31% over 100%. Nearly 1/5 of the patients were getting help (professional, volunteered or from a family member) for daily health care, due to health problems. Mean percentages for absenteeism and presenteeism were 24% and 15%, respectively. General work productivity loss (absenteeism plus presenteeism) was found as 39%.
Conclusion: DM is a disease that affects work and daily productivity of both the patients and their companions, negatively. Productivity loss of patients' and their companions can be prevented with better management of DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P670

### The Relationship between Cognitive Function and Microalbuminuria in Elderly Type 2 Diabetic Patients

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Objective: The prevalence and incidence of diabetes mellitus (DM) are increasing at all ages, including older populations. Several studies have shown that elderly diabetics have impaired cognitive function compared to age-matched non-diabetics, as well as a higher risk of dementia. In the current study we aimed to analyze relationship between microalbuminuria and cognitive dysfunction in elderly patients with type 2 diabetes.
Material and Methods: One hundred four diabetic patients who are over 60 years and diagnosed at least 6 months ago and forty four healthy volunteers who are over 60 years were included in this study. Medical history taking, physical examination, biochemical analysis and collection of urine specimens to determine microalbumin-creatinine ratio were performed. Cognitive function was evaluated by the Standardized Mini Mental State Examination (SMMSE). The patients were evaluated in three groups: type 2 diabetic patients without microalbuminuria (group 1), type 2 diabetic patients with microalbuminuria (group 2) and the control (group 3).
Results: One hundred four diabetic patients (42 males, 62 females; mean age: 65.8 ± 5.1 years) and 44 healthy volunteers (17 males, 27 females; mean age: 65.4 ± 5.0 years) were enrolled in the study. SMMSE scores significantly lower in diabetic patients compared with the control group ($P < 0.05$). The mean SMMSE scores of normoalbuminuric (60 patients) and microalbuminuric (44 patients) patients were 22.3 ± 4.6 and 22.6 ± 4.9 respectively. Hemoglobin level ($P = 0.044$) and educational status ($P < 0.01$) were positive, systolic blood pressure ($P = 0.032$), diastolic blood pressure ($P < 0.01$) and hemoglobin A1c ($P = 0.01$) were negative correlated with SMMSE scores. However, there was no statistically significant relationship between microalbuminuria and SMMSE scores. Decreased cognitive functions related with diabetic neuropathy.
Conclusion: Cognitive functions are lower in diabetic elderly patients when compared with healthy elderly volunteers. There were no significant relationship was found between cognitive function and microalbuminuria.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P671

### Is skin tag associated with diabetic macro and microangiopatyh? Preliminary Report

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Introduction
Although skin tag is associated with diabetes mellitus, no data in the literature show that the presence of skin tag is associated with diabetic macro and microangiopathy. The purpose of this study was to investigate frequency of hypertension, dyslipidemia, obesity, macro and micro angiopathy in type 2 diabetic patients with and without skin tag.
Methods/Design
We evaluated 99 (40 female and 59 male) type 2 diabetic patients. All patients were evaluated for blood pressure, body mass index, lipids, HbA1c, macroangiopathy (peripheral vascular disease, cerebrovascular disease and coronary hearth disease), microangiopathy (neuropathy, nephropathy, retinopathy) and skin tag.
Results
Mean HbA1c was 8.1 ± 2.0% and body mass index was 30.5 ± 6.4 kg/m2. The frequency of skin tags 53.5%, dyslipidemia 68.7%, hypertension 69.7%, obesity 39.4%, macroangiopathy 61.6% (peripheral vascular disease 12.1%, cerebrovascular disease 16.2%, and coronary heart disease 49.5%), microangiopathy 63.6% (neuropathy 21.2%, nephropathy 38.4%, retinopathy 38.4%) were detected. Higher body mass index (p: 0.04) and frequency of obesity (p: 0.03) were detected in patients with skin tag than without skin tag. HbA1c (p:0.4) and the presence of dyslipidemia ($P = 0.4$), hypertension (p: 0.6), macroangiopathy (p: 0.2), and microangiopathy (p: 0.9) were not different in patients with and without skin tag.
Conclusion
We conclude that presence of skin tag is merely related to obesity and may not be strongly associated with macro- and microangiopathy in type 2 diabetic individuals. Further studies with large patient population are required to elucidate the association between the presence of skin tag and diabetic angiopathy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P672

### Incidence and mortality of diabetic ketoacidosis in Benghazi-Libya

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Background: Diabetic ketoacidosis is a frequent cause of morbidity and mortality among diabetics. The annual incidence and the mortality from this condition in Libyan diabetics have not been studied before. Aim and objectives: The aim of this study was to estimate the incidence of diabetic ketoacidosis and the its mortality rate at Benghazi city. Patients and methods: a descriptive retrospective analysis of the records of all patients admitted with diabetic ketoacidosis to all Benghazi hospitals (governmental and private) between 1st of January and 31st of December 2007. Results: the annual incidence of diabetic ketoacidosis was 41.7 episode/100 000 populations with a mean age of 33 ± 20.1 years (2–93). Around 52% of all the episodes occurred in males and 2.6% of adult DKA occurred in pregnant females. Type-2 diabetics were responsible for 27.7% of all episodes. The commonest precipitating factor in the whole study group was dose disruption (35%) followed by infection (20%). The commonest presenting symptoms were the gastrointestinal, whilst 3.5% of the patients were comatose at presentation. The overall mortality was 11.7% and there was no significant difference in mortality between males and females (11% vs. 12.6%, $P = 0.6$) however type-2 diabetics showed a significantly higher mortality rate (29.3% vs. 4.9%, $P = 0.000$). The died patients were significantly older with longer duration of diabetes and higher rate of co-morbidities and they had significantly faster respiratory rate, lower blood pressure, higher urea and platelet count at presentation. Conclusion: diabetic ketoacidosis is a common condition in Libya with a high mortality rate and type-2 diabetics constitute a considerable number of the cases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P673

**Drugs or insulin used in diabetics not associated with increased risk of malignancy**

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Aim
The aim of the present study is to determine the effects of the diabetes mellitus (DM) treatment regimens on malignancy retrospectively.
Material-Method
655 DM patients were enrolled in this study. Patients receiving diabetes therapies for at least one year were assessed with physical examination, detailed medical and family history, habits, demographic characteristics and laboratory tests and divided into two groups according to the diabetes treatment type (using oral agent or insulin and using metformin or not). Insulin users were grouped according to insulin regimens (intensive, basal and mixed) and the insulin type (glargine, detemir, human insulin, biphasic analogue). Cancer cases were identified at the first visit. And if there was a cancer suspicion, clinical examination was made for clearing the diagnose. Patients who have not administrative data of cancer or benign tumor and diagnosis of cancer before date of diagnosis of diabetes mellitus, were excluded from the study.
Results
655 DM patients with 13 (%2) Type 1 DM, 642 (%98) Type 2 DM, 379 (%58) female, 276 (%42) male were enrolled. 36 cancers (4 colorectal, 1 lung, 4 larinx, 1 biliary duct, 5 breast, 4 prostate, 4 bladder, 3 leukemia/lymphoma, 1 urogenital, 2 hepatic, 5 thyroid, 1 renal, and 1 unknown origin) and 76 benign tumors were observed. Patients with and without a cancer were compared for diabetes treatment regimens and types of agents. No significant differences was observed between groups using oral agent or insulin (p:0.429) and using metformin or not (p:0.119). Also no significant differences was observed between groups according to insulin regimens (p:0.059) and the insulin type (p:0.418).
Conclusion
Between the types of pharmacological treatments of diabetes, there was not any significant differences in regard to cancer risk. But in subgroup analysis, patients using metformin had lower rates of benign tumor compared to metformin non-users.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P674

**Polyneuropathy in diabetic patients based on the clinical and paraclinical findings**

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Introduction
Prevalence of diabetic neuropathy is between 10 to 90% in different studies base on the method of it evaluation(clinical or paraclinical).
Objective
Evaluation of frequency of diabetic polyneuropathy indiabetic persons in endocrinology clinic of mashad endocrinand metabolic research center based on symptoms,signs and electrodiagnostic study.
Method
The study was cross-sectional; 110 diabetic patients were enrolled.patients with other causes of polyneuropathy such as hypothyroidism, collagen-vasculardiseases,renal failure were excluded. Neuropathic symptoms (paresthesia, hyperesthesia,neurotic pain,…) were recorded in questionnaire. Physical exam included evaluation of superficial sensation (by 10 gram monofilament), vibration(by 128Hz fork), position, pain, temperature and deep tendon reflexes. Electrodiagnostic tests conducted in proneal, sural and sensory branch of radial nerve.
Results
Frequency of neuropathy based on symptoms, signs and electrodiagnostic tests were 55.4%, 59.09% and 70% respectively. Most frequent symptom was paresthesia, most frequent sign was abnormal DTR and most frequent finding of electrodiagnostic tests was decreased amplitude of proneal and sural nerves. only 16 of 77 patients with abnormal electrodiagnostic findings didn't have any symptom and sign.
Discussion
Frequency of symptoms, signs and abnormal electrodiagnostic findings were consistent with several other studies. The percentage of abnormal electrodiagnostic findings were low in patients without both signs and symptoms.
Conclusion
It seems history and physical examination has an important role compared to expensive electrodiagnostic tests.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P675

**Not all neuropathies in people with diabetes are due to diabetic neuropathy - a case of chronic inflammatory demyelinating polyneuropathy(CIDP) in a type 1 diabetic**

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46yr old gentleman. Recently diagnosed with late-onset type 1 diabetes.HBA1C of 12% (108 mmol/molHb) at diagnosis. Also Vit B12 deficient. Normal TSH, Normal 9am cortisol, Normal TTG antibodies. On Novomix 30 Insulin.
No diabetic end-organ complication. Smokes 8g tobacco/day. Moderate alcohol intake
Presented with a year history of painful neuropathy,started with his left toes and progressed in an ascending manner to involve the whole foot ant the lower leg. Shortly afterwards, his right leg became involved in exactly the same manner. At a later stage, both hands became involved spreading to the forearms.
Associated with these was marked wasting of his Quadriceps,calves, tricep and bicep muscles.
Neurological examination showed normal strength but quite significant wasting especially in both quadriceps. There were patchy areas of decreased sensation all over his body with impaired proprioception distally in his right leg. Lower limb reflexes were absent.
Rest of systems examination were normal.
Results
Polyneuropathy Screen -
Normal U & E, folate and ferritin levels
Normal FBC
TSH Normal
Purkinje Cell Antibodies NEGATIVE
Cryoglobulins Not detected
Serum ACE 45 (Ref: 8–70 U/L)
Myeloma screen - Negative
EMG - Evidence of mild neurogenic change in the right lower limb
Nerve Conduction Report - Reduced CMAP(compound muscle action potential) amplitude and conduction velocity compatible with a primarily demyelinating polyradiculoneuropathy - Likely CIDP
Treatment
Currently has had IV Immunoglobulin treatment with good response i.e marked improvement in muscle strength and pain
Discussion
CIDP is suspected in patients who develop a sub-acute and progressive predominantly motor neuropathy with proximal weakness, which is distinguished from the "typical" diabetic neuropathy that is predominantly sensory and length-dependent. Incidence of diabetes in CIDP and vice versa may be higher than in the general population. Diagnostic criteria for CIDP in diabetes is similar to CIDP in general. However, making a diagnosis of CIDP in diabetics can be rather difficult as nerve conduction abnormalities are common in diabetes along with axon loss and demyelination. This distinction is of importance, as these patients can respond to immune therapies similar to patients with CIDP without diabetes
Declaration of interest
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## P676

**High burden of comorbid disease and complications in underprivileged type 1 diabetes children**

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This multicentric cross sectional study, conducted in northern India, aimed to assess the clinical profile of socioeconomically underprivileged children and adolescents, aged below 18 years, with type 1 diabetes. A structured questionnaire was distributed to OPD patients, after taking informed written consent. These patients hailed from six states of India, thus providing a comprehensive picture of the burden of disease. Data was collected regarding their clinical history and laboratory investigations.

Of the 70 patients studied, 47 were male. This skewed gender ratio is typical of this region of India. Age ranged from 2.5 to 17 years, with a duration of diabetes ranging from 1 month to 9 years. Six patients had experienced an episode of DKA in the past 3 months, while none had experienced severe hypoglycemia. Only one patient was overweight, and she had severe acanthosis nigricans. Surprisingly, one boy (aged 7) had acute balanoposthitis, while two premenarcheal girls had pruritis vulvae. Three children complained of painful neuropathy. Four children were found to have short stature, as per Indian standards. Seven girls were hypothyroid, and two boys had subclinical hypothyroidism. Two patients exhibited syndrome of limited joint mobility, two girls had coeliac disease, while four boys had delayed puberty.

HbA1c varied from 6.6% to 16.7%, with 53 patients having an A1c $>9.0\%$. While 56 patients were on a traditional basal-bolus regime, 7 were on a three dose regime (premixed- regular-premixed: 4, or regular-regular-premixed: 3), and 7 were on premixed biphasic insulin twice daily. None of the patients were found to have microalbuminuria or retinopathy.

This abstract highlights the unique clinical features of type 1 diabetes in an underprivileged cohort of children and adolescents in northern India. It quantifies the burden of acute and chronic complications of diabetes in this group of patients. There is a high prevalence of hypothyroidism, diabetic ketoacidosis, genitourinary infections and chronic complications.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P677

**Effects of acute hyperglycaemia on ANP levels in diabetic patients**

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Introduction

Mechanism underlying glucose-mediated development and progression of diabetic complication are incompletely understood. Atrial natriuretic peptide (ANP) is a hormone released by atrial myocytes in response to acute and chronic extracellular volume expansion. Moderate hyperglycaemia ($>400$ mg/dl) in diabetic rats is associated with an elevation in circulating ANP levels and in GFR (glomerular filtration rate), (1) and play a role in the development of microalbuminuria (2).

Elevated plasma ANP concentrations have been reported in human diabetic subjects with poor glycaemic control although these findings have not been confirmed by other studies (3).

Materials and methods

We included 21 diabetic patients, with ages (X $\pm$ DS) 61.3 $\pm$ 12.6 years and 8.4 $\pm$ 9 years of diabetes progression. They were 2 type 1 diabetes and 19 were type 2. We analyzed levels of ANP levels by RIA.

Results

-There was no change in the levels of ANP in hyper-or normoglycaemia (90.52 $\pm$ 47.48 pg/ml vs. 86.98 $\pm$ 37.74 pg/ml). $P=0.56$.

-The average levels of glucose in hyperglycaemia were 294.85 $\pm$ 75.19 mg/dl and 134 $\pm$ 34.16 mg/dl in normoglycaemia.

Conclusion

-Hyperglycaemia did no induce changes in the ANF levels in our study.

-This result could be influenced by the higher prevalence of type 2 diabetics in our group with higher basal levels of ANF.

-Furthermore, the level of hyperglycemia was not too high in our work and this can be a stimulus course shorter than in other published studies with more marked hyperglycaemia.

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P678

**Hyperhomocysteinemia as Risk's Factor of Diabetic Nephropathy in the Patients with Diabetes Mellitus Type 1 and Depression**

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The aim was to evaluate the space of hyperhomocysteinemia presence with depression and diabetic nephropathy (DN) in diabetic mellitus type 1 (DMT1) patients.

Materials and methods

it was examined 147 patients with DMT1 (57.1% of males, 42.9% - females) and duration of disease was 11.17 [9.07;12.26] years. Moderate age was 40.08 [30.44; 48.79] years. DN of 3–5 stages was diagnosed in 43 patients (29.3%). To provide analysis it used blood serum creatinine level, total cholesterol (TC) and homocysteine (HC) determining. The hospital scale of anxiety and depression was used for detection of depression.

Results

HC level in DMT1 patients with depression was 10.50 mmol/l vs 9.95 mmol/l in the the patients without depression ($U=1066.0$; $P=0.085$). HC level correlated with duration of disease in DMT1 patients ($r=0.44$; $P=0.02$).

HC level was 11.20 [8.80;11.65] mmol/l at the presence of DN vs 9.41 [7.96;11.30] mmol/l at the patients without DN ($U=605.00$; $P=0.034$). Creatinine concentration was higher at DN presence 81.50 [72.50;86.5] mmol/l vs 76.00 [69.00;83.00] mmol/l at the patients without DN ($U=614.50$; $P=0.004$). TC level was 5.60 [4.40;6.60]mmol/l at the patients with DN vs 4.70 [4.00;5.60] mmol/l at the patients without it ($U=582.00$;$P=0.002$).

Using quartile value of HC level it was pointed, that creatinine blood level was decreased at the patients with HC level less than 8.35 mmol/l. Analyzing the frequency of DMT1 complications we estimated higher frequency of DN complicated by arterial hypertension ($\chi 2=3.78$; $P<0.05$) in group of patients with HC level more than 12.55 mmol/l to compare the patients with HC level presented less than 8.35 mmol/l.

Conclusion

Increasing DMT1 duration leads to increasing of blood HC level. HC level got the tendency to growing in the patients with D1TM and depression.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P679

**No influence of the chronic poor clycaemic control on ANP levels in diabetics with microvascular illness**

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Introduction

Atrial natriuretic peptide (ANP), a member of the natriuretic peptide family, regulates several physiologic parameters including diuresis and natriuresis, and lowers arterial, blood pressure.

It has been reported that plasma concentrations of ANP rise in response to acute hyperglycaemia in diabetes (1) (2) and this may indicate a neurohormonal response to limit target organ damage (3).
We investigate if in situation chronic poor glycaemic control, determined by measuring Hba1c levels, there might be changes in concentrations of plasmatic ANP as regards a metabolic state of aggression y patients with microvascular disease.
Materials and methods
The study analyzes a group of 7 diabetic patients affected by microvascular disease compared with a control group of 34 patients. The control group were 21 women and 13 healthy men, aged 46±21.1 years (range 24–78 years). In the diabetic group had 4 females and 3 males, aged 37±16 years (range 17–61), 5 with type 1 diabetes and 3 type 2. Poor glycemic control groups were those with chronic HbA1c >9%. Microangiopathy was defined as the presence of renal, ocular or neuropathic disease. Statistical analysis was carried out by the statistics department of the Centre, through SPSS. The determination of ANP levels was performed by RIA.
Results
No differences were found between the levels of ANP from the group of diabetic microangiopathy with HbA1c >9% compared to HbA1c <9%.
However we could demonstrate significantly higher levels of ANP in the diabetic group than the control group.
Conclusion
-No evidence of elevated ANP levels in relation to chronic poor glycemic control in diabetic patients suffering from microvascular disease.
-ANP levels are higher in diabetics with microvascular disease than in healthy controls without diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1** ANP levels in diabetics according to the metabolic control

| | Control group ($n=34$) | Diabetics with microangiopathy ($n=4$) HbA1c<9% | Diabetics with microangiopathy ($n=3$) HbA1c>9% |
|---|---|---|---|
| ANP levels (pg/ml) | 5.84±3.96** $p<0,05$ | 20.12±18.68 | 13.08±11.82 |

## P680

### Nephropathy could be a main factor in the increased levels of ANP in diabetes

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Introduction
Its has been suggested that atrial natriuretic peptide (ANP) may contribuye to the development of microalbuminuria in diabetes mellitus.
Plasma ANP concentration have been reported to be elevated in association with microalbuminuria, all of wich predict the future development of overt nephropathy in diabetes (1).
Key players in neurohormonal activation include neuropeptides and their receptors wich include the ANP (2).
Targeting neuropeptides and their signalling pathways might thus serve as new therapeutic interventions in the treatment of complication associated with diabetes.
In addition, the magnitude of the albuminuric response to ANP is greater in diabetic subjects than in healthy volunteers, suggesting increased renal sensivity to the albuminuric action of ANP (3).
Materials and methods
We performed a study of 16 diabetic patients, 10 women and 6 men, aged 37.4± 16.2 years (range 17 to 61) and an evolution of diabetes of 13.6±7.7 years. Of the total group 11 were diabetic type 1 and 5 were type 2 diabetics. In the group only 12% of patients had with any degree of renal lesion and 56% did not had any vascular complication. Microangiopathy was defined as the presence of renal, ocular or neuropathic disease- The determination of ANP levels was performed by RIA.
Results
In our group, with a low incidence of nephropathy, we found no differences in the levels of ANP between patients with and without microvascular disease (anp 20.12±18.69 pg/ml vs 9.24±5.29, $P=0.12$ (ns).
Conclusion
-The presence of renal hyperfiltration, increased albumin permeability and target organ damage may determine the elevation of ANP in diabetes, therefore in our group, with low incidence of nephropathy, did not show a clear increase in the levels of this neuropeptide.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P681

### Quality of life and its dimensions among the type2 diabetes patients referred to the diabetes center of Zanjan University of medical science (Vali-e-Asr Hospital)-2010

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Introduction
One of the most important methods for evaluation of treatment and care of diabetic patients is to assess the quality of life (QOL). The aim of this study is assess QOL and this dimensions and relational pattern between demographic characteristic.
Method
This study is cross-sectional study wit convenience random sampling. The sample consisted 161 patients with type2 diabetic who refferd to diabetes center of Zanjan. Research instruments was standard instrument of QOL related to health included: physical, psychological, social and demographic Aspect of diabetic patients. Data were analyzed with SPSS software.
Finding
The finding indicated that physical dimension (%49.1) and psychological dimension (%50.3) and social dimension (%9.9) and specific dimoinsion (%17.4) were desirable. The finding showed that independent variables (age, marriage, family income, sex, educational level) were meaningful relationship with QOL.
Conclusion
Personal, social and economic factors can affect QOL in diabetic patients. It is suqqested that managers should take care of bio-psycho-social support in such groups for improving quality of life in diabetic pationts.
Keywords: diabetes, quality of life.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P682

### Sexual weakness: Different demographic and clinical features & cost-effective management

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Introduction
The inability to achieve and maintain an erection sufficient for satisfactory sexual intercourse is a distressing and common symptom in Bangladeshi Diabetic subjects.
Aims and Objectives
The purpose of this study is to present a framework for cost effective evaluation & treatment. Because many of the diabetic subjects usually suffers from psychological stress since he is detected as diabetic and many of them believe in Myths that diabetes makes a person impotent.

Materials and Methods
Diagnosis of erectile dysfunction was based on patient self-reporting. Clinical information was collected by participating physicians. The depressive symptoms was assessed using visual analogue scale. Minimal lab investigations are suggested.
Results
255 diabetic, male were interviewed and Investigated. Age 43.88±11.38. BMI 24.5±3.3 and duration of diabetes 5.69±5.01 years. 31% smoker and 36% subjects having moderate to severe stress. Age of spouse(wife) ranges 35.32 ±10.88 years. Their age differences are <5 years 23.8%, and >10 years 21%. Among them libido was normal only in 50%. 66.7% cases have premature ejaculation. More than 50% have extramarital affairs. 70.7% subjects were HbA1c > 8% (Mean 10.54±3.32 SD). 33.3% haves. Testosterone < 3.4 ng/l. Serum PRL was higher in 6.7% cases. Associated IHD was detected (by ECG change) in 7.2% and Enlarged Prostate in 3.4% subjects.
Conclusion
Interplay of clinical and psychological factors determines the risk of erectile dysfunction in type 2 diabetics. Greater attention is needed to detect demand of the patient. All are not having Erectile problems. Cost of hormonal investigations may be lessen by clinical judgment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P683

**Role of education in prevention and delay of complications in type 2 diabetic patients**

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Aim: To assess the impact of education of people with T2DM and complications. Patients and methods. Observational study, 100 T2DM patients of age 35–75 years, both sex, duration of DM >1 year.Results. From 100 T2DM 47% were male sex and 53% female. Mean age 60.45±8y, duration of DM 10.45y±3 y. Average A1c 8%±0.6, BMI 27%, systolic BP 140±6 mmHg, TG 2.2± 0.3 mmol/L, LDL 4.0±0.6 mmol/L. Education (diet, physical activities, drug compliance) for DM was 67% for male and 54% for female. Education for diabetic foot care (inspection, foot hygiene, appropriate shoes) 51% for male and 49% for female.
Conclusion
Metabolic risk factors are poorer in no educated than in educated T2DM, People who had not satisfactory education for foot care have higher risk for foot ulceration.
Education, diabetes, complications, foot care
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P684

**Structured outpatient education and treatment program in type 1 diabetes patients during preconception care and pregnancy**

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Our aim was to evaluate the efficacy of a structured outpatient education and treatment program (SOETP) in type 1 diabetes patients (T1DM) patients during preconception care (PC) and pregnancy, and its effect on pregnancy outcomes. Study Methods: In total 173 women with T1DM were supervised. Maternal age was 28±8 yrs and diabetes duration - 12±7yrs. SOETP combined group and individual training. Stage 1 - preconception care (group and individual training). Stage 2 - individual education once per week throughout the pregnancy; group discussion once per month. SOETP included: diabetes physiology in pregnancy, managing hypoglycemia, diet, insulin therapy, glucose self-monitoring, relaxation, exercise, non-stress tests and diabetes complications. Specific Knowledge Assessment Questionnaire (65 questions) was completed by all patients pre- and post-program.
Gr. 1 - 88 women went through Stage 1 and 2; Gr. 2 - 85 women - only through Stage 2. Results. At conception women from Gr. 1 were well-controlled (HbA1c - 6.0 (0.31)%, postprandial glycemia - 142.2 (10.6)mg/dl), though HbA1c and PPG levels in women from Gr. 2 were statistically higher (8.4 (0.51)%, P=0.000; 184.8 (13.3) mg/dl, $P=0.000$ respectively). By the end of the 1st trimester those indices dropped. All patients were tested to evaluate knowledge. At entry number of correct answers was very low (28%); post-program scores were significantly higher (96%). Spontaneus abortions were revealed in 2.2% (Gr. 1) vs 5.8% (Gr. 2); preeclampsia- in 6.8% (Gr. 1) vs 14.1% (Gr. 2); perinatal mortality - in 1.1%(Gr. 1) vs 3.5% (Gr. 2).
Conclusion
SOETP may be effectively used for T1DM patient education during PC and pregnancy; it significantly reduced rates of spontaneous abortion rates, mortality and morbidity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P685

**A Randomized controlled trial of the effect of internet based mentoring program for type 1 diabetes patients with inadequate glycemic control**

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Introduction
Widespread availability of internet makes it an attractive communication tool among patients and providers. However, the clinical benefit of telemedical support on diabetes care remains inconclusive. This study is to determine whether an internet based mentoring program can improve glycemic control in type 1 diabetes (T1DM) patients with inadequate glycemic control.
Methods
Patients with T1DM on intensive insulin therapy and with HbA1c ≧ 8.0% were randomized to mentoring (glucometer transmission with feedback from mentors) or control (glucometer transmission without feedback) for 12 weeks. Five mentors (3 male and 2 female) were interviewed and selected from an internet community for Korean T1DM people (i.e. JahkEunSon(small hands)'s Type 1 Diabetes Cafe; http://cafe.naver.com/dmtype1.cafe). Two of them were T1DM patient themselves and three of them were parents of at least 1 child diagnosed with T1DM for at least 5 years. They were all university graduates except for one with 2 years of college education.
Results
A total of 57 T1DM patients with mean diabetes duration 7.4 years were recruited from Samsung Medical Center in Seoul, Republic of Korea. In total, the mentoring group and control groups login to the website 764 and 203 times, respectively. There was a reduction in fasting plasma glucose from 231.03± 79.39 mg/dL to 191.63±81.38 mg/dL in the mentoring group after 12 weeks. In addition, treatment satisfaction score (from 28.94±7.26 to 33.21±4.89) and number of self blood glucose monitoring (from 3.96±1.91 to 5.02±2.36) improved in the mentoring group. There was no significant reduction in fructosamine and HbA1c in the mentoring after completion of the study. However, these differences in changes in metabolic parameters and questionnaire scores between groups were not statistically significant.
Conclusions
Internet based mentoring program for T1DM patients with inadequate glycemic control has not proved to be superior to the usual follow-up.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P686

**Type 1 diabetic women treated with insulin pumps or multiple daily injections of insulin: pregnancy outcome and glycemic control**

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Introduction
Pregestational diabetes is a threatening condition for pregnancy; a strict glycemic control can decrease some pregnancy complications. That control can be achieved while on multiple daily insulin injections (MDI) treatment or with subcutaneous insulin infusion systems (CSII). Up to now no benefits of CSII treatment over MDI on pregnancy outcomes have been proved.
Design
Case control retrospective study, comparing glycemic control and pregnancy outcomes between type 1 diabetic pregnancies CSII or MDI treated (1996–2010). Selected variables were compared with X2 or Student-T or Mann-Withney tests. A $P<0.05$ was considered significant.
Results
Women treated with MDI were older; to get comparable groups, we excluded some women (disease for <7 years), then obtaining 206 pregnancies (138 women): 50 pregnancies treated with CSII and 156 with MDI. Age, duration of diabetes, retinopathy and nephropathy were not significantly different. Insulin pumps were mostly installed while on pregnancy planning; thus, planned pregnancies were much more common in the CSII group (84% vs 40.4%, p <0.000). Preconception HbA1c was significantly lower for CSII group (6.78±0.68 vs 7.32±1.36, p 0.015); for the 1st and 2nd terms no differencies were found, and for the 3rd term HbA1c was even better for MDI group (6.50±0.64 vs 6.16±0.74, p 0.015). Severe hypoglycemic events and ketoacidosis, miscarriages, gestational hypertension, preterm birth, cesarean sections, congenital anomalies, big babies, respiratory distress, hypoglycemia or jaundice did not show significant differences between groups, either.
Conclusions
As other authors, we were not able to show that CSII treatment improved metabolic control or pregnancy outcomes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P687

**DPP-4 inhibitor vildagliptin reduces urinary albumin excretion in type 2 diabetic patients with microalbuminuria**

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Introduction
A potent and selective DPP-4 inhibitor improves islet function by enhancing α- and β-cell responsiveness to glucose in the diabetic patients. Renal impairment is a common complication of type 2 diabetes mellitus (T2DM). Vildagliptin is used to treat T2DM as monotherapy or in combination with other antidiabetic drugs, since that it efficiently decreases glycated hemoglobin (HbA1c) values. Additionally, vildagliptin has been shown to be safe in patients with moderately impaired kidney function.
Designs
We studied 107 T2DM patients with diabetic nephropathy, the mean ages are 62.2± 10.4 y; early nephropathy stage with microalbuminuria ($n=37$), apparent nephropathy stage with daily urinary albumin excretion more than 300 mg/g of albumin-creatinine ratio or eGFR less than 60 ml/min ($n=50$), renal failure stage with increased serum creatinine concentration ($n=16$) or dialysis stage ($n=7$). All patients were treated with vildagliptine at least 3 months before entry of this study. We monitored body weight, urine albumin excretion or eGFR of the patients during 6 months.
Results
Vildagliptin-treatment had no influence on the body weight or eGFR in all stages of diabetic nephropathy. In the patients in early nephropathy stage, vildagliptin therapy significantly decreased the concentrations of albumin. Patients in other stages exhibited no differences in concentration of urinary albumin between before and after administration of vildagliptin. There were no severe side-effects of vildagliptin in these patients. Four patients in renal failure stage and 5 dialysis patients were able to convert to monotherapy with vildagliptin from insulin therapy.
Conclusion
Vildagliptin 50 mg qd is well tolerated and efficacious in T2DM patients with severe renal impairment. Otherwise vildagliptin therapy is effective to reduce microalbuminuria in the patients with early diabetic nephropathy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P688

**Insulin protocol for hospital management of diabetes: a retrospective evaluation one year after the implementation**

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Introduction
Protocols for identifing and managing hyperglycemia and diabetes in hospital are necessary. Glycemic goals in non critical inpatients are:140 mg/ml(preprandial) and 180(posprandial),oral antidiabetic drug(OADs) withdrawn, avoid hypoglycemias, basal/bolus insulin (nor sliding scale) and request glycosylated haemoglobin.
Aims
To evaluate the implementation of a protocol for management of diabetes(PHD) during the years 2009 and 2010 in our Hospital.
Methods
In January2009 we presented a PHD for non critical patients. Educational meetings were given the first 6 months of the year2009. A nurse supervised the protocol fulfillment. We evaluated HbA1c requested, used treatment and variables of results (glucemias for 3–6 correlative days) in a sample of 1367 patients.
Results
PHD was implanted in 70. 5% of the patients evaluated in 2009: 17.7% had sliding scale,5.4% other insulin treatments and 6.4%OADs. In 2010 the proportions were 61.8%, 19.2%, 11.3% and 7.7% respectively. The average glycemias in the first and last day of registered treatment were 182.0±69.1 mg/dL and 162.4±67.8 mg/dL in the group PHD, 164.5±51.4 mg/dL and 161.5±55.2 in the group Insulin in scale, 185.1±64.8 and 169.6±81.6 mg/dl in the group of other insulin treatments, 133.8±35.2 mg/dL and 133.2±38.6 mg/dl in the group OADs, being the difference between glucemias −19.6±64.6 mg/dl, −3.0±48.1, −15.5±85.6 and -0.6±42.7 mg/dL respectively($P<0.01$). HbA1c was determined in 37% of patients in 2009 and 47.6% in 2010.
Conclusions
Protocol implementation was better in the first year of evaluation. Less than 50% of diabetic patients had HbA1c during the hospitalization. PHD patients had better control than the others. The results show that it is not enough to design a PHD, it is necessary to supervise and evaluate it constantly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P689

**Analysis of fasting glycemia variability in patients with type 1 diabetes mellitus undergoing different types of basal insulin therapy: glargine vs. NPH**

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The goal of this research was to analyze fasting glycemic variability in patients with DM1 depending on the type of basal insulin therapy - insulin NPH versus Glargine.
Material and methods
The glucose levels 24-hour monitoring was performed applying the CGMS. Glargine group - 19 and NPH group - 24 patients.
Results
In the glargine group there have been no episodes of severe fasting hypoglycemia (<2.8 mmol/L), LBGI=2.42±1.26; in the NPH group the relative duration of period of severe nocturnal hypoglycemia was 15.41±23.12%, LBGI=9.18± 8.21. Intra-day variability indices: SD - 1.98±1.01 and 2.71±1.39 mmol\l; VC −24.32±15.49 and 31.51±16.21%; IQR −3.21±1.74 and 5.16±2.75 mmol\l;

amplitude of glycemic excursions -7.31±3.42 and 11.24±5.58 mmol\l, CONGA1 - 1.60±0.72 and 2.71±1.62 mmol/l×h-1, $P<0.05$; for the glargine and NPH groups respectively. The predictors of high fasting glucose variability are frequent short-time (<1h) severe hypoglycemic episodes.
Both groups revealed a strong positive correlation between MODDf (analogous to MODD, but calculated for fasting period only) and difference of mean fasting blood glucose values: in the glargine group - rs=0.78; in the NPH - rs=0.895; $P<0.001$. Glargine group - MODDf=2.30±1.12; NPH group - MODDf=5.01±2.62, $P<0.001$.
In the NPH group only, high inter-day variability of blood glucose levels at 3 AM is a reliable predictor of high values of the difference of mean fasting blood glucose values (B=0.662; $P<0.001$). According to the equation of the linear regression with an increase of the difference of blood glucose values at 3 AM on 1 mmol/L, the difference of mean fasting blood glucose values increases by 0.662 mmol/L. In the Glargine group day-to-day blood glucose values at the control points - 3, 5 and 7 AM - were much more stable.
Conclusion
The use of glargine leads to a significant reduction in daily variability of fasting glycemia, minimizes the risk of hypoglycemia and provides a greater reserve in dosage increase in comparison with NPH insulin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P690

**To evaluate and compare the outcome of pregnancies in women with type 1 diabetes treated with continuous subcutaneous insulin pump or multiple insulin injections**

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[2]Joshi Clinic, Mumbai, India.

The study was aimed to compare the outcome of pregnancies in women with type 1 diabetes treated with continuous subcutaneous insulin infusion pump (CSII) or multiple insulin injections (MDI). Total 14 patients were treated with insulin pump and 20 patients were treated with multiple injections; were mainly investigated for HbA1c, incidence of hypoglycemia, fetal outcome, rates of pregnancy induced hypertension and cesarean section. HbA1c with insulin pump was significantly better from that obtained with multiple injections. Hypoglycemic events were significantly less in CSII group as compared to MDI group. Moreover, severe hypoglycemia was not seen in CSII group, whereas there were numerous episodes of severe hypoglycemia in MDI group, few of them required hospitalization. Rate of early or mid pregnancy abortion was 10% treated with multiple insulin injections, while no abortion was seen in CSII group. Fetal prognosis was also better in pump treated patients, macrosomia was seen in 4 new borns in pump group whereas 12 in MDI group. The occurrence of pregnancy induced hypertension was similar in both groups. The rate of cesarean section was not influenced by therapeutic device, similar in both groups.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P691

**Antihyperglycemic effects of sodium orthovanadate and Trigonella foenum graecum in diabetic rats**

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Objectives
Oral administration of vanadate to diabetic animals have been shown to stabilize the glucose homeostasis and restore altered metabolic pathways. The present study explored the prospect of using low doses of vanadate with Trigonella foenum graecum, seed powder (TSP), another antidiabetic agent, and to evaluate their antidiabetic effect in diabetic rats. The effect of these antidiabetic compounds was examined on general physiological parameters, monoamine oxidase activity, calcium homeostasis, membrane fluidity and distribution of lipofuscin accumulation in liver, brain and heart tissues. Expression of glucose transporter (GLUT4) protein was also examined by immunoblotting method in experimental rat heart after three weeks of diabetes induction.
Methods
Diabetes was induced by administration of alloxan monohydrate (15 mg/100g b.wt.) and rats were treated with 2IU insulin, 0.6 mg/ml SOV, 5%Trigonella in the diet and a combination of 0.2 mg/ml SOV with 5% Trigonella separately for 21 days.
Results
Diabetic rats showed high blood glucose levels. Activity of monoamine oxidase increased in diabetic brain, liver and heart. Diabetic rats exhibited an increased level of calcium with lipofuscin accumulations and decreased membrane fluidity. GLUT4 distribution was also significantly lowered in heart of alloxan diabetic rats. Treatment of diabetic rats with insulin, TSP, vanadate and a combined therapy of lower dose of vanadate with TSP revived normoglycemia and restored the altered level of monoamine oxidase, calcium homeostasis, lipofuscin and membrane fluidity and also induced the redistribution of GLUT4 transporter. TSP treatment alone is partially effective in restoring the above diabetes-induced alterations.
Conclusion
Combined therapy of vanadate and TSP was the most effective in normalization of altered metabolic parameters and GLUT4 distribution without any harmful side effect.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P692

**Therapy with analogous of glp 1 and basal insulin in patients with diabetes mellitus type 2. Our experience.**

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Objectives
Evaluate the impact of the therapy with GLP-1 analogues (exenatide) toguether with basal insulin in glycemic control and weight of patients with type 2 diabetes mellitus poorly controlled.
Materials and methods
We analyzed 37 patients with type 2 diabetes with poor glycemic control and obesity, treatment with exenatide and basal insulin. As pre-treatment 56.7% of our patients were with basal insulin, 10.8% with insulin mixtures and 32.4% with basal-bolus regimen. The patients with basal insulin exenatide were added exenatide. The patients with insulin mixtures were passed to basal insuline more exenatide. Finally to the patients with basal bolus insulin we replaced rapid insulin by exenatide, always maintaining the metformin unless contraidicated. At the start we reinforce the diabetes education and we analyze the variables: HbA1c, weight and the insulin requeriment in 3 months. We did statistical analysis with SPSSv18 throught t-student for paired data.
Results
Our series had a mean age of 51.8±11.5 years, 26 men (70.3%) and 11 women (29.7%).They had about 8.0±7.5 years of disease progression and poor metabolic control expressed as HbA1c of 8.27±1.71% and BMI of 40.3±6.0 kg/m2. In our series 22 patients (59.5%) were on glargine and 15 patients (40.5%) with detemir. We observed a decrease clinically relevant and statistically significant in the average HbA1c at 3 months of 8.27% to 6.95% (1.33%) ($P<0.001$). At the same time there is a average weight loss of 5.3 kg at 3 months ($P<0.001$). We also analyzed the insulin needs to appreciate an average reduction in dose of 0.38 IU/kg to 0.31 IU / kg (−0.07 IU / kg) at 3 months ($P<0.05$). As Adverse effects of interest were reported gastrointestinal disturbances (nausea, vomiting) in 6 patients causing 2 retired. We did not observed episodes of hypoglycemia.
Conclusions
The association of GLP-1 analogues with basal insulin in patients with type 2 diabetes, who need insulin therapy for their poor control, is an interesting therapeutic option and to consider for the improvement in glycemic control and weight loss. This also contributes to the decrease of the insulin requirements in their treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P693

**Variations in insulin daily dose and weight with continuous subcutaneous insulin infusion therapy**

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Introduction
Intensive diabetes type 1 management can be achieved either with multiple daily insulin injection therapy or with continuous subcutaneous insulin infusion (CSII). The former is becoming increasingly popular do to its positive effects on glycemic control.
Objectives
Evaluate the evolution of total daily insulin dose (TDID) and weight on patients with CSII.
Methods
Patients with CSII of our department were included. Data regarding glycemic control, anthropometric parameters and TDID was collected before beginning of CSII, and 6 and 12 months after. Paired sample T-test was used to compare means and Pearson correlation coefficients were calculated, a value of $P<0.05$ was considered significant.
Results
We studied 64 patients (24 men; 39 women) with a mean HbA1c before CSII initiation of 8.2%±1.4, mean diabetes duration of 24.1±12.7 years and mean age in the moment CSII initiation of 33.6±11.2 years. We found a significant reduction in weight 12 months after the beginning of CSII (69.9 kg ±10.3 vs 68.3 kg ±11.1; $P<0.001$) and in HbA1c (8.2%±1.4 vs 7.4%±1.1; $P=0.002$). A reduction of TDID was also found but without statistical significance (52.6±14.9 vs 49.0±11.3; $P=0.058$). There was a moderate correlation between the variation of TDID with the duration of diabetes ($r=0.56$; $P=0.007$). No correlation was found between diabetes duration and weight variation or between weight and DTID.
Conclusions
Continuous subcutaneous insulin infusion therapy seems to be associated not only with the improvement of glycemic control, but also with reduction in body weight and in TDID. Diabetes duration seems to play a role in the individual response to CSII.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P694

**Insulin therapy requirement in ESRD patients (Our experience)**

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Diabetes (DM) is the leading cause of end-stage-renal disease (ESRD). Majority of T2DM patients (Pts) with ESRD need insulin. Kidneys play significant role in exogenous insulin metabolism. Chronic renal disease is associated with alterations in insulin metabolism and glycemic control. As renal function starts to decline, insulin requirement needs to be adjusted to reach euglycemia.
A good metabolic control is not only important in the early phase of diabetic nephropathy but also in diabetic Pts with ESRD. Glycemic control can reduce the progression of atherosclerosis and improve the survival in Pts treated with hemodialysis (HD).
Zahra nephrology center is located close to Tripoli (40 Km) with 180 Pts on HD (~8% of total HD Pts).
Aim
Evaluate the effect of HD on pre to post dialysis in insulin requirements to achieve euglycemia.
Method
11 Pts (6 male) were included. Average age 59.7 years, mean duration of DM 20 years, 75% were on insulin (pre mixed) prior to HD. Mean duration of HD was 4.5 years, 3 sessions a week.
Pts were admitted to our hospital, insulin physiological regimen (basal & bolus) was applied to maintain euglycemia (BS=100–140 mg/dl). Insulin is adjusted hourly pre,during and post-HD, and investigation compared day-to-day insulin requirements.
Result
Early results should that ESRD due to diabetic nephropathy is counted for 15% in our center. Since HD was started, insulin requirements were reduced in 91%. 3 Pts are off treatment since HD (2 pts were in oral therapy). Insulin was reduced by 75 to 50% in 5 Pt and by 40 to 25% in the rest of the Pts. Only one Pt kept in the same dose.
All Pts are off insulin on HD day, and all of them were experienced hypoglycemic attacks.
Rest of results well be published by the meeting time when are completed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P695

**Comprehensive approach to Paediatric diabetes management: NGO initiatives in developing country India.**

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Issues
In developing nations diagnosis of diabetes brings mental-trauma/depression in family. Focused treatment for pediatric age-group is unavailable in developing-countries. 26% of diagnosed diabetics are children's. Adequately trained physicians/Nurses in issues of pediatric-diabetes provide continuity of care, relief from depression and smooth transition from diagnosis to treatment. Qualitative collaborative relationship between these makes diabetics life bearable. Our NGO-project highlights significance of relationship between nurses and diabetic-children in community clinic setup of rural India.
For Diabetes, its assumed that depression is inevitable sequel to diagnosis. Retrospective analysis of past studies shows—counselling improves QOL & attitude towards diabetes-treatment.
Aims
To describe care issues in diabetic-children's. Observe/modify nature of relationship between nurse and child. To evolve comprehensive treatment plan for patients and families.
Methods
A retrospective analysis of data base from 7 rural health-clinics. Specialized therapy/support to pediatric-age-group not available at any centre. Total 117 children's [4–13 years] diagnosed with diabetes. 23 had additional endocrine/metabolic problems. Nursing/medical care plan analyzed. No specialized trained personal in rural/tribal India. Opinion/needs from patients families collected on feedback questionnaire. Then we trained 10 nurses & 2 physicians for handling pediatric cases [4 weeks training].
Results
Out of 117, 41 discontinued Rx due to improper counseling/guidance. 3 died. Patient/family's feedback highlights: Better access to newer drugs-delivery-systems, psychosocial support, follow-up-plan. Nurses/physician be sensitized in pediatric care-issues. Main issues of concern were: [1] illness and coping with their feelings. [2] Initial impact of diagnosis and a search for solution? Expectations for future life & its quality? [3] Concerns of cost of RX [4] Availability of proper follow-up centers in rural areas of developing nations.
Conclusion
Multifaceted Relationship between physician/nurse and Diabetics childrens is crucial. This relationship provides better continuity of treatment. We show concerns/difficulties while working in Asian set-up to experts/seniors at ECE-2012-congress.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P696

**Renal sodium dependent glucose co-transporter 2 (SGLT2): A neoteric target for treatment of type2 diabetes mellitus**

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Contemporary therapies to rationalize the hyperglycaemia in type 2 diabetes mellitus (T2DM) generally involve insulin-dependent mechanisms and lose their effectiveness as pancreatic b-cell function decreases to a greater extent. Kidney emerges out as a novel and potential target to trim down the T2DM. The filtered glucose is reabsorbed principally through the sodium glucose co-transporter-2 (SGLT2), a low affinity transport system, which are present at the luminal surface

cells that covers the first segment of proximal tubules. Competitive inhibition of SGLT2 therefore represents an innovative therapeutic strategy for the treatment of hyperglycaemia and/or obesity in patients with type 1 or type 2 diabetes by enhancing glucose and energy loss through the urine. Selective inhibitors of SGLT2 reduce glucose reabsorption, causing excess glucose to be eliminated in the urine; this decreases plasma glucose. SGLT2 inhibitors are coupled with osmotic dieresis and loss of weight which aid in reducing the blood pressure. The observation that individuals with familial renal glycosuria maintain normal long-term kidney function provides some encouragement that this mode of action will not adversely affect renal function. This novel mechanism of targeting kidney for the treatment of T2DM is reasonably valuable and is independent of insulin and clutch with the low risk of hypoglycemia.

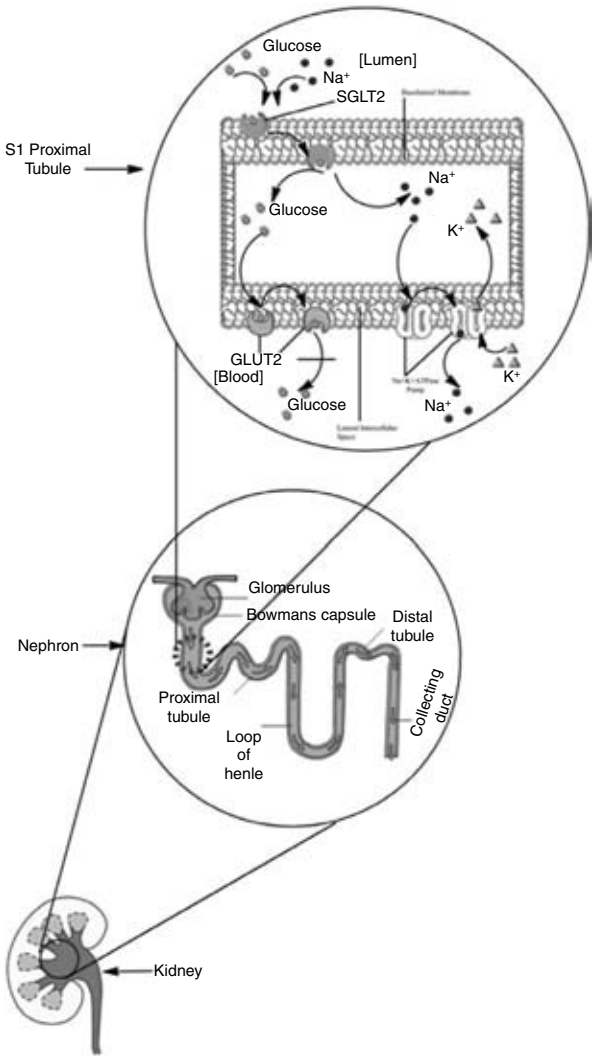


**Fig.** Reabsorption of glucose from the renal proximal tubules by the sodium glucose cotransporters SGLT2. Almost all of the glucose entering glomeruli in the afferent glomerular arterioles is filtered into the nephron fluid of the promimal renal tubules. Most (upto 90%) of this filtered glucose is reabsorbed in the initial proximal convoluted segment (SI) by SGLT2 located at the luminal surface of proximal tubular cells. Remaining glucose is reabsorbed from the filtrate in themore distal convoluted and straight segments by SGLTI. Glucose within the proximal tubular cells is then transported back to the interstitial compartment and thence to the plasma by the facilitative glucose transporters GLUT2 in the SI segment, respectively. In normal individuals with an average plasma glucose concentration of 5–5.5 mmol/L (90–100mg/dL), approximately 160–180 g of glucose is filtered daily, with less than 0.5 g/day of glucose appearing in the urine. Based on Bakris et al. and Bays

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P697

**Management of Diabetes in Hospital**

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Introduction
People with type 1 or type 2 diabetes mellitus are frequently admitted to a hospital, usually for treatment of conditions other than the diabetes. Whether in hospital or not, glycemic control is likely to become unstable in these patients because of the stress of the illness or procedure, the concomitant changes in dietary intake and physical activity, and the frequent interruption of the patient's usual antihyperglycemic regimen. Once in the hospital, the length of stay and cost are greater for people with diabetes than for those without it. Efficient treatment of diabetes in hospital may be an important factor in limiting the costs of care.
Aim
To assess diabetes management in general wards of hospital.
Methods
This study was a retrospective cross sectional descriptive one which accessed diabetes management criteria in 120 hospitalized people with diabetes in a private hospital in Tehran during 6 months in 2011. The data gathered from patients' recorders including Hb A1C, FBS, BS, TG, Chol, and Cr.
Results
The finding showed 60% of the samples were women and 40% were men. The mean duration of hospitalization was 6 days. In addition the mean amounts of different diabetes management criteria were HBA1C: 8/14%, FBS: 165, BS: 181, TG: 145, and CHOL: 170.
Conclusion
However the results special mean of FBS and BS were reasonable for hospitalized people with diabetes; better outcomes for people with diabetes following admission to hospital can be improved in the hospital as well.
Keywords: Diabetes Management, Hospital, People with diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P698

**Relation between glucose daily profile (CGMS) and graft function after islet transplantation for type 1 diabetes mellitus**

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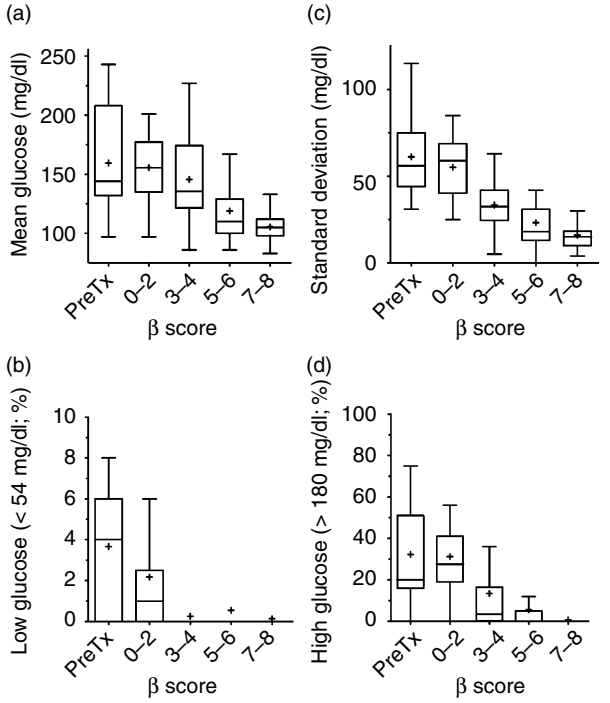
Context
The influence of beta cell replacement on daily glucose profile in Type 1 diabetes mellitus (T1DM) is not firmly established.
Objective
To examine the influence of islet transplantation (IT) on the various component of dysglycemia in T1DM patients.
Design, setting and patients
Single arm open labeled study. Twenty-three consecutive patients with T1DM, 11 males and 12 females, 14 non uremic and 9 uremic with a previous kidney graft, receiving IT at a referral center from 2003 to 2007. All participants completed a 3-year follow-up.
Intervention
IT consisting of 2 or 3 intra-portal allogenic islet infusions over 3 months with the Edmonton immuno-suppression protocol.
Outcomes
Glucose daily profile was assessed during 72 hours by continuous glucose monitoring system (CGMS) prior to transplantation, at 6 months and yearly during 3 years after transplantation. Outcomes were mean glucose (MG), glucose standard deviation (GSD), the percentage of time spent in hyperglycemia

>180 mg/dL (HYPER), and the percentage of time spent in hypoglycemia < 54 mg/dL (HYPO). Graft function was estimated with beta score, a previously validated index (range 0–8) based on insulin or oral treatment requirements, plasma C-peptide, blood glucose, and A1C. Analysis was per intention to treat.
Results
At 3 years, 19 patients had a functional graft and 10 remained insulin independent with A1c ≤6.5%. Median A1c in the whole cohort was 6.7% (interquartile range[IQR], 5.9%-7.7%) vs. 8.3% (IQR, 7.3%-9.0%) at baseline ($P<0.01$). The four CGMS outcomes were significantly improved vs. baseline ($P<0.01$), in a close relation with graft function ($P<0.001$). Partial function (beta score 3) was sufficient to abrogate HYPO but optimal function (beta score 8) was necessary to normalize MG, SD, and HYPER.
Conclusion
Glucose daily profile significantly improved during the 3-year period post-IT and reached normal values when optimal graft function was achieved.




Relation between graft function and the various outcomes of continuous glucose measurement during follow-up (a, mean glucose; b, glucose standard deviation; c, percentage of time with glucose >180 mg/dL; d, percentage of time with glucose <54 mg/dL). Tukey box plots indicates median interquartile range and upper and lower range. ($P<0.0001$; test for linear trend).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P699

**Evolution of macro-angiopathy 5-years after islet transplantation (IT) in type 1 diabetes**

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Long-term benefit-risk ratio of IT remains poorly evaluated. The aim of this work was to determine the evolution of macroangiopathic complications 5 years after IT.
21 out of the 36 prospectively followed islet-transplanted patients in a single center, had at least 5 years of follow-up and were included. Their initial features were: duration of C-peptide negative diabetes: 28±9years; age: 43±7years; BMI: 24±0.2 kg/m2; 8/21 kidney-transplanted patients for 23±10 months; immunosuppression: Edmonton. These 21 patients had a systematic screening for macroangiopathy before transplantation, and then yearly for 5 years. Three «islet-alone » and 2 « islet-after-kidney» patients had lost their islet function at 5 years. Nine patients were insulin-independent, 8/9 with HbA1c ≤ 6.5% (38% with both criteria). The mean HbA1c level was 8.1±1.0% before vs. 7.1±1.0% 5 years after IT ($P<0.01$). Analyses were performed in intention-to-treat. There was no loss of follow-up.
The mean level of cholesterol total (1.86±0.37vs.1.76±0.36g/l), HDL (0.71 ±0.18vs. 0.73±0.27g/l), LDL (0.98±0.32vs.0.88±0.30g/l), triglycerides (0.79±0.33vs.0.83±0.34g/l) and mean blood pressure (124±17/72±7 vs. 127±13/72±8 mmHg) did not differ before vs. after transplantation (patients treated with statins: 28% vs. 65% and with ≧ 1 antihypertensive drug: 38% vs. 75%). Three patients among whom 2 « islet-after-kidney» had macroangiopathic complications before transplantation (2 femoral by-passes among which 1 with amputation, 1 coronary stent). There were no clinical acute cardiovascular events during these 5 years., Macroangiopathic screening was abnormal before vs. after transplantation for: carotid ultrasound: 37%vs.52%; lower limb ultrasound: 36%vs.85% (22 vs 25% if mediacalcosis was excluded), non-invasive test for cardiac ischemia: 19% (with 3 normal coronarographies except for 1 calcification) vs. 24% (among which 4 stented). Conclusion: IT was not associated with acute clinical cardiovascular event in these 21 patients with long-lasting diabetes, among whom 8 kidney-transplanted at the expense of systematic screening and treatment of macroangiopathic complications.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P700

**Sustained Exendin-4 secretion through gene therapy targeting salivary glands in Zucker fa/fa rats and high fat diet fed mice.**

I. Dicembrini[1], G. Di Pasquale[4], L. Raimondi[2], C. Pagano[3], J. Egan[4], A. Cozzi[2], L. Cinci[2], E. Manni[2], A. Loreto[2,3], S. Berretti[2], A. Morelli[2], B. Baum[4], M. Maggi[2], R. Vettor[3], J. Chiorini[4], C. Rotella[1] & E. Mannucci[1]
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Background
Exendin-4 (Ex-4) is a GLP-1 receptor agonist approved for the treatment of Type 2 Diabetes (T2DM). Aim of this study was to characterize the effects of Ex-4 expressed continuously from salivary glands (SG), following adeno-associated virus-mediated (AAV) gene therapy in two different model of obesity and T2DM. Several trials support an AAV good safety and little toxicity profile. Serotype 5 (AAV5) presented enhanced gene transfer activity in SG and an efficient protein secretion into the bloodstream. Materials and methods: a recombinant AAV vector was produced using a four-plasmid procedure. Following 5x1012 DRP/ml vector (encoding Ex-4 or empty) administration, efficacy and metabolic effects in high fat diet (HFD) fed mice ($n=20$) and Zucker fa/fa rats ($n=10$) were evaluated.
Results
Ex-4 levels averaged 138.9±42.3 pmol/L at day 42 in treated mice and 238.2± 72 pmol/L at day 30 increasing to 3 nmol/L at day 60 in treated rats. Transduction specificity was confirmed through a qPCR amplification using specific primer in SG, liver, spleen and pancreas. Treated animals reached a significantly lower weight gain in comparison to controls at day 42 in mice (16.5±2.7 vs 19.5±1.9 g; $P<0.05$) and by day 35 in rats (156.8±17.5 vs 186.2±18.6 g, $P<0.05$) throught the study. Transient reduction on food intake was reported. Furthermore, treated mice presented: significant lower leptin circulating levels (2.24±0.39 vs 5.89±1.07 ng/ml; $P<0.01$) and visceral adipose mRNA expression (3.43±0.48 vs 8.28±0.72 Arbitrary Unit; $P<0.01$) and at day 41, a greater insulin-induced reduction in glycaemia during an intraperitoneal insulin tolerance test. A significant reduction in HbA1c (4.7±0.1 vs 4.9±0.1%; $P<0.05$) and glycosuria (4 cases vs 0) was reported in treated rats.
Conclusion
This study suggests an alternative approach delivering Ex-4 in the treatment of T2DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector

## P701

**A Hydrophilic Derivative of Probucol, Probucol-(glutaric branched-triglycerol)$_2$ (ProBGL$_2$) Ameliorates Glucose Tolerance and Insulin Sensitivity by Independent Mechanism of the Canonical Potency of Probucol in HFD-fed mice.**

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Probucol is a traditional anti-hyperlipidemic agent harboring marked antioxidant activity. While probucol is highly hydrophobic, its succinic acid ester, succinobucol, was reported as a water-soluble derivative and was in clinical trials. Succinobucol was shown to be more potent in lipid-lowering, anti-inflammatory and anti-atherogenic effects than probucol in animal models. In addition, succinobucol reduced HbA1c in diabetic patients. Although the clinical trials finally ended in failure, it appears a promising strategy for drug discovery to modulate water-solubility of established active compounds. Meanwhile, we have reported chemical modification of lipophilic agents with branched origo-glycerol as a suitable approach to intensify hydrophilicity. Thus we synthesized highly hydrophilic derivative of probucol, ProBGL$_2$ and evaluated its effects on glucose and lipid metabolism in HFD-fed mice. 1 g/kg/day of probucol or ProBGL$_2$ were orally administered for a week to male ddY mice fed HFD (24 wk), and then ipGTT was performed. Plasma insulin and lipid parameters were also determined. While body weight and food intake were not affected, glucose tolerance was much improved in probucol and ProBGL$_2$ groups (AUC; -50%). Fasting plasma insulin levels and HOMA-IR in the both groups were 70% decreased, suggesting increase in insulin sensitivity. However, ProBGL$_2$ did not change plasma total cholesterol concentration though probucol lowered it more than 50%. Furthermore, TBARS assay revealed ProBGL$_2$ did not possess antioxidant activities in contrast to probucol. Plasma TG and NEFA levels were not altered. Our data demonstrate that probucol and its hydrophilic derivative, ProBGL$_2$ ameliorate glucose intolerance and insulin resistance in HFD-fed mice and those are independent of the canonical potency of probucol such as lipid-lowering or antioxidant activities. ProBGL$_2$ should be a novel potent anti-diabetic agent.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This work was supported, however funding details unavailable.

## P702

**Simultaneous Pancreatic Kidney Transplant Experience in The South West of Ireland between 2001–2011**

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Introduction

Simultaneous pancreatic kidney transplantation (SPK) has become the therapy of choice in patients with type 1 diabetes mellitus (DM) and end stage diabetic nephropathy.

Methods

An analysis was performed of type 1 diabetic patients with end stage diabetic nephropathy who received treatment with SPK between 2001–2011 in the South West of Ireland, population 663,176 with a prevalence of type 1 DM in this region estimated to be 0.3%. Data was obtained from the South West of Ireland nephrology database.

Results

Between 2001 and 2011, 35 type 1 diabetics with end stage diabetic nephropathy were treated in the South West of Ireland. 14 have undergone SPK and 8 have undergone kidney transplant alone. 3 patients are currently awaiting SPK and 3 awaiting kidney transplant alone. Seven out of 35 died while receiving dialysis. The mean age of the SPK group was 41 years $\pm$9.80. The mean duration of disease at time of SPK was 29.58 years $\pm$9.48. The average waiting time from being placed on the transplant pool to transplantation was 19 months $\pm$12.38. No mortality was reported post SPK. There was loss of pancreatic allograft in 4 patients: 2 in the immediate post-operative period ($<$ 10 days post op), 1 at 9 years post-transplant and 1 at ten years post-transplant. The mean pre-operative HbA1C was 9.6% and 12 months post-operatively was 6.2% in the 12 functioning pancreatic allografts. Twelve month mean post-operative GFR in the 14 patients was 55mL/min/1.73m2.

Conclusion

SPK is the treatment of choice in type 1 diabetics with end stage diabetic nephropathy. SPK is performed in a single centre in Ireland, with 12 month pancreas allograft survival rates in our cohort of 79% which is comparable to international centres of excellence.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P703

**Implementation of the principles of the personalized medicine in the clinical practice: a pilot study for type 2 diabetes**

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Background

Many patients with type 2 diabetes (T2D) are not reaching glycemic targets even by using different combinations of antidiabetic drugs, including insulin. On the other hand, intensified multidrug therapy is associated with increased risk of severe side effects and individualized selection of medication is essential. The aim of our study is to evaluate efficiency of personalized pharmacotherapy according to the genotype and phenotype of T2D patients.

Methods/design

95 treatment naive T2D patients were involved in prospective clinical trial. Genotyping by GoldenGate Genotyping Assay with VeraCode technology (Illumina, Inc.) of 192 SNP in previously reported genes influencing T2D treatment efficiency and tolerability (OCT1,2,3, MATE1,2, PMAT and others) was performed in all patients.

Results

First results of treatment efficiency and tolerability are currently available in 61 patients. Decrease of the mean HbA1c from 7.7$\pm$2.0% to 6.7$\pm$0.8% and the mean BMI from 34.6$\pm$5.9 kg/m$^2$ to 34.1$\pm$5.6 kg/m$^2$ was measured. SNP located in region between genes SLC22A2 – SLC22A3 was associated with HbA1c decrease after 3 month metformin therapy and the commonly carried A allele was more often detected in patients who responded to metformin therapy. Unadjusted association test ($P$=9.29e$-$005, OR (CI 0.95)=4.971[2.163–11.43]) identified association between genetic polymorphisms and response to metformin therapy. Association maintained statistical significance after Bonferroni correction ($P$=0.01793).

Conclusions

Genotyping of large number of known polymorphisms in genes responsible for efficiency of T2D therapy may help to individualize treatment schemes. Genetic polymorphisms in SLC22A2 – SLC22A3 gene region are associated with efficiency of short-term metformin treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This work was supported, however funding details unavailable.

## P704

**Pathway Integration of Pro-Inflammatory Cytokines, 12-Lipoxygenase and NADPH Oxidase in Beta Cell Dysfunction; Protection by Novel Selective 12-Lipoxygenase Inhibitors.**

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In this study we explore and integrate a pathway for loss of functional beta cell mass in diabetes. Inflammatory cytokines induce human islet dysfunction, elevate expression of 12-lipoxygenase, increase cellular reactive species and induce NADPH oxidase. These pathways are elevated in islets from diabetic donors. NADPH oxidase-1 (NOX-1) is significantly induced in either human donor islets, primary mouse islets or homogeneous beta cell lines following stimulation with a cocktail of inflammatory cytokines (TNF$\alpha$, IL-1$\beta$, INF$\gamma$) ($P<0.05$). Beta cell dysfunction was concomitantly induced by the pro-inflammatory cytokine (PIC) stimulation as measured by the loss of glucose stimulated insulin response ($P<0.05$), elevated expression of MCP-1 ($P<0.01$), increase in cellular reactive oxygen species (ROS) ($p<0.05$) and induced cell death. Inhibitors of NADPH oxidase, apocynin, diphenylene iodonium or selective NOX1/4 inhibitors, block ROS generation ($P<0.01$) and inhibit expression of MCP-1 ($P<0.05$) induced by PICs. In islets from diabetic donors, expression of NOX-1 is elevated ($P<0.05$, $n=5$).

12-lipoxygenase (12-LO) has been shown to regulate NADPH oxidase in non-islet models. Does this linkage occur in islets? Stimulation with PICs increases the expression of 12-LO in human islets, and 12-HETE (12-hydroxyeicosatetraenoic acid), a product of 12-LO activity, stimulates NOX-1 expression 12±4 fold ($P<0.05$) in human islets. Validating an association between 12-LO and NOX-1 in islets, we show that a selective inhibitor of 12-LO (NCTT-956) blocks 80% of NOX-1 expression ($P<0.01$) and inhibits procaspase 3 cleavage ($P<0.05$) induced by PICs in homogeneous beta cells. An inactive structural analog (NCTT-695) did not exert these effects.
This report unifies 12-LO and NOX-1 in beta cell dysfunction induced by PICs, a pathway not previously described in islet beta cells. Inhibition of this pathway is a candidate target to preserve and protect beta cell mass in diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P705

**GLP-1 agonists enhance stress-induced corticosterone release**

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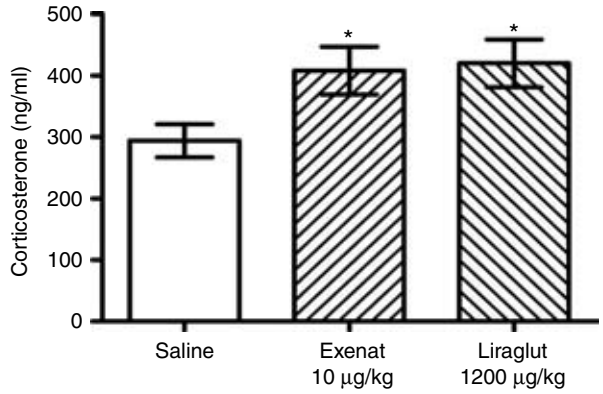
Introduction
Currently, there are two glucagon-like peptide 1 (GLP-1) receptor agonists approved for the treatment of type 2 diabetes.
While the primary pharmacological actions of GLP-1 agonists are antihyperglycaemic effect and weight reduction, these drugs may influence other neural and hormonal processes. Animal studies have indicated that GLP-1 receptors are involved in the regulation of stress, anxiety, learning and memory as well as regulation of corticosterone and aldosterone release.
Objective
The aim of the current study was to compare the behavioural and hormonal effects of glucaemically equipotent doses of exenatide and liraglutide in mice after acute and chronic treatment.
Methods
The effects of GLP-1 receptor agonists were determined on anxiety level in the light-dark compartment test, the motor activity in automated activity cages and finally, the forced swimming test (used to detect antidepressant-like effects) was performed. The plasma levels of corticosterone and aldosterone were also measured.
Results
Both exenatide (1–20 μg/kg s.c.) and liraglutide (200-1200 μg/kg s.c.) induced very similar behavioural and hormonal effects after acute administration: there was no change on anxiety level or immobility time, however, both drugs dose dependently suppressed motor activity. Remarkably, GLP-1 agonists stimulated corticosterone release even when combined with the swimming stress. After chronic dosing of GLP-1 agonists (exenatide 10 μg/kg bid; liraglutide 1200 μg/kg qd for 2 weeks) both drugs still enhanced stress-induced corticosterone release but did not change the locomotion of animals. The effects on aldosterone levels are also reported.
Conclusion
We conclude that exenatide and liraglutide induce very similar suppression of motor activity and stimulation of corticosterone release in mice. Interestingly, tolerance developed to hypolocomotory effect but not to corticosterone stimulating effect of GLP-1 agonists during 2 weeks of treatment.




Effect of chronic treatment with exenatide and liraglutide on plasma corticosterone Results are expressed as mean ±S.E.M. $n=9$–10. *$P<0.05$ vs. saline (Newman-Keuls test)
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
This work was supported, however funding details unavailable.

## P706

**Bile acid binding resins affect diverse molecules to improve metabolic control.**

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Introduction: Bile acid binding resins (BABRs), anion exchange resins developed as medication for hypercholesterolemia, have been reported to improve glycemic control in patients and animal models with type 2 diabetes mellitus. Although the mechanism has not been clarified yet, we suspect that diverse factors including peripheral bile acid composition and incretins play significant roles in the effect. Besides BABRs, bile acids (BAs) administration has been recently reported to ameliorate metabolic status. In our study, we also administered BAs to metabolic syndrome model mice, to show similarities and differences of BABRs and BAs, to elucidate the mechanism of their anti-metabolic syndrome effects. Methods: We evaluated the metabolic effects of BABR and BA by administering colestimide and cholic acid to male C57BL/6J and KK-Ay mice, at 6 weeks of age, fed normal or high-fat diet supplemented with / without colestimide or cholic acid. A dipeptide peptidase-4 (DPP-4) inhibitor had been also administered alone or combined with colestimide or cholic acid. We performed animal studies including body weight gain, food intake, OGTT, IPGTT, IPITT, serum GLP-1 and insulin concentrations. Morphological study of major tissues, bile acid composition analysis, and gene expression analysis were conducted with samples from the model mice. Results: BABR administration increased energy expenditure to induce weight reduction and insulin sensitization. The effect was similar to that of BA administration on BA composition and thermogenesis. BABR and BA also stimulated GLP-1 secretion, and additional administration of DPP-4 inhibitor augmented antidiabetic effect of BABR and BA. Conclusion: Our data suggest that BABRs could be useful for the management of not only hypercholesterolemia but metabolic syndrome. Futhermore, Combination uses of DPP-4 inhibitor with BABRs or BAs could exhibit great efficacy, providing clues to elucidate the effect of BABRs, and proposing new combination of established medications.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P707

**A novel beneficial effect of liraglutide: the reduction in subclinical atherosclerosis in patients with type-2 diabetes**

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Introduction
There is currently high interest in the non-glycemic effects of incretin-based therapies, specifically glucagon-like peptide 1 (GLP-1) analogues, such as those on cardiovascular system. Liraglutide has been approved in Italy since one year to be prescribed in combination with oral hypoglycemic agents; it has several non-glycemic properties, but its effect on carotid intima-media thickness (IMT), a recognized marker of subclinical atherosclerosis, is still unknown.
Methods
We evaluated in our Italian center the effect of liraglutide on carotid IMT as assessed by B-mode real-time ultrasound, in a prospective study of 33 patients with type 2 diabetes (58% males, age: 59±9 years), when added to metformin at a fixed dose of 1500/daily. Patients were newly diagnosed or previously treated subjects with oral hypoglycemic agents. The dose of liraglutide was 0.6mg/daily for the first 2 weeks, followed by a dose of 1.2 mg/daily. Statistical analysis was done using paired t-test and the Spearman correlation method.

Results
At baseline patients weighted 82±9 Kg, with average fasting glucose 9.5± 1.4 mmol/L and HbA1c 8.3±0.6%. The following significant changes in these parameters were recorded after 4 months of therapy: weight decreased by 3.1 kg, fasting glucose by 2.3mmol/l, and HbA1c by 1.8% ($p<0.0001$ for all). In addition, carotid IMT decreased from 1.55±0.45 mm to 1.36±0.31 mm ($P=0.0003$) after 4 months of treatment. Changes in carotid IMT did not correlate with changes in body weight, fasting glucose, or HbA1c; yet, we cannot exclude that this maybe due to the small sample size.
Conclusions
Liraglutide has desirable actions on carotid IMT, a surrogate marker of subclinical atherosclerosis, in type 2 diabetes after only a few months of therapy. This result may be at least partially independent of the effect of liraglutide on glucose metabolism. Future studies are needed to further explore this topic.
Declaration of interest
I fully declare a conflict of interest. Details below:
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## P708

**The Sodium Glucose Cotransporter-2 Inhibitor Empagliflozin Does Not Alter ECG Endpoints in a Thorough QT (TQT) Study**

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Empagliflozin is a potent, highly selective sodium glucose cotransporter-2 inhibitor in development for treatment of type 2 diabetes mellitus. This randomized, placebo-controlled, double-blind study assessed the effects of empagliflozin on the QT interval. Thirty healthy subjects (14/16 female/male; mean [range] age 34.5 [18–52] years) received single doses of 25 mg (within therapeutic range) and 200 mg (supratherapeutic) empagliflozin and 400 mg moxifloxacin as positive control. A new 5-period crossover, 'double-placebo period' design was utilized that reduced the sample size (from 40 to 30 subjects) and the total number of treatment periods compared with the classical 4-period TQT-design. Triplicate 12-lead 10-s-ECGs were recorded at baseline and selected time points over 24 h after dosing. Placebo-corrected mean change from baseline (MCfB) in population heart rate (HR)-corrected QT interval (QTcN) 1–4 h after dosing (primary endpoint) was 0.6 (90% CI: −0.7, 1.9) ms and −0.2 (−1.4, 0.9) ms for 25 and 200 mg empagliflozin, respectively – below the ICH E14 non-inferiority margin of 10 ms. Maximum placebo-corrected MCfB in QTcN between 0.5–24 hours after dosing were 2.2 (−0.3, 4.7) ms and 1.6 (−0.4, 3.5) ms for 25 and 200 mg empagliflozin, respectively. 90% CIs for placebo-corrected MCfB in HR 1–4 h after dosing were within −1.8 and 0.8 beats per min for both doses, indicating no relevant empagliflozin-related changes. Assay sensitivity was confirmed by placebo-corrected MCfB in QTcN 2–4 h post dose of 12.4 (10.7, 14.1) ms with moxifloxacin. Empagliflozin tolerability was rated 'good' for all subjects. Adverse events (AEs) were reported for 23.3% of subjects receiving empagliflozin and 27.6% receiving placebo. The most frequent AE was nasopharyngitis (4 subjects on empagliflozin, 5 on placebo). In conclusion, single doses of empagliflozin 25 and 200 mg were not associated with QTcN prolongation or changes in HR, and were well tolerated.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P709

**Safety and efficacy of linagliptin in type 2 diabetes (T2D) patients (pts) with common renal and cardiovascular risk factors**

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Introduction
Vascular complications are the leading cause of morbidity and mortality in T2D. Hypertension and/or microalbuminuria predict renal and cardiovascular outcome. This large pooled analysis from a global development program aimed to evaluate the safety and efficacy of linagliptin in this particularly high-risk population.
Methods
Data of 1982 pts from 3 randomized, double-blind, placebo(PBO)-controlled phase 3 trials were analyzed. Pts with microalbuminuria (urine albumin-to-creatinine ratio [UACR] 30-300 mg/g) and hypertension (systolic BP ≧140 and/or diastolic BP ≧90 mmHg; and/or taking antihypertensive drug; and/or history of hypertension) at baseline were eligible for this analysis. Pts were treated with linagliptin 5 mg qd or PBO as monotherapy (18 wks), add-on to metformin (MET) or MET + sulphonylurea (SU) (24 wks). Efficacy endpoints were change from baseline in HbA1c and fasting plasma glucose (FPG) after 18 or 24 wks. Descriptive analysis for safety was performed, including adverse events (AE), serious AE, and hypoglycaemia episodes.
Results
340/1982 pts met screening criteria; 251 linagliptin, 89 PBO. Baseline demographics and characteristics were similar between groups (mean [SD] HbA1c, 8.3 [0.9]%; FPG, 171 [46] mg/dL; age, 59.9 [10.2] yrs; eGFR (MDRD) ≧90/60–<90/30–<60 mL/min, 53.2%/38.5%/8.2%). Linagliptin significantly lowered HbA1c and FPG vs PBO (Table). Overall, the incidence of AE and serious AE with linagliptin were similar to PBO (AE 65.7% vs 66.3%; serious AE 3.2% vs 6.7%, respectively). Hypoglycaemic event rates were increased only when linagliptin was administered with SU (linagliptin 13.5% vs PBO 2.2%). The hypoglycaemic event rate with linagliptin was <1% as monotherapy or add-on to MET.
Conclusion
In T2D pts with hypertension and microalbuminuria, linagliptin was well tolerated and achieved significant, clinically meaningful improvements in glycaemic control. Linagliptin may support long-term preventive strategies to reduce risk of cardiovascular events and declining renal function.

**Table 1** Red blood cell fatty acid composition in volunteers (% of total FA)

| Adjusted mean change from baseline (SE) | | Linagliptin | Placebo | Linagliptine Placebo |
|---|---|---|---|---|
| HbA1c (%) | Wk 18† | −0.43 (0.08) | 0.19 (0.10) | −0.62 (0.12)*** |
| | Wk 24‡ | −0.55 (0.09) | 0.04 (0.12) | −0.59 (0.13)*** |
| FPG (mg/dL) | Wk 18† † | −5.12 (3.33) | 6.29 (4.77) | −11.41 (5.06)* |
| | Wk 24‡ ‡ | −3.76 (4.36) | 15.95 (6.15) | −19.72 (6.35)** |

*$P<0.05$, **$P<0.01$, ***$P<0.0001$ vs placebo †Linagliptin: n=246, placebo: n=87; ‡linagliptin: n=218, placebo, n=69. † †Linagliptin: n=212, placebo: n=63; ‡ ‡linagliptin: n=208, placebo: n=64.

Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P710

**Effects of flaxseed oil on anti-oxidative system and membrane deformation of human erythrocytes in high glucose level**

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Aim
The erythrocyte membrane lesion is a serious diabetic complication. Although most available data demonstrated by earlier studies that n-3 fatty acid was a potent agent in this complication of diabetes. Realization about protection effects of flaxseed oil on antioxidative capacity and against to membrane deformation of human peripheral blood erythrocytes in high glucose model is limited.
Main methods
In this study, erythrocytes were treated with 50 mM glucose to mimic hyperglycemia in the presence or absence of three doses of flaxseed oil (50, 100 and 200 μM) in the medium at 37 °C for 24 h. The malondialdehyde and L-glutathione were checked by HPLC and LC/MS respectively. The phospholipids symmetry and membrane fatty acid composition of erythrocytes were demonstrated by flow cytometer and gas chromatograph (GC). The morphology of erythrocyte was illuminated by ultra scanning electron microscopy.
Key findings
Our results illustrated that the flaxseed oil could prevent lipid peroxidation and delay the antioxidant capacity decreasing in erythrocytes. Also, the results of GC demonstrated that flaxseed oil groups contained higher C22:5 and C22:6 than 50 mM glucose group. These data indicated that flaxseed oil could reduce lipid asymmetric distribution and membrane perturbation.
Significance
This study provides new insights that the flaxseed oil supplementation may prevent the lipid peroxidation and membrane dysfunction of erythrocytes in diabetes.

**Table 1** Red blood cell fatty acid composition in volunteers (% of total FA)

| | 50 μM flaxseed oil +50 mM glucose | 100 μM flaxseed oil +50 mM glucose | 200 μM flaxseed oil +50 mM glucose | glucose group (50 mM) | PBS (0.01 M) |
|---|---|---|---|---|---|
| C14:0 (Myristic acid) | 2.90±0.3* | 3.70±0.5** | 4.20±0.7** | 1.90±0.2 | 6.80±0.4## |
| C16:0 (Palmitic acid) | 36.44±1.8* | 27.25± 2.9** | 24.22± 1.9** | 38.10±2.7 | 19.70±2.2## |
| C17:0 (Internal standard) | 0.61±0.1 | 0.62±0.2 | 0.67±0.1 | 0.62±0.1 | 0.64±0.2 |
| C18:0 (Stearic acid) | 17.16±3.0* | 14.81± 3.0** | 10. 44± 3.3** | 21.30±3.9 | 5.50±1.7## |
| C18:1 (Vaccenic acid) | 21.11±2.2* | 19.01± 4.1** | 16.71± 2.9** | 24.40±2.4 | 14.40±0.9## |
| C18:2 (Linoleic acid) | 2.20±0.3* | 4.90±0.6** | 6.30±0.8** | 1.40±0.2 | 9.60±0.5## |
| C22:0 (Behenic acid) | 0.97±0.2* | 1.29±0.2** | 2.00±0.4** | 0.90±0.1 | 3.90±0.3## |
| C22:5 (Docosapentaenoic acid) | 0.77±0.2* | 1.40±0.3** | 1.90±0.3** | 0.70±0.1 | 2.80±0.2## |
| C22:6 (Docosadienoic acid) | 0.79±0.1* | 1.10±0.1** | 2.10±0.1** | 0.60±0.1 | 3.20±0.6## |
| RBC fatty acids total concentration (pmol/mg Hb) | 18.29±1.4* | 20.10± 2.2** | 27.89± 2.7** | 17.04±2.5 | 32.33±3.1## |

Note: Three concentrations of flax seed oil effect on fatty acid composition of membrane. *: $P<0.05$, ** and ##: $P<0.01$ are significantly different from 50 mM glucose group. (n=24)

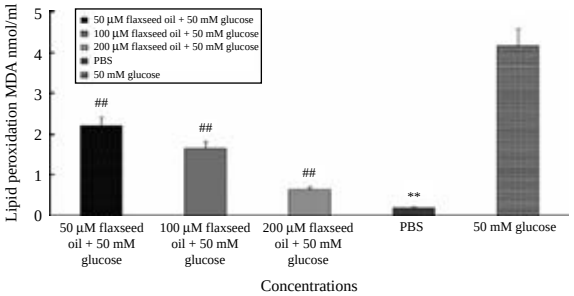


**Figure 1** demonstrated flaxseed oil reduced MDA level in erythrocytes. The MDA level in three concentrations of flaxseed oil and PBS groups were remarkably lower than glucose group. The ## and ** were significant difference from 50 mM glucose group ($n=24$, $P<0.01$).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P711

### Rapidly and strong suppression of human acylated ghrelin serum concentrations during infusion of des-acyl ghrelin in obese diabetic subjects.

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Aim
To assess the effects of a continuous overnight infusion of two doses of UAG (3&10 μg UAG/kg.hr-1) versus placebo in a double blind cross-over study on AG levels, as well on glucose and insulin response to a standard breakfast meal (SBM) in 8 overweight patients with type 2 diabetes (HbA1c 6.5-7.5%).
Methods
During these 3 admissions, in randomized order, all subjects received either placebo, or UAG 3 μg/kg.hr-1 or UAG 10 μg/kg.hr as a continuous venous infusion. The admission were divided by a wash-out period of >7 days. Serum glucose were assessed at regular intervals and via a continuous glucose monitor. Continuous infusion of either placebo or UAG started at 22.00 until 13.00 hr the next day. At 08.00–08.15 hr a standard breakfast meal (SBM) was used. SBM consisted out of 714 kcal (17% proteins; 46% fat; 37% carbohydrates).
Results
Morning AG levels (before SBM) dropped significantly from 21.01±8.9 (mean±SD) to 3.0±6.7, to 1.4±3.2 pg/ml after placebo, 3 and 10 mcg/kg.hr UAG infusion, respectively. After breakfast AG levels drop from 14.03±9.4, 0.8±1.8, to 0.8±1.8 pg/ml after placebo, 3 and 10 mcg/kg.hr-1 UAG infusion, respectively.
UAG levels, increased from 105.9±31.4, 10,998±2,623, 12,085±1,616 pg/ml after placebo, 3 and 10 mcg/kg.hr UAG infusion, respectively.
During continuous glucose monitoring, post-SBM, overnight infusion of 10 mcg/kg.hr UAG significantly decreased glucose levels. The AUC decreased from 1618, 1601, to 1540 mmol/3 hrs of placebo, 3 and 10 mcg UAG infusion.
A significant decrease in peak serum glucose levels after SBM during 10 mcg UAG infusion was observed.
Conclusion
UAG is a potent inhibitor of ghrelin levels in obese mild diabetics. Additionaly, UAG improves postprandial glycemia, especially in whom preprandial AG levels are high, which makes UAG (analogs) strong potential candidates for the development of drugs in the treatment of metabolic disorders or other conditions in which a disturbed AG/UAG ratio has been found.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P712

### Educating educators - the way to improve health outcomes of people with diabetes

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Background and Aims
Diabetes causes immeasurable personal suffering and costs to the society, though diabetes and its complications are largely preventable. There are proven, affordable preventions available, that can stop or delay diabetes/complication development. Political Declaration adopted by UNO stresses importance of educational measures to promote health education and literacy. IMAGE Project/Toolkit on Type 2 Diabetes Prevention in Europe outlines education as cornerstone of motivation creation. To provide high quality Therapeutic Patient Education (TPE) /motivation, education/training of educators (ETE) is essential. TPE is voluntarily practiced in Easter-European countries (EECs) for around 20yrs, though systematic ETE is rarely performed, besides, lack in education materials (EM) is experienced. Our aim was to train healthcare providers (HCPs) using Conversation Map Education Tools (CMapT) and provide them with EM.
Materials and Methods
Totally, 21 HCPs from 7 EECs participated in 2-day education course using CMapT. Training was carried out under the aegis of IDF-Global and IDF-Europe. CMapT are developed by HealthyInternational, Canada. HCPs were endocrinologists, pediatrician-endocrinologists, cardiologists/endocrinologists, psychologists, GPs, family medicine/nursing trainers. Agenda included 4 lectures and 7 Small Group Activities (SGA).
Results
Participants highly appreciated possibility to discuss problems and share views, they showed interest in CMapT and their implementation, ranging from HCPs education to school staffs and various civil services training/awareness-raising campaigns. Participants stated that CMapT make education flexible; interesting both for HCPs and people with diabetes; help to avoid professional burnout; create strong motivation; solve problems with EM.
Conclusion
IDF Global Diabetes Plan includes continuous self-management education and support in its core components. This training was important from standpoint of Political Declaration and interest paid by EEC Governments to TPE. Our challenge is to reduce human and financial costs of diabetes; TPE/ETE is an important tool in achieving this goal. Education of educators needs further development to make progress sustainable.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P713

**Reducing Residual Vascular Risk Through combination Therapy with Fenofibrate and Alpha-Lipoic Acid in Patients with Ischemic Heart Disease and Type 2 Diabetes Mellitus**

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Purposes
To investigate effects of combination therapy with fenofibrate and α-lipoic acid (ALA) on atherogenic dyslipidemia, which determines the residual vascular risk and endothelial dysfunction, levels of proinflammatory mediators in patients with ischemic heart disease (IHD) and type 2 diabetes mellitus (T2DM).
Methods
We examined 42 patients with IHD and T2DM (19 males, age 60.5±4.7 years). Baseline characteristics of patients included history of IHD (7.2±2.3 years), T2DM (4.7±0.5 years). The level of HbA1c was less than 7.5%. All patients were divided into 2 groups: the 1st ($n=22$) - received the standard therapy, the 2nd ($n=20$) in the standard therapy received combination of fenofibrate 145 mg once daily with ALA 600 mg once daily. In all patients were determined the levels of total cholesterol, low-density lipoprotein cholesterol (LDL), triglycerides (TG), high-density lipoprotein cholesterol (HDL) by enzymatic colorymetric method, and proinflammatory mediators (TNF-α, hsCRP) by ELISA method at baseline and in 6 months.
Results
As compared with baseline, combination therapy with fenofibrate and ALA substantially lowered plasma levels of TNF-α by 7±2% ($P<0.05$) and hsCRP from 1.58±0.19 to 0.98±0.17 pg/ml ($P<0.05$) compared to the 1st group. Furthermore, combination therapy increased plasma levels of HDL on 12% (0.13 mmol/L), decreased total cholesterol, LDL and TG levels on 7%, 9% and 12% respectively (all $P<0.001$).
Conclusions
Combination therapy with fenofibrate and α-lipoic acid significantly reduced total cholesterol, LDL, and TG, proinflammatory mediators, increased HDL and as a result reducing residual vascular risk in patients with IHD and T2DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P714

**Antidiabetic therapies and malignancy: a retrospective study in oncological cases.**

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Introduction
Despite the relation between Type 2 Diabetes Mellitus (DM) and development of malignancy is well known, data on antidiabetic drugs and cancer risk is contradictory. Thesis which have suggested that analogue insulins, especially glargine, increase cancer risk, has not been validated. The present study aimed to evaluate the possible contributions of diabetes durations and medications to the type and course of malignancy in patients with both diabetes and malignancy.
Methods
Among patients registered in the Oncology department of Baskent University Hospital, those who had DM before the oncological diagnoses were enrolled. The course of diabetes, antidiabetic medications and their relevance to the oncological diagnoses were examined.
Results
Totally, 246 patients were enrolled. Some characteristics of the group were summarized in Table 1. Among 69 patients receiving insulin, no relation was determined between duration of DM, basal glycohemoglobin values and stage of malignancy at diagnosis and the course of malignancy. There was no relation between the type and duration of antidiabetic therapy used (including metformin, glargine insulin) and the type of malignancy, stage at diagnosis and the final state of malignancy.
Conclusion
Despite this retrospective study may not give idea about possible cancer initiating or promoting potential of antidiabetic medications, it may suggest that therapies put forward to be protective (such as metformin) or risk increasing (such as glargine) might not have any influence on the type and biological behavior of the developing malignancy. Prospectively designed research is required to exhibit these relations clearly.
Some features and results of the study

**Table 1**

| | |
|---|---|
| N (female / male) | 246 (132/114) |
| Age (years, mean±S.D.) | 70.3±11.3 |
| Distribution of antidiabetic therapy*, N (%) | No treatment-29 (11.8) OAD- 148 (60.2) Insulin- 69 (28) |
| Malignancy types, N (%) (most frequent) | Breast- 40 (16.2) Prostate- 27 (11) Thyroid- 24 (9.8) Colorectal- 20 (8.1) Renal cell- 15 (6.1) Other- 120 (48.8) |
| Distribution of antidiabetic therapy until diagnosis of malignancy **, N | No treatment- 29 Metformin- 196 Other OAD- 208 Insulin- Non-analogue- 37 Glargine- 7 Other analogue- 25 |
| Time between diagnosis of DM and malignancy, (years, mean±S.D.) | 16.1±11.0 |
| Glycohemoglobin level at the time of diagnosis of malignancy (%, mean±S.D.) | 8.0±1.9 |
| Duration of metformin use, years (minimum-maximum) | 1–38 |
| Duration of insulin glargine use, years (minimum-maximum) | 2–10 |

*Those using insulin for at least 6 months between DM and malignancy diagnoses were included in insulin group.** Those drugs used for at least 6 months were included.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P715

**Adding Liraglutide to insulin treated obese type 2 diabetics improves glycaemic control with sustained weight reduction and low hypoglycaemia rate**

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Aims
To evaluate the effect of Liraglutide on clinical parameters in obese Type 2 diabetic patients with inadequate glycemic control despite treatment with oral hypoglycaemic agents (OHAs) and insulin.
Methods
Three hundred and fifty three (M:232, F:121) obese (according to Asian-Indian criteria) type 2 diabetic patients with inadequate glycemic control (HbA1c>7% & <10%) attending outpatients clinic on insulin and OHAs were initiated on liraglutide. Weight, Blood pressure, BMI, HbA1c, fasting lipids, high sensitivity C-reactive protein (hsCRP), and insulin doses (basal, prandial & total) were recorded at baseline, 3, 6 and 12 months. Side effect profiles were recorded.
Results
Ten patients discontinued therapy because of nausea. The remaining patients demonstrated a reduction in mean HbA1c by 0.9±0.21% ($P<0.001$), weight reduced by 10.7±0.7 kg ($P<0.001$) and insulin doses were reduced from 83.1+3.9 U to 52.2+5.3 U ($P<0.001$). There was a reduction in serum total cholesterol by 12.4±4.1% ($P=0.03$), triglycerides by 30±4.6% ($P=0.01$), systolic blood pressure by 10.2±2.5 mm Hg ($P=0.01$), and hsCRP by 29±11.3% ($P=0.02$). There was also a reduction in glycemic excursions at the end of study period. Hypoglycemia was seen in 4 patients. Nausea, diarrhoea, vomiting, headache, and constipation were main adverse events noticed in patients.
Conclusions
The addition of Liraglutide effectively treats obese type 2 diabetics on insulin, leading to weight loss and reduction in levels of HbA1c, systolic blood pressure, tri¬glycerides, hsCRP & a significant reduction in insulin dosages. Liraglutide could be considered in a subset of obese type 2 diabetics with poor glycemic control who might gain more weight and be exposed to hypoglycaemia with increasing doses of insulin in striving to achieve adequate glycemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P716

**Clinical and Patient Reported Outcomes over the 24 Months of Insulin Therapy in Patients with Type 2 Diabetes in Greece: Data from The Instigate Study**

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Aims
We present final Hellenic data from the INSTIGATE study which assessed direct costs, resource utilisation, and clinical outcomes in the first 24 months of insulin therapy in type 2 diabetic (T2D) patients.
Methods
INSTIGATE was a prospective European observational study of patients with type 2 diabetes who had initiated insulin during usual care. Data were collected at baseline, when subjects initiated insulin (visit T1), then 3 (T2), 6 (T3), 12 (T4), 18 (T5) and 24 months (T6) later.
Results
The data presented here is for the 237 patients with Baseline, 6 and 12 month visits; at 18 and 24 months data were available from 229 and 227 patients respectively.
Following insulin initiation mean HbA1c improved from (SD) 9.65(1.63)% at baseline to 7.44(1.06)%, 7.39(1.15)%, 7.28(0.96)% and 7.14(0.85)% at 6, 12, 18 and 24 months respectively. Mean fasting blood glucose (FBG) improved from (SD) 12.8(3.9) mmol/l to 7.8(2.2) mmol/l, 7.8(2.4) mmol/l, 7.5(2.1) mmol/l and 7.3(2.1) mmol/l over the same period. Mean BMI (SD) was 28.2(4.7) kg/m2 at baseline up to 29.4(4.6) kg/m2 at 24 months. Patients reporting at least one hypoglycaemic episode were 3.0% (in the three months prior to insulin initiation) 29.1% (in the six months after initiation) and above 20% thereafter. Health status, measured by EQ-5D visual analogue scale score, improved from baseline to 6, 12, 18 and 24 months, the highest improvement being from baseline up to 6 months after insulin initiation.
Conclusions
Greek patients initiated insulin when HbA1c is by much higher than recommended by international guidelines; yet their glycaemic control improved in the 6 months following initiation, the best improvement being observed from baseline to 6 months. Mean health-related quality-of-life scores also improved, however there was an increase in the number of patients reporting hypoglycaemia.
Declaration of interest
I fully declare a conflict of interest. Details below
Funding
This work was supported, however funding details unavailable.

## P717

**The outcome of Beta cell function after early insulin therapy in the recently diagnosed type 2 diabetes (in Egyptian population) our experience in EL-Minia university hospital**

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The purpose of this prospective cohort study was to evaluate whether early insulin therapy is more advantageous in achieving long-term optimal glycemic control with improved B cell function than oral drugs in the recently diagnosed type 2 diabetes mellitus. Methods: Sixty consecutive patients with recently diagnosed type 2 diabetes mellitus were divided into 3 groups.The 1st group received 2 SC injections of premixed insulin. The 2nd group received bed time NPH and 3 injections of regular insulin before meals. The 3rd group received metformin and/or sulphonylureas. The treatment continued for 3 months till euglycemia was reached. Then, all medications were stopped and the patients were followed up till the end of the year. BMI, FBG, PPG, HbA1C, fasting level of insulin, proinsulin, C-peptide, HOMA-IR, HOMA-B, serum cholesterol, triglycerides were estimated. Results: Six patients from group I (30%), nine from group II (45%), and only one from group III (5%) succeeded to maintain euglycemia without further therapy for 9 months after stoppage of treatment. The mean HbA1C level was 6.5% in group I, 6.1% in group-2, and $>7$% in the group-3. Level of HbA1C in the succeeded patients declined significantly in comparison to the failed patients. Markers of β-cell function of the succeeded patients showed a statistical significant increase regarding the C-peptide, insulin and HOMA-B. Meanwhile, proinsulin level and proinsulin / insulin ratio declined ($P=0.0001$). The total serum cholesterol and triglycerides of insulin treated groups reduced significantly ($P=0.001$). Conclusions: Short-term insulin therapy in a newly diagnosed type 2 diabetes naive patients can preserve β-cell function and insulin secretion, allowing long-term glycemic control without medication and may improve glycemic responses to supplemental oral treatment if needed.

Keywords: Type 2 DM, early insulin therapy, B cell function
Abbreviations: BMI: Body mass index, FBG: fasting blood glucose; PPG: postprandial glucose, HbA1C: Glycosylated haemoglobin, OADs: oral antidiabetic drugs; HOMA-B: homeostasis model assessment of B-cell function; HOMA-IR: homeostasis model assessment of insulin resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P718

**Gender differences in metabolic outcomes of continuous subcutaneous insulin infusion therapy**

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Introduction
The continuous subcutaneous insulin infusion (CSII) is an alternative to multiple daily injection therapy in type 1 diabetes and its use is increasingly common due to the beneficial effects on the glycemic control of the patient.
Aims
To find differences on outcomes of CSII therapy between genders. Patients and methods: Patients on CSII therapy in our department were included in the study and we recorded outcomes regarding the following set points: immediately before beginning of CSII therapy, 12 months after inclusion and in the last appointment. For statistical analysis, we used the Student's t-test and Pearson correlation, and considered significant a value of $P \leq 0.05$.
Results
We studied 64 patients (24 men; 39 women) with a mean HbA1c before initiation of $8.2\% \pm 1.4$; mean age at the time of placement of $33.6 \pm 11.2$ years; and mean time of follow up after placement of $2.1 \pm 1.9$ years. Men had significantly lower levels of HbA1c than women ($7.8 \pm 0.8\%$ vs $8.4 \pm 1.7\%$, $P<0.05$). The reduction of HbA1c on the last consultation was not sustained in the group of men, whereas in women there was a persistent tendency to reduction of HbA1c ($\Delta$HbA1c $0.1 \pm 1.2\%$ vs $\Delta$HbA1c $-1.3 \pm 1.6\%$, $P=0.01$). There was no significant correlation between duration of diabetes, age, gender and weight change. When adjusted to gender, there was a negative correlation between previous HbA1c and its reduction by the end of follow-up ($R=-0.555$, $P=0.021$) and a positive correlation between weight gain and HbA1c reduction at 12 months ($R=0.505$, $P=0.04$). There was a significant correlation between duration of diabetes and reduction of HbA1c at 12 months ($R=0.600$, $P=0.01$).
Conclusions
In our study we found better results of CSII therapy in women than men, probably reflecting the fact that patients with higher baseline HbA1c benefit most of this diabetes therapeutic modality.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P719

**Continuous subcutaneous insulin infusion systems: experience in 92 patients with type 1 diabetes**

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Background
Continuous subcutaneous insulin infusion (CSII) using an external pump is an alternative intensive diabetes therapy recognized to improve metabolic control and glycemic instability in selected type 1 diabetic (T1DM) subjects. The aim of this study was to examine the clinical effectiveness and safety of CSII systems over a 5-year follow-up period in T1DM patients.
Materials and methods
We performed a retrospective observational study of T1DM patients previously treated with multiple daily insulin therapy who initiated CSII until December

2010. We analyzed A1C, weight and total daily insulin dose (TDD) at the start of therapy, 6 months after and then annually for 5 years. We examined the occurrence of hypoglycemic and diabetic ketoacidosis (DKA) episodes and local problems at infusion site.
Results
We followed 92 patients treated with CSII during 4.08±3.01years, 62% female, mean age 28.73±11.7years-old, diabetes duration 15.63±8.97 years. The main reasons for starting CSII were: poor glycemic control in 47.7%, glycemic instability 31.1%, frequent and unnoticed hypoglycemia 12.1% and dawn phenomenon in 4.5%. Baseline A1C was 8.79±1.62% and decreased to a minimum of 7.58±1.0% at 6 months ($P<0.05$). Compared to baseline, A1C remained lower in all follow-up period ($P<0.05$). Patients followed during 5 years maintained lower A1C (8.95±1.6% vs 7.60±0.94%, $P<0.05$), although we verified a slight increase between 1 and 4 years (7.35±1.08% vs 7.75±0.91%, $P<0.05$). Insulin requirements reduced from 57.7UI/day to 41.3UI/day ($P<0.05$) at 6 months and we found no statistically weight difference (59.5 vs 58.3 kg). We registed 18 severe hypoglycemia (incidence 0.057/patient/year), 12 DKA (0.038/patient/year) and 11 recurrent infections at the infusion site. Eight (8.7%) patients quit pump therapy mainly because of maladaptation.
Conclusion
In this study CSII improved glycemic control during long term follow-up and reduced total daily insulin requirements. The rate of major complications was low and similar to those reported in other studies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P720

**A 52-week efficacy and safety profile of sitagliptin in Japanese type 2 diabetic patients in clinical settings**

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Background of Study
Some medications for the treatment of type 2 diabetes mellitus fail to achieve a continuous favorable glucose control in the long run during clinical use. Therefore we examined the durability of dipeptidyl peptidase-4 (DPP-4) inhibitor, sitagliptin which is relatively new to Japanese type 2 diabetic patients in clinical settings.
Objectives and Methods
117 subjects (M/F=65/52, Age: 66±10 y.o., duration of diabetes 12±8 y. body weight 62 kg) received sitagliptin 50 mg q.d. and were followed up for 52 weeks. We observed HbA1c, body weight, blood pressure, and lipid profile. The percentage of concomitant use of sulfonylureas, biguanides, tiazolidinediones, alpha glucosidase inhibitors, and glinides are 63%, 45%, 28%, 20%, and 6% individually.
Results
Five subjects terminated medication due to loss of hypoglycemic potency and worsening patients' condition. In the rest of 112 subjects, HbA1c was decreased from 7.89 to 7.00% ($P<0.001$), while there were no significant changes in body weight (62.2 to 62.2 kg), systolic blood pressure (133 to 131 mmHg) and lipid profiles (LDL-C: 111 to 108 mg/dl, HDL-C 55 to 56 mg/dl). The rate of achievement of HbA1c less than 6.9% was increased from 5 to 41%,and the frequency of decrement more than 0.4% was 66%. Contribution of HbA1c before the treatment was significant to the decrement of HbA1c, while there were no contributory effect of sex, age, the duration of diabetes, weight, combination medications and existence of complications. There were no serious adverse events.
Conclusions
Sitagliptin is a good therapeutic additive option to improve glycemic controls continuously in Japanese type 2 diabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P721

**Short-term liraglutide therapy as compared with basal insulin therapy on restoration effect of insulin secretion in Japanese patients with type 2 diabetes**

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Objective
To investigate whether restoration of insulin secretion and long-term optimal glycemic control can be achieved by short-term liraglutide (a glucagon-like peptide-1 receptor agonist) therapy compared with basal insulin therapy in Japanese patients with type 2 diabetes.
Research Design and Methods
Japanese subjects with type 2 diabetes had a short-term, 3 week course of basal insulin therapy ($n=114$) or liraglutide therapy ($n=29$) to achieve a target two hours postprandial glucose of less than 140 mg/dL, then they were discontinued. Efficacy and safety of short-term liraglutide and basal insulin were retrospectively compared in terms of restoration effect of insulin secretion evaluated by fasting C-peptide immunoreactivity (CPR) (ng/mL) / fasting blood glucose (FBS) (mg/dL), two hours postprandial CPR / two hours postprandial blood glucose, insulinogenic index and urinary CPR.
Results
Fasting CPR / FBS was improved from 0.014±0.007 to 0.021±0.008 in liraglutide group, favorably compared to the improvement in basal insulin group (from 0.011±0.006 to 0.020±0.006). Moreover, liraglutide also improved two hours postprandial CPR / two hours postprandial blood glucose from 0.027±0.024 to 0.052±0.028, insulinogenic index from 0.18±0.16 to 0.50±0.39, and urinary CPR from 103.3±69.4 to 66.8±39.5 μg/day. Blood glucose levels of liraglutide-withdrawn patients were kept in long-term good control without medication or with only oral antidiabetic drugs. Gastrointestinal side effects were occasional but tolerable, and hypoglycemic episode was never observed in liraglutide group.
Conclusion
Short-term liraglutide and basal insulin therapy induced the same degree of improvement on insulin secretion in Japanese type 2 diabetic patients. A long-lasting protective effect on pancreatic beta-cell function can be expected by short-term liraglutide therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P722

**Vitamin B-12 deficiency associated with metformin treatment of type 2 diabetes mellitus in daily practice: Clinical consequences**

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In a recent study, vitamin B-12 deficiency was observed in 10% of metformin-treated type 2 diabetic patients, but neurological status of the patients was not defined. Thus, potential consequences of this observation remained subject to controversy. Therefore, we decided to examine to which extent a vitamin B-12 deficiency can be detected in metformin-treated diabetic patients in daily practice and which impact vitamin B-12 deficiency – if present – could have on the development of diabetic neuropathy in these patients.
88 consecutive type 2 diabetic patients with metformin treatment for >1 year (37 females, 51 males, age, 66±2 years [SEM], duration of diabetes, 10±2 years, metformin, 1450±120 mg/day for 6±2 years) were included in this cross-sectional study. We measured vitamin B-12 levels, folate, 25-OH-vitamin D and PTH by enzyme immunoassay, additionally we performed a routine screening of all patients for signs or symptoms of diabetic neuropathy.
10% of the patients had vitamin B-12 deficiency, a total of 29% of the patients had vitamin B-12 levels<200 pmol/l. Patients with decreased vitamin B-12 levels received higher doses of metformin for longer duration than patients with vitamin B-12 levels >200 pmol/l ($P<0.05$). Moreover, they exhibited an increased prevalence of diabetic neuropathy, had decreased folate levels and decreased vitamin D levels ($P<0.05$). In patients with decreased vitamin B-12, diabetic neuropathy developed after shorter duration of diabetes and despite better HbA1c levels than in patients with normal vitamin B-12 ($P<0.05$).
In this cross-sectional study in daily practice, there is evidence for neurological consequences of metformin-associated vitamin B-12 deficiency, which therefore

must be considered as eventually harmful for metformin-treated patients. Thus, patients should be regularly screened and vitamin B-12 replacement started as early as necessary, together with the replacement therapy of mostly associated vitamin D-deficiency.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P723

**A comparison of pharmacokinetics and pharmacodynamics of insulin aspart, biphasic insulin aspart 70, biphasic insulin aspart 50 and human insulin: a randomized, quadruple crossover study**

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Aims
To compare the pharmacokinetic and pharmacodynamic profiles of insulin aspart, biphasic insulin aspart 70 (BIAsp70), biphasic insulin aspart 50 (BIAsp50) and soluble human insulin under experimental conditions.
Methods
In this randomised, four-period crossover study, 19 type 1 diabetic patients received subcutaneous injections of identical doses (0.2 U/kg) of either insulin aspart, BIAsp70, or BIAsp50 immediately before a standardized meal, or human insulin 30 min before meal. Plasma glucose and serum insulin were measured for 12 h postprandially.
Results
The pharmacokinetic and pharmacodynamic profiles of human insulin differed from insulin aspart, BIAsp70 and BIAsp50. The 3 different aspart preparations had easily distinguishable features as regards onset and duration of action. Insulin aspart preparations were, on average, absorbed twice as fast as human insulin. In the initial phases (0–4 h and 0–6 h), AUCins was significantly higher during insulin aspart treatment as compared to the others, whereas insulin aspart had a significantly lower AUCins over the last 6 h ($P<0.05$). BIAsp70 and BIAsp50 provided comparable insulin coverage to that of human insulin over the last 6 h. Insulin aspart had the most pronounced onset of action and the shortest duration. Comparing with insulin aspart and BIASp70, BIAsp50 revealed a closer treatment ratio to human insulin on pharmacodynamic endpoints.
Conclusions
BIAsp70 and BIAsp50 injected immediately before a meal are at least as effective as human insulin injected 30 minutes earlier in controlling postprandial glycaemic excursions. BIAsp50 showed the greatest similarity to human insulin as regards pharmacokinetic and pharmacodynamic profiles.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P724

**Resource utilization and patient satisfaction associated with switching insulin in Greece (Subanalysis of the SWING study).**

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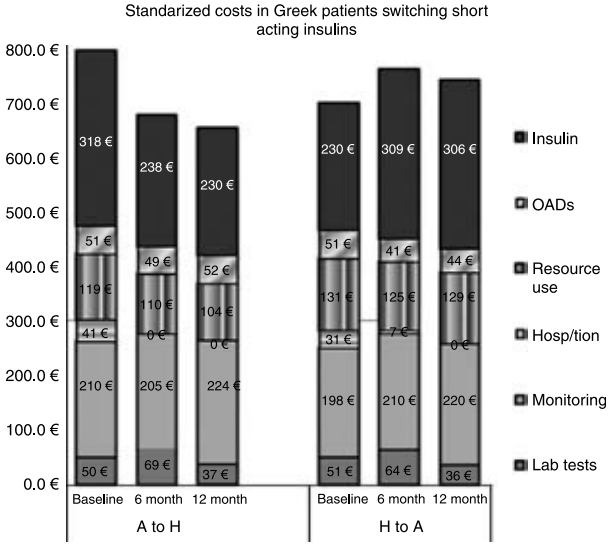
Purpose
Diabetic patients (T2D) switch from short-acting insulin to rapid-acting analogue (H-A) and less commonly from A-H. The SWING study was designed to assess treatment costs, glucose control, satisfaction, and quality of life in T2D following H-A or A-H switches.
Methods
SWING was a multinational, prospective, observational study of previously mentioned parameters associated with switching in either direction between any rapid-acting analogue insulin therapy and short-acting human insulin therapy over 12 months following the switch. Data were collected at baseline (switch) and at approximately 3, 6 and 12 months post-switch. Descriptive analysis of the Hellenic subgroup data is presented.
Results
309 Greek patients switched H-A [N = 270, mean(SD) age 65.3(11.9), Body Mass Index (BMI) 28.8(4.5) kg/m2, diabetes duration 15.6(9.0) years] or A-H [N = 39, age 64.4(12.6), BMI 28.8(5.4) kg/m2, diabetes duration 13.7(7.9) years] insulin. Mean (SD) HbA1c at baseline was 8.4(1.3) % (H-A) and 8.4(1.4)% (A-H) respectively and decreased over 12 months: −1.2(1.1) units H-A, −1.3(1.3) units A-H.
Reported hypoglycaemia incidence (recalled over the 6 months preceding baseline) was 45.9% for H-A and 28.2% for A-H.
Mean (SD) direct diabetes-related costs for standardized 6 month periods prior to switch, 0–6 months and 6–12 months following switch were €692.2(293.1), €755.3(235.7) and €734.6 (231.9) for H-A, and €788.9(402.0), €670.5(170.0) and €646.8(171.5) for A-H respectively. Compared to baseline, intra-patient standardised costs at 0–12 months post switch were mean (SD) €31.0(257.9) higher in H-A ($n=246$) and €168.6(342.2) lower in A-H ($n=36$).
Conclusions
In a cohort of Greek diabetic patients switching short-acting insulin A-H, total mean direct costs were lower in the 6–12 month period post-switch than in the 6 month period prior to the switch, although standard deviations are wide. Mean HbA1c decreased following switch in both groups. Substantially more patients were switched to analogues. Results should be interpreted in the context of an observational study.
Figure 1
Declaration of interest
I fully declare a conflict of interest. Details below.
Funding
This work was supported, however funding details unavailable.




## P725

**Inpatient Hypoglycaemia Management**

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Introduction
Inpatient hypoglycaemia management remains a significant challenge. An audit in 2009 revealed poor understanding ("recognition" by frontline staff) of the need to treat and manage hypoglycaemia. Consequently a management protocol, documentation sheet and hypoboxes with standardized quantities (15 g) of dextrose in various forms were introduced throughout the hospital.
Aims
We re-audited inpatient hypoglycaemia management and the impact of the standardized hypo-boxes and guidelines.
Methods
For the purposes of these audits, hypoglycaemia, was defined as capillary blood glucose (CBG) $\leq 4$ mmol/l and "Recognition" by staff as identifying the low CBG and initiating treatment. On a single day, 126 diabetes inpatients were identified, of whom $n=33$ had $\geqq 1$ episode(s) of hypoglycaemia totaling 62 events, as evidenced by their CBG. Data relating to 'recognition' and

management of the event(s) by the staff was collected and compared with the 2009 audit.
Results
Similar numbers of inpatients with diabetes experienced hypoglycaemia in 2009 ($n=32/120$) and 2011 ($n=33/126$), but the total episodes decreased from 79 to 62. Frontline staff failed to "recognize" hypoglycaemia in $n=12$ (36% of 33). Only 64% of the initial and 27% of the subsequent management was appropriate despite the new management tools. Recognition and management was better when events were recurrent (100% at $>4$ recurrences). Recovery from hypoglycaemia was confirmed in 24% in 2011 vs only 6% in 2009. Documentation in notes; ranged from 12% with one event and 55% with $\geqq 4$ events compared to 3% and 13% in 2009. Medication-review and diabetes-team referral improved by 6% and 15% respectively. Introduction of the hypo-box was considered beneficial by 73% of frontline staff.
Conclusions
Introduction of hypoboxes and guidelines have led to improved reassessment and documentation after treatment and the benefit reflected by the fewer number of recurrent hypos. Further guidelines to proactively decrease the incidence of hypoglycaemia are planned.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P726

**Study of the outcomes of application of ISPAS versus ADA guidelines of diabetic Ketoacidosis in type 1 diabetic children**

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Diabetic ketoacodosis (DKA) is a serious complication of diabetes mellitus, especially type 1, and its secondary consequences account for a large proportion of diabetes-related hospitalizations and mortality in children with type 1 diabetes. Aim of the work: The aim of this study was to compare between the outcomes of application of ISPAD (International Society of Pediatric & Adolescence Diabetes) versus ADA, (American Diabetes Association) guidelines for management of diabetic ketoacodosis in type 1 diabetic children attending the National Institute oof Diabetes & endocrinology in Cairo, Egypt (NIDE). Each protocol had been applied on 100 dibetic children with DKA with no significant difference between both groups as regarding, age, sex, acidity represented by ph. and serum bicarbonate and anion gap or coma score. The results showed that there was no statistical difference as regarding all outcome results as duration of recovery of acidosis or dehydration or the morbidity or mortality results. While the results of the change in potassium was better in ISPAD protocol. The Net results showed that there is no great difference between both groups. It could be recommended to apply any of the ISPAD or ADA protocols for managment of DKA in type 1 diabetic children but it will be more benificial to delay the insulin therapy for 1 hour after giving the intravenous fluid therapy as had been recommended by the ISPAD guidelines.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P727

**Subcutaneous use of rapid insulin analog: an alternative treatment for patients with mild to moderate diabetic ketoacidosis**

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NIDE, Cairo, Egypt.

Background
Diabetic ketoacidosis (DKA) is a life-threatening condition that requires hospitalization in children with type 1 diabetes. Many reports have indicated that low-dose insulin therapy is quite effective regardless of the route of administration, whether, intramuscular, or subcutaneous.
Aim of the work
The aim of this study was to look for technical simplification and economic efficiency in the treatment of diabetic ketoacidosis (DKA) with subcutaneous use of the rapid-acting insulin analog and compare its use with regular intravenous insulin treatment.
Subjects & methods
A total of 80 consecutive patients admitted with DKA were randomly classified into 4 groups: Group 1: Patients with DKA on regular insulin by infusion pump, (infusion pump, $n=20$), group 2: Patients with DKA on subcutaneous rapid onset of action-aspart insulin analog (Novolog; Novo Nordisk) / 2 hrs, sc-2 hr, $n=20$), group 3: Patients with DKA on subcutaneous rapid onset of action-aspart insulin analog (Novolog; Novo Nordisk) / 1 hrs, (sc-1 hr, $n=20$), and group 4: Patients with DKA on rapid insulin analog by continues subcutaneous insulin pump (CSII, $n=20$).
Results
The results of this study showed that there was no statistical difference between the 4 groups as regarding age in years, blood glucose in mg/dl before the starting of management of DKA, pH, serum HCO3, serum K, anion gap, urine acetone and time needed for resolution of DKA by hours.
Conclusion
It could be concluded that management of any patient with mild to moderate DKA with good tissue perfusion can be treated with subcutaneous rapid insulin analog every 1 or 2 hours or to be treated by continuous insulin infusion pumps, if available with same results as giving regular insulin by intravenous infusion pumps.
Diabetic ketoacidosis (DKA), Insulin analog and continues subcutaneous insulin infusion (CSII).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P728

**Planning Treatment of Diabetic Patients with Chronic Renal Disease Using Pre-mixed Insulins Having Higher Proportion of Soluble Insulin**

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Diabetes mellitus is a leading cause of chronic renal insufficiency and later end-stage renal failure; on the other hand, chronic renal disease has been linked with alterations in carbohydrate and insulin metabolism. The pharmacokinetics of various insulin preparations has not been well studied in patients with varying degrees of renal dysfunction. There exist sparse guidelines regarding appropriate dosing adjustment of insulin. Further, chronic renal insufficiency is associated with variably impaired insulin sensitivity. These patients may show about 2.5-fold increase of insulin elimination half-life. There are erratic insulin secretory patterns, as well. In summary, CRF is associated with a complex disruption of the processes of insulin release and resistance; its metabolism and elimination.
These facts are important while treating the diabetic patients suffering from a variable chronic renal deficiency, because they will entail adequate therapy adjustments in a patient with declining renal function decline. The rational insulin therapy will improve glycaemic control, a reduced incidence of hypoglycaemia and a retarded progression to end-stage renal disease. The adequate glycaemic control has also been associated with a reduced cardiovascular morbidity and mortality in these patients.
There exists opinion that in patients with end-stage renal disease, long-acting insulin preparations should be avoided because of increased hypoglycaemic events. A rapid acting insulin preparation on the other hand may entail less than desirable uniform blood sugar control. In this background, treating the diabetic patients with chronic renal disease using premixed insulin preparations having a higher proportion of soluble (rapid acting) insulin may be most plausible option.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P729

### Study on cases with hypoglycemia carried to the emergency room in the last two years

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Recently, many cases with hypoglycemia who take oral hypoglycemic agents and/or insulin therapy are carried to the emergency room in our hospital due to hypoglycemia. Pharmacotherapy for patients with diabetes mellitus occasionally can causes severe hypoglycemic attack. In this study, we investigated 69 type 2 diabetics on clinical features (26 outpatients of our hospital, 43 outpatients of other clinics) among 100 patients who were carried to the emergency room due to hypoglycemia from October, 2009 to September, 2011 to reveal the cause of hypoglycemia that required the treatment in medical facilities. Age (79.2±8.8 vs 70.4±12.6 years old) and the prevalence of pharmacotherapy with sulfonyl urea (SU, 65.8 vs 26.7%) are significantly higher in cases of HbA1c<7.0% than in cases of HbA1c≧7.0%. Patients needed to be hospitalized have significantly higher than those needed not to be hospitalized in age (78.1±9.8 vs 71.9±12.8 years old), and in the prevalence of pharmacotherapy with SU (64.7 vs 31.4%). Outpatients of other clinics have significantly lower HbA1c value than outpatients of our hospital (6.64± 0.93 vs 7.55±1.33%), and rate of the prevalence of pharmacotherapy with SU significantly higher than outpatients of our hospital(69.8 vs 11.5%).
Pharmacotherapy with SU is widely practiced for the treatment with type 2 diabetics. However, in elder patients strict glycemic control by SU may increase the risk of hypoglycemia. It is suggested that we should pay attention to the risk of hypoglycemia due to pharmacotherapy, especially with SU, and effort to direct the patient's attention to the risk of hypoglycemia and guide how to take measure to hypoglycemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P730

### Treatment of obese type 2 Diabetes Mellitus patients with CSII Humulin U-500 Regular Insulin infusion vs. CSII Aspart insulin infusion coupled with basal insulin and metformin

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We describe 10 obese patients with longstanding type 2 diabetes mellitus treated with a combination of basal insulin coupled with insulin Aspart and oral metformin. Average age was 59.5 years. Average BMI was 31.82 and Hemoglobin A1c 8.71%. Average Total Daily insulin dose (TDD) was 157.1 IU. EGFR was >60 in all patients. No patient underwent weight reduction surgery.Patients were randomized into 2 groups of 5 patients each. One group(1) received only Humulin U-500 Regular insulin by (CSII) via Animas pump. The other group(2) was randomized to receive 2/3 of the basal insulin dose coupled with CSII insulin Aspart via Animas pump and continuation of metformin. The patients in group1 also received the CGMS Dexcom sensor. Duration of the study was 15 months. At the end of the study, the average BMI was 33.47, average A1c hemoglobin 7.14% and TDD was 145 IU in group 1 vs. 35.28 BMI, 7.26% A1c hemoglobin and 149.2 TDD in group 2. (table 1). Average incidence of hypoglycemia was 8% in group1 vs. 4% in group 2.
Conclusion: Treatment of significantly obese type 2 diabetes mellitus patients with Humulin U-500 Regular insulin via insulin pump can lead to signicant improvement in glycemic control and at a statisticallly significant weight advantage over a combination of continous insulin Aspart coupled with adjuvant basal insulin and metformin. The additional hypoglycemia could be prevented by adding a CGMS to the treatment algorithm.
TDD is expressed in 100 unt/ml (for U-500 units divide by 5) A+L+M=Aspart CSII +Lantus or Levemir+Metformin WCM=White Caucasian Male AAM= African American Male Lantus, Levemir, Humulin U-500, Animas, Dexcom are all Registered Trademarks.

**Table 1** Trial Data U-500 Insulin CSII VS. Aspart CSII+Basal Insulin+Metformin

| Patient | Race/ sex | Initial BMI | Initial a1C | Initial TDD (IU/) | BMI Post Treat-ment | A1c post treat-ment | TDD post treat-ment | Treatment type |
|---|---|---|---|---|---|---|---|---|
| 1 | WCM | 29.04 | 8.0 | 135 | 31.50 | 7.2 | 142 | U-500 |
| 2 | AAM | 30.0 | 9.6 | 190 | 34.52 | 7.5 | 168 | A+L+M |
| 3 | WCM | 32.0 | 9.2 | 180 | 34.20 | 7.5 | 160 | U-500 |
| 4 | WCM | 31.5 | 8.5 | 150 | 33.68 | 7.3 | 138 | A+L+M |
| 5 | AAM | 33.0 | 8.7 | 168 | 33.51 | 6.9 | 158 | U-500 |
| 6 | WCM | 31.0 | 9.8 | 115 | 33.81 | 7.4 | 148 | A+L+M |
| 7 | WCM | 33.4 | 8.9 | 175 | 32.50 | 6.8 | 135 | U-500 |
| 8 | WCM | 32.8 | 7.9 | 148 | 36.81 | 7.2 | 152 | A+L+M |
| 9 | WCM | 31.5 | 8.4 | 138 | 35.66 | 7.3 | 130 | U-500 |
| 10 | WCM | 34.0 | 8.1 | 172 | 37.58 | 6.9 | 140 | A+L+M |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P731

### Effects of DPP-IV inhibitors on the changes of glycemic and lipid control of type 2 diabetes patients at the great east Japan earthquake

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Purpose
The effects of the Great East Japan Earthquake, a life-threatening event, on glycemic and lipid control in type 2 diabetic patients were examined. The influence of the DPP-IV inhibitors on glycemic and lipid levels was studied.
Method
Three hundred fifty type 2 diabetes outpatients (age 63 years old) who followed careful diet regimen on a normal condition were divided into the two groups: the administrated group (A group), 175 patients who were given DPP-IV inhibitors (Sitagliptin, Vildagliptin, Alogliptin) at least three months before the earthquake and the non-administrated group (NA group), 175 patients were given not given. Sulfonylurea and/or α-glucosidase inhibitor were used in some patients in both groups. All patients were able to have meals and visit our hospital before and after the earthquake. Their meal contents were confirmed by hearing and glycemic and lipid levels were examined.
Results
Before the earthquake, there were no differences in levels of fasting plasma glucose (FPG), HbA1c, triglyceride (TG), HDL-C, LDL-C in the two groups. One month after the earthquake, in both groups, the 77% patients increased intake of carbohydrates compared with the normal condition due to emergency food and stress. The transient increases of FPG and TG occurred after one month of the earthquake. However, the increases of the values were significantly lower in A group than those in NA group (0% vs. 3%, 6% vs. 17%, $P<0.05$), and the HDL-C significantly increased in A group. The LDL-C, HbA1c and body weight revealed tendencies of decline in the two groups.
Conclusion
These results suggest an association between life-threatening stress and the worsening of metabolic control in the type 2 diabetes patients. The DPP-IV inhibitors may possess ability to improve the unfavorable changes of FPG and TG to some extent.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P732

### Exenatide versus insulin glargine in type 2 diabetes inadequately treated with metformin

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Introduction
The recent ADA and EASD algorithm for the treatment of type 2 DM suggests addition of basal insulin or GLP-1 agonist to pre-existing treatment when glycemic control is not achieved.
Aim
Compare efficacy and safety of exenatide (E) versus insulin Glargine (G) added after metformin monotherapy.
Patients and method
Our study was open labeled, non- randomized, retrospective and included 48 patients, 17 men/ 31 women, mean age 61.98±8.8 years, mean duration of DM 11.8±7.25 years, and mean HbA1c 8.34±1.63 at baseline. All patients were on

metformin (1700 mg/day) for at least 3 months when E or G was added. Body mass index (BMI), systolic (SBP) and diastolic (DBP) pressure, frequency and severity of hypoglycemic episodes, gastrointestinal side effects, HbA1c and lipid profile were determined at baseline and after 24 weeks.
Results
HbA1c reduction was similar in both groups (E: $P=0.006$ vs G: $P=0.010$). G group had more hypoglycemias ($P=0.039$). E group had greater BMI reduction than G ($-2.5\pm1.8$ vs $0.1\pm1.4$; $P=0.002$). GI side effects and changes in SBP/DBP were insignificant in both groups. Total cholesterol was reduced (E: $P=0.010$ vs G: $P=0.014$). E group had higher HDL $P=0.021$, lower LDL ($P=0.012$) and triglycerides ($P=0.016$) at the end of the study.
Conclusion
Exenatide equals Glargine in glycemic control with fewer hypoglycemias and better metabolic parameters. In order to form selection criteria between the two strategies further testing is needed with randomized studies, longer observation period and greater numbers of patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P733

**Vitamin D3 deficiency effect on the HbA1c in type 2 diabetic Population**

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Introduction
Aim is to determine vitamin D level in patients with Type 2 DM and to evaluate the relationship between vitamin D level and glycemic control.
Methods
We evaluated 107 women and 63 male patients with Type 2 DM whose mean age was $55\pm0.81$. Patients fasting blood glucose (FBG), postprandial blood glucose (PPBG), HbA1c and 25(OH)D3 level were measured. The cases were divided into three groups according to measured 25(OH)D3 level (Vitamin D value $\leq$20 ng/ml, between 20–30 ng/ml, $\geqq$30 ng/ml). DM less than 6 months duration, hypercalcemia or hypocalcemia, existing diagnoses of hyperparathyroidism or hypoparathyroidism, receving vitamin D treatment were excluded.
Results
Vitamin D level was found $\leq$20 ng/ml in 41 cases, between 20–30 ng/ml in 50 cases and $\geqq$30 ng/ml in 79 cases. Mean 25(OH)D3 level was found as 38.10 ng/ml (11 to 68.7 ng/ml) in men and 24.20 ng/ml (5.8 to 70.7 ng/ml) in female ($P$:0.001). Between the vitamin D groups, no significant difference was founding in terms of the FBG, PPBG and HbA1c levels ($P$:0.97, 0.96, 0.44). Between the vitamin D groups, in regard to age, BMI and waist circumference, significant differences were detected ($P<0.001$). There was a significant difference between calcium (Ca) levels between these 3 groups ($P$: 0.02). Vitamin D level was higher in the group with HbA1c $\leq7$ than the group with HbA1c$>$ 7 ($P$: 0.115).
Conclusion
A53% of the patients with Type 2 DM was found to have vitamin D deficiency and insufficiency. Vitamin D deficiency was more evident in women than in men. Although there was not any difference in terms of blood glucose regulations between the patients with Vitamin D deficiency or insufficiency and without deficiency, vitamin D level was found to be higher in patients with HbA1c$\leq$7.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P734

**Salsalate effect on BRACHIAL FLOW-MEDIATED DILATION in early diagnosed Diabetes mellitus**

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Objective
Obesity and fat deposition in tissues along with inflammatory response may induce insulin resistance and finally type 2 diabetes mellitus Salsalate, a prodrug form of salicylate can inhibit IKKβ and NF-kappaB inflammatory pathway as a potential pharmacologic target in diabetes. The aim of this study was to determine the efficacy of salsalate as an anti-inflammatory drug to resolve endothelial dysfunction in diabetic patients.
Methods
This was a double blind controlled trial study. Forty newly diagnosed type 2 diabetic patients (30 to 45 years of age) were randomized in the drug and placebo groups. The drug group received 3 g Salsalate per day(two 750 mg tablets every 12 hours orally) for one month. The placebo group received identical placebo. Fasting plasma glucose level was assessed in two groups before and after treatment period. Endothelial function was assessed via flow mediated dilation (FMD) of the brachial artery following reactive hyperemia before and after treatment period in two groups.
Results
Thirteen patients in the drug group and 15 ones in the placebo group finished the study. At baseline, there was no significant difference in mean fasting plasma glucose level (120 vs. 122 mg/dl, $P=0.621$) and FMD ($10.5\pm5.2$ vs. $10.2\pm5.4\%$, $P=0.19$) between drug and placebo groups, respectively. Salsalate reduced the fasting glucose level in the drug group (18 mg/dl) significantly, in comparison with the placebo group ($P<0.05$). At the end of the trial, FMD in the salsalate and placebo group was $11.5\pm5.6$ vs. $10.1\pm5.3\%$, respectively ($P=0.09$).
Discussion
This study showed that daily use of 3 grams salsalate for one month reduced 15.5% of baseline blood glucose level in diabetic cases. However, endothelial dysfunction did not change significantly. It might be because of the short duration of the study. We suggest further studies with longer treatment duration and controlling other factors of insulin resistance, should be done to investigate the role of salsalate in resolving the endothelial dysfunction in diabetic patients.
Conclusion
These data demonstrate that salsalate improves glucose homeostasis, but endothelial dysfunction did not change.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P735

**Differences in health related resource use and costs before and in 24 months after insulin initiation in patients with type 2 diabetes: final data from instigate study**

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Aims
We present final Hellenic data from the INSTIGATE study on direct costs of care and resource use, over the 24 months after insulin initiation, in type 2 diabetic (T2D) patients.
Methods
INSTIGATE, a prospective European observational study involving T2D patients who initiated insulin during usual care, assessed direct costs of care over the 6 months prior to and for the intervals from baseline to 6 months, 6–12, 12–18 and 18–24 months after insulin initiation.
Costs of health related resource uses, ancillaries, oral anti-diabetic medication-s/insulin and the cost of hospitalization were analyzed after collecting data on individual resource use and assigning local unit costs (from 2006).
Results
237 patients at baseline, 6 and 12 month visits and 229 and 227 patients at 18 and 24 months respectively were followed.
Mean total contacts with Health Care Professionals were 7.5 per patient during the 6 months prior to insulin initiation and 9.8, 5.4, 4.8 and 4.4 during the baseline to 6, 6–12, 12–18 and 18–24 month periods respectively. Although mean total contacts decreased from baseline to 24 months, increase in visits to specialists was shown (mean from 1.9 to 3.2 visits).
Numerical increase in median total costs per patient was observed at the first six months following insulin initiation. Cost of insulin was 199–224€. In the first 6 months median costs of oral anti-diabetic treatment reduced from baseline to 6 months (from 197 to 17€) and remained low while costs for Blood Glucose Monitoring increased sharply (from 60 to 146€) but somewhat lower at 24 months (121€).
Conclusions
In the first 6 months following insulin initiation, we observed more frequent use of specialist care and increase of diabetes related costs. After the first 6 months health care resource use as well as total cost of diabetes care slightly decreased.

Declaration of interest
I fully declare a conflict of interest. Details below
Funding
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## P736

**Clinical Findings on the Effect of Early Insulin Therapy in Newly Diagnosed Type 2 Diabetes**

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Several recent studies have shown that early intensive insulin therapy can improve β-cell function in newly diagnosed type 2 diabetes. However, in clinical practice, insulin therapy has not been widely used in out-patients due to the fact that insulin therapy is perceived by the patients as the strongest and the last remedy and patients fear that, once on that therapy, they will have to remain on it for life. In fact, based on our clinical data, out-patients, who were newly diagnosed type 2 diabetes with HbA1c > 9% or fasting plasma glucose > 250 mg/dl and agreed to insulin therapy, could successfully shift to oral antidiabetic drugs within 6 months' of insulin therapy. After the termination of insulin therapy, almost all of those patients can maintain optimal glycemic control (HbA1c < 7.0) for many years with metformin only and some with combination use of metformin and sitagliptin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P737

**The effect of nighttime glargine with morning NPH in type 2 DM.**

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Recently basal insulin is the first recommended step when failed with oral medication, and adding morning NPH is one of step 1 intensive insulin treatment regimen when failed with basal insulin alone and post-lunch glucose level is high. But morning NPH or premixed insulin alone was the usual first step before basal insulin was available. When once a day morning insulin failed to reach target, next option was splitting twice a day injection. But many patients don't agree to pre-supper insulin because they frequently eat out due to their social environment. Also frequent nighttime hypoglycemia was troublesome when pre-supper insulin was added. Long acting basal insulin is very efficient in controlling fasting glucose and safe in terms of hypoglycemia because fasting glucose is not variable when same amount of it was given at same time compared to NPH or premixed insulin. So we tried to evaluate the effectiveness and compliance of morning NPH with night-time basal insulin in type 2 DM.
We recruited 2 groups of morning NPH and nighttime glargine therary.
The group 1 was 45 type 2 DM who failed target with NPH alone and their HbA1c was above 7.5%. They were asked to add nighttime glargine with morning NPH. The dose of glargine was adjusted by fasting glucose below 120 mg/dL.
The group 2 was 63 who failed target with glargine alone and HbA1c level above 7.5%. They were directed to add morning NPH to control postprandial glucose and NPH dose was changed to control post lunch glucose level below 180 mg/dL. Their HgA1c level 6 months after changing was compared with HgA1c level just before changing and incidence of hypoglycemic event was also compared.
In 44 out of 45 group 1, HgA1c was improved by between 0.3% and 3.5%. The minor hypoglycemia was increased in some patients (30% of all), especially when their previous fasting glucose was not so high. Their compliance to twice a day insulin injection was very good and they rarely skip nighttime insulin injection. In 61 out of 63 group 2, HgA1c was improved by between 0.2% and 2.1%. The frequency of daytime hypoglycemia was increased in most patients after adding morning NPH, but major hypoglycemia was rare.
In conclusion, morning NPH and nighttime glargine therapy is very efficient in improving glucose control and safe in major hypoglycemic events.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P738

**Telemedicine and Type 1 diabetes: A pilot project**

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Type 1 diabetes is among the top rising problems in public health, since its incidence is growing in almost all series studied, all over the world. The gold standard for its control is based on achieving good metabolic control, through insulin, diet and regular physical activities, and it is delivered by a multi-professional team in tertiary centers, which have enough resources (material and personnel). Scheduled visits are ideally programmed three to four times annually, but many patients, especially in a country like Brazil, with great distances between small villages and towns without specialists and tertiary centers make the task very hard to accomplish, with direct effect on the metabolic control of these patients. The availability of a structure for videoconferences between a tertiary center and health centers in geographically distant regional relevant cities allowed the project to be designed. It is based in training small teams with updated protocols of assistance to diabetic children and adolescents, and monitoring its assistance by following its register in computerized registers, that will be shared electronically with the tertiary center. There will be also weekly live videoconferences between the tertiary center and the health provider, in order to discuss specific issues, difficult cases and even update protocols. This project is about to start its activities, since the chosen city (Caicó), which is about 280 km away from Natal (our tertiary center), has already received all the hardware, while the local teams' training is scheduled to early 2012. We firmly believe that this project has a strong potential to change the reality of many diabetic patients that are even without basic follow-up. After the implementation of this program, other pathologies (from endocrinology or other pediatric areas) can also benefit from this project in the near future.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P739

**The State of Therapeutic Patient Education in Diabetes**

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Background: It is impossible to provide qualified diabetes care without patient education (TPE). Effect of education on treatment outcomes was demonstrated in 1972. Unfortunately in 1970-ies we lived in society, where many progressive ideas were not acceptable. Though progress at the end of 1980-ies leading Centers started to implement TPE. Methods: In Georgia first "5-day in-patient education course" was initiated. It was carried out at our Center. The Course was based on Berger/Jorgens program. Books for type 1 and 2 DM (T1/T2 DM) were first translated into Georgian. Past 20-yrs were hard period for Georgia - economic, energy, political crises, healthcare system reorganization; no insurance system slowed integration into global processes. Due to objective reasons, regular, obligatory TPE in T2DM is not carried out, though individual training is performed. Since 2000 we realized that only TPE could not improve treatment outcomes; professional education (medical students, Society) also need attention. Results: In 2009–2010 Ministry of Health of Abkhazia carried out a 2-yr. Screening/Management Program for T1/T2DM. Around 100 people were enrolled in total. Education was an integral part of the program. Conversation Maps (CM) were used during a 4-day education course. To accelerate the process of establishing TPE and training educators, an Expert Training for healthcare providers from Georgia and other Eastern European countries is initiated this year together with IDF-Global and IDF-Europe. Number of Expert Trainers will be trained for each country. It will permit to create a basis for professional diabetes education in our country and in the region. Conclusion: Diabetes education is a complex system, where all elements are equally important. If only TPE is addressed - we will face reality where "educated patient is too expensive". Thus, main goal is to change mentality of the whole society. This needs time and effort.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P740

**Diabetes mellitus management in elderly over 80 years old.**

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Introduction
The prevalence of diabetes mellitus increases with age in all population. With the demographic transition and population aging, diabetes mellitus will be a major public health problem in all countries.
Aims: to study the specific management of diabetes in very old subjects with multiple co morbidities and functional disabilities.
Patients and Methods
We described a population of 199 diabetic patients aged 80 years and over consecutively admitted in the department of endocrinology from August 2007 to August 2011.
Results
patients are hospitalized in emergency and particularly with diabetic ketoacidosis or hyperglycemic hyperosmolar state. 12 patients were newly diagnosed with type 1 diabetes mellitus On 104 patients who were hospitalized for chronic complications of diabetes mellitus 75 subjects had diabetic foot ulcers.
We described the characteristics of diabetes and its management and therapeutic goals in the elderly over 80 years old...
The management of very elderly patients with any type of diabetes requires careful consideration. Advancing age and changes in health status can have on the competing risks and benefits of therapeutic interventions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P741

**Improving health numeracy: utilizing objective parameters to improve shared decision making for insulin initiation**

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Background
Health numeracy is the ability to understand, and act upon quantitative data, in an appropriate and effective manner. Health numeracy is distinct from health literacy. Health numeracy is associated with better self-management.
Aims and objectives
This study was performed at two endocrine centres in north India, to assess and improve the health numeracy of patients with type 2 diabetes, with an aim to improving the quality of shared decision making (SDM).
Material and methods
A three months long campaign was begun to explain the meaning of HbA1c, vibration perception threshold and mean plasma glucose to 1000 patients presenting with T2DM in the OPD. This study was carried out by laboratory technologists, by means of semi-structured training, at each OPD visit.
Results
At baseline, 24.5, 11.8 and 45.0% of patients understood the meaning of HbA1c, mean vibration perception threshold (VPT) and mean plasma glucose (MPG) respectively. At three months, these proportions had increased to 56.6, 90.0 and 67.3% respectively.
During these three months, 351 patients were initiated on various regimes of insulin, of which 250 consented to fill a pre structured questionnaire. 60.0% of them felt that understanding their HbA1c value had played a major role in motivating them to begin insulin. Similar response was given by 80.0% for mean VPT and 100% for MPG.
Conclusion
This paper highlights the efficacy of improving health numeracy as a means of enhancing shared decision making, and facilitating insulin initiation in type 2 diabetes. It reveals the higher importance of mean plasma glucose values, rather than HbA1C and mean VPT in motivating patients to accept insulin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P742

**Challenges and problems in the management of glycemia in patients with co-existing diabetes mellitus and a malignant disease in the Gerontology Institute - Hospice "Sue Ryder"-Skopje in the period of 2009–2011**

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Gerontology Institute 13 November Skopje, Skopje, The Former Yugoslav Republic of Macedonia.

Objectives
Pointing out all the problems and challenges that the medical staff encounters in the management of the patients with a terminal malignant disease with co-existing diabetes mellitus.
Methods
Medical documentation, laboratory results, US (Ultra Sound) etc.
Results
The study included patients hospitalized in the Gerontology Institute - Hospice "Sue Ryder"-Skopje, with a capacity of 75 beds, in the period of 2009–2011. During that period the institute hospitalized 212 patients, 35 of which had co-existing diabetes mellitus and a malignancy. All of the 35 patients were on an insulin therapy (Novomix, Mixtard 30, Insulatard, Levemir etc.) and a diabetic food regime. Most of the patients were with disseminated metastatic deposits in the lungs, liver, brain and bones.
Conclusions
Despite the insulin therapy and the diet regime it was not possible to regulate the glycemia in these patients with a severe illness. The glycemia reached a score of 29,0 mmol/L in some patients. There were no sings of hyperglycemia, which is unlike to happen in patients with Diabetes mellitus without co-existing malignancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P743

**Efficacy and Safety of Insulin Glargine in Type-2 Diabetes Mellitus Patients with Moderate Renal Failure**

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Background and aims
Due to increasing risk of hypoglycemia, tight control of glycemia is difficult to achieve in the diabetic patients with diabetic nephropathy. Also, there are no absolute guidelines defining appropriate dosing adjustments of insulin in such patients. This study was aimed to determine the safety and efficacy of insulin glargine in type 2 diabetic patients with diabetic nephropathy.
Methods
A total of 89 subjects with type 2 diabetes (mean age $62.9 \pm 10.7$ and diabetes duration $13.9 \pm 7.6$ years) who had moderate renal failure (mean GFR $34.4 \pm 11.6$ ml/min) were included in the study. All the patients had been receiving insulin glargine at bedtime after discontinuing all oral hypoglycemic agents and other types of human insulin. Doses were begun with 0.1 U/kg and adjusted to obtain target fasting glucose (5–7.2 mmol/l). The medical records obtained before and 2 and 4 months after beginning insulin glargine.
Results
At the end of 4 months of treatment, significant reduction in HbA1c was observed (from $8.4\% \pm 1.6$ to $7.7\% \pm 1.2$) ($P < 0.001$). The treatments were associated with significant reductions in fasting glucose levels (from $159.7 \pm 67$ to $119.4 \pm 28.4$ mg/dl) ($P < 0.001$). No increment in body mass index (BMI) was seen at the end of 4 months of therapy ($26.2 \pm 3.9$ and $26.2 \pm 3.8$ kg/m2) ($P = 0.966$). Mild symptomatic hypoglycemia was seen in 12.5% of subjects. No other side effects were noted throughout the study.
Conclusion
Insulin glargine improved HbA1c at short-term and proved to be safe and well tolerated in type 2 diabetic patients with diabetic nephropathy.
Keywords: Insulin glargine, diabetic nephropathy, hypoglycemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P744

**The weight reduction could be achieved on DPP-IV inhibitors in very obese T2D patients**

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Incretin-based therapy can improve glucose control in type 2 diabetes (T2D) with no weight gain. Usually dipeptidyl peptidase-IV (DPP-IV) inhibitors considered as weight neutral and glucagon-like peptide-1 receptor agonists are associated with weight loss. Some trials showed non-remarkable weight-lowering effect on DPP-IV inhibitors. We aimed to identify patients on DPP-IV inhibitors which are likely to achieve both outcomes either good glycaemic control or weight loss.
We studied 89 (58F/31M) T2D drug-naïve patients treated with metformin 1000–2000 mg/24 plus sitagliptin 100 mg/24 for 26 weeks. The age was 57.1 + 6.5 y (M + SD), A1c − 8.4 + 1.0%. 44 patients with weight more then 100 kg (Group 1) were compared with 45 controls from Group 2 (weight less then 100 kg). Groups were matched for gender and age.
At the beginning patients from Group 1 on average were approximately 25 kg heavier: 113.3 + 6.49 kg vs. 88.2 + 6.13 kg in Group 2 (BMI 39.1 + 3.25 kg/m2 vs. 30.4 + 3.28 kg/$m^2$ respectively). After 26 weeks of treatment A1c not differed between groups (7.20 + 0.18% vs. 7.15 + 0.20%). At the same time change in weight in Group 1 reached (−)3.2 kg (from 113.27 + 6.49 kg to 110.05 + 6.96 kg) and was significant. In Group 2 twice less weight lowering effect was observed (from 88.22 + 6.13 kg to 86.56 + 6.23 kg) and there was no significant difference. Moreover, weight reduction more then 3% (from 4 up to 14 kg) was seen in 18 from 44 patients (40.9%) in Group 1 and only in 10 (from 3 to 6 kg) from 45 patients (22.2%) in Group 2 ($P < 0.05$).
We concluded the glycaemic control in T2D patients on DPP-IV inhibitors could be associated with remarkable weight loss. The composite outcome is more likely to be achieved in very obese persons.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P745

**Diabetes Mellitus Control Status of Turkish Diabetic Patients**

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Introduction
Our aim was to collect demographic data of diabetes mellitus (DM) patients to determine the patients education, follow-up and treatment priorities.
Material-Method
500 patients with diagnosis of DM followed up at least 6 months were included the study. Datas containing patients demographic characteristics, treatment modalities, biochemical parameters were retrospectively reviewed. On the day of admission patients; fasting blood glucose (FBG), postprandial blood glucose (PPBG), and glycated hemoglobin (HbA1c), creatinine, albumin/creatinine ratio electrolytes (Na, K), and lipid parameters were recorded
Results
Mean age and BMI was 56.6 ± 11.8 years, and 31.7 ± 6.9 kg/m respectively. 97% of cohort had Type 2 DM. DM duration was 9.3 ± 7.3 years in Type 1 and 8.1 ± 7.2 years in Type 2. 20% of them were being treated with insulin, 26.5% with insulin and oral antidiabetics (OAD) and 53.5% with OAD. There was a significant relationship between the form of treatment and duration of diabetes ($P < 0.001$). Insulin usage increases with increased DM duration. 31.9% retinopathy, 50.6% neuropathy, 37.1% nephropathy, 70.8% hypertension (HT), 69.8% hypercholesterolemia was detected. Chronic complications of DM were seen lesser in patients receiving OAD (63.4%) than receiving insulin or insulin and OAD (%85). Complication rates were increasing with aging ($P < 0,001$) and longer duration of DM ($P < 0,001$). The mean HbA1c was 8.1 ± 2. Only 32% of patients had HbA1c < 7%. As duration of DM prolonged, the ratio of well metabolic control were decreased ($P < 0.001$). Metabolic control of the insulin users was worse than others ($P < 0.001$).
Conclusion
These findings shows that metabolic control levels and follows-up of diabetics are generally poor. There is numerous medical and social factors that affect metabolic control so both of the patients and health personel should give adequate consideration to this matter.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P746

**Insulin analogues in the treatment of diabetes**

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Nowadays the human insulines and insuline analogues are used in the treatment of diabetes.
The Advantages of modern insulines
Better glycemic control;
Reduction of hypoglycemia
The reduction of the weight;
Low variability of developing of the complication.
The objective of the survey is to come up to the specific functional benefits about modern insulines through the data analysis, and through researching of
Hemoglobine A1C
The presence og hypoglycemia
Fasting blood glucose.
There were analyzed 40 patients aged 50–80 years in the period of 5 months, who were transferred from a human to modern insulin analogues. Of these, 22 were men and 18 were women.
Conclusion
- The research was done on 40 patients (22 males, 18 females).
- The transfer from human to modern insuline was done on 37 patients although on 3 patients that transfer was done to insuline glargine.
The reason of this transfer was the enormous increasing of their weight.
- According to the hypoglycemias there was reduction of their number despite their glycemic control.
Besides this only two patient complained on the major hypoglycemia and the main reason for this was not taking their meal during the day.
The measurement and the values of AB A1C showed that only 5 patients had hemoglobine A1C higher then 9.
Fasting blood glucose refered from 6.5 mmol/l to 7.9 mmol/l.
Keywords: insuline analogues, Hemoglobine A1C, hypoglycemia, hyperglycemia.
Declaration of interest
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**Table 1** Major and minor hypoglycaemia

| hypoglycemia | Number of patients (at beginning) | Number of patients (after 20 weeks) |
|---|---|---|
| Minor | 26 (65%) | 35 (87.5%) |
| Minor and major | 13 (32.5%) | 2 (5%) |
| Does not had | 1 (2.5%) | 3 (7.5%) |
| Total | 40 | 40 |

## Endocrine Disruptors

### P747

**Effects of octylphenol and bisphenol A on the expressions of calcium transport genes during pregnancy in mice**

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Introduction
Octylphenol (OP) is a degradation product of alkylphenol ethoxylates that are widely used to in rubber, pesticides and paints. Bisphenol A (BPA) is an organic compound with two functional phenol groups and used to make polycarbonate plastic and epoxy resins, along with other applications. OP and BPA are known as endocrine disruptors that can induce inappropriate estrogenic action, and may disturb natural calcium metabolism. In the present study, the effects of OP and BPA on the calcium levels of serum and urine, and calcium transport genes were investigated in the duodenum, kidney and placenta of the pregnant mice.
Methods
From 6.5 to 16.5 days after post coitus (dpc), pregnant mice were orally given with ethylestradiol (EE, 0.2 mg/kg/day), OP (15, 45, or 135 mg/kg/day) or BPA (5 or 50 mg/kg/day) dissolved in corn oil. The duodenum, kidney, placenta, blood and urine were obtained from mice at day 18.5 of pregnancy.
Results
As a result, serum and urinary calcium levels were decreased by OP and BPA in a dose dependent manner. The expression levels of calcium transport genes, TRPV6, TRPV5 and CaBP-9k were decreased in the kidney after treatment with OP and BPA, while duodenal expression of TRPV6 was reduced by high a dose of BPA. In the placenta, the gene expressions of TRPV6 and CaBP-9k were induced in the mice treated with OP, whereas it was not altered by BPA.
Conclusion
OP and BPA altered gene expressions of calcium transport in the pregnant mice, which may cause reduced serum and urine calcium levels. These results suggest that estrogenic actions of OP and BPA may lead to influence the calcium levels during pregnancy in the mice.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### P748

**Effects of Quercetin on mRNA expression of Steroidogenesis genes in Primary Cultures of Interstitial Leydig cells treated with Atrazine**

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The mechanisms of reproductive malfunction of male mammals caused by atrazine (ATZ) remain unknown. To explore the effects of ATZ on the expressions of StAR (steroidogenic acute regulatory protein), CYP (cytochrome-P450) 11A1, CYP17A1, 3β-HSD (3β-hydroxysteroid dehydrogenase), INSL-3 (insulin-like factor 3), AR (androgen receptor), ER (estrogen receptor), LHR (luteinising hormone receptor) and INH-α (inhibin-alpha), we isolated Leydig cells from healthy immature rats (18–20 days Wistar rats), set up Leydig cell cultures, evaluated the toxicity, and measured the expression levels of mRNA of tested genes by real-time polymerase chain reaction method after cultured Leydig cells were exposed *in vitro* to ATZ (232 μM) for 6 h. The results showed that the survival rate of Leydig cells decreased sharply with ATZ which could be blocked by a phytochemical agent, quercetin (50 μM). The mRNA expression of the tested genes increased with ATZ treatment which could be block by quercetin except AR and ER expressions. Furthermore, the phytochemical agent (15–50 μM) caused a dose-dependent increase in both AR and ER mRNA expressions, suggesting stimulatory effects of QT on AR and ER-responsive signaling. The expressions of these genes were unaffected by cyclic-AMP when the stimulatory effects of ATZ on the tested genes were sustained. These findings suggest that ATZ may stimulate the expression of the tested steroidogenesis genes via a mechanism independent of cyclic-AMP signalling which could be blocked by QT. In addition, QT might promote the survival of interstitial Leydig cells exposed to ATZ.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### P749

**Octylphenol and triclosan induced proliferation of human breast cancer cells via an estrogen receptor-mediated signaling *in vitro***

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The class of endocrine disrupting chemicals (EDCs) is comprised of both naturally occurring and man-made compounds which are structurally similar or distinct to estrogen, and can interfere with the actions of an endogenous steroid hormone via estrogen receptors (ERs). Octylphenol (OP) is alkyl phenol chemical, which is considered to be a low-level endocrine disruptor owing to its tendency to mimic estrogen. It has been reported that triclosan can cause endocrine disruption and has weak estrogenic properties with binding to ERs. In this study, we examined that these EDCs, which have an estrogenic activity, can induce cell growth of human breast cancer cells via an ER-dependent signaling pathway by using the cell proliferation assay, semi-quantitative RT-PCR and Western blot analysis. Treatments of MCF-7 breast cancer cells with OP and triclosan resulted in the stimulation of their cell gorwth and induced the alteration of transcriptional and translational levels of cell cycle-related genes. These OP and triclosan increased the proliferation of MCF-7 breast cancer cells in a dose-dependent manner, similar to 17-beta estradiol (E2). We also found that they caused the induction of cyclin D1 gene and reduction of p21 gene at tranlational levels. It can be assummed that the alterations of genes associated with G1/S transition in MCF-7 cells by OP and triclosan may result in the increase of cell growth. However, cell growth and alteration of genes caused by OP and tricalosan can be reversed by an ER antagonist, ICI 182,780, in these cells. An activation of an ERa signaling pathway by propyl pyrazoletriol (PPT) can promote the stimulatory effect of OP and triclosan on the alteration of transcriptional regulation in cell cycle. To ensure the effect of these EDCs on ERa, we knowdowned ERa by siRNA in MCF-7 cells. When ERa level was knockdowned in these cells, the effects of these EDCs on the upregulation of cyclin D1 and downregulation of p21 disappeared. Taken together, OP and triclosan can lead to stimulate breast cancer cell growth via the alterations of cyclin D1 and p21 genes in human breast cancer cells through an ER-mediated signaling pathway. A further study is required to determine the effects of EDCs on breast cancer carcinogenesis *in vivo*.
Declaration of interest
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### P750

**Endocrine disrupting chemicals induced alteration of cell cycle related genes resulting in proliferation of human breast cancer cells via an estrogen receptor-mediated signaling pathway *in vitro***

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Endocrine disrupting chemicals (EDCs) have brougt the growing concern about the potential to interfere with the endocrine system and to cause health problems. Among EDCs, bisphenol A (BPA) and 1,1,1-trichloro-2,2-bis(4-methoxyphenol)-ethane (methoxychlor, MXC) can bind to estrogen receptors (ERs) and lead abnormality of ER depenent signaling pathways, resulting in human health problems such as dysfuction of reproduction and carcinogenesis. In this study, we investigated whether BPA and MXC promote transition of cell cycle and the cell growth by ER binding in human breast cancer cells, MCF-7 cells. We further examined the alterations of the cell cycle -related genes in MCF-7 cells following treatments with BPA or MXC, compared to a vehicle (EtOH). Both BPA and MXC induced up-regulation of cyclin D1 and down-regulation of p21. To determine whether BPA and MXC increase the growth of MCF-7 cells though an ER signaling pathway, we co-treated MCF-7 with agonists of ER signaling pathways (propyl pyrazoletriol, PPT, and diarylpropionitrile, DPN) or an antagonist of ER (ICI 182,780) in the presence of BPA or MXC. The effect of BPA and MXC on the breast cancer cell growth was enhanced in the presence of PPT in MCF-7 cells. The expressions of cyclin D1 and p21 were altered by BPA or MXC in the presence of PPT, but it was less altered by them in the presence of DPN. We knockdowned ERa gene expression via siRNA in MCF-7 cells before EDCs treatment. When the expression level of ERa was knockdowned in MCF-7 cells, effects of BPA and MXC were lost on the expression levels of cyclin D1 and p21 genes. Taken together, these results indicate that BPA and MXC altered the

gene expressions associated with cell cycle, especially in G1/S transition, and resulted in the stimulation of breast cancer cell growth via an ERa signaling pathway. These collective results confirm that these EDCs may have the carcinogenicity via an ER-mediated signaling pathway in vitro. A futher study is required to determine whether EDCs may have a potency to be carcinogenic in human breast cancer cells.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P751

**Bisphenol A and phthalate stimulated the growth of human prostate cancer cells and altered downstream target genes of ras signaling pathway**

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Prostate cancer have been most cause of cancer death to men in recent, but there are not effective treatments for this cancer. In growth of prostate cancer, steroid hormones such as androgen play a role in their growth and survival. However, endocrine disrupting chemicals (EDCs) intend to bind the hormone receptor and induce the abnormal hormone response. EDCs have potency to activate estrogen receptor (ER) and androgen receptor (AR) mediated signaling pathways, and they cause the human health problem including hormone-dependent carcinogenesis. Bisphenol A (BPA) has the phenolic structure with the effect on the endocrine system and promotes human breast cancer. Recent studies have reported that phthalates have a potency to disrupt reproductive system in male rodents. Thus, we identified in this study whether BPA and dibutyl phthalate (DBP) stimulate the cell growth of prostate cancer cells, LNCaP cells, which have both ERs and ARs. We evaluated proliferation of LNCaP cells following BPA and DBP treatment using a cell viability assay. Both BPA and DBP promoted LNCaP cells proliferation over two-fold at $10^{-7}$ M to $10^{-5}$ M compared to a vehicle. Further, we examined the alteration of the downstream target genes of ras signaling pathways by using the semi-quantitative RT-PCR. Like 17b-estradiol (E2) and dihydrotestosterone (DHP), the treatments with BPA and DBP resulted in a significant increase in the transcriptional levels of c-myc and c-fos in LNCaP cells at 30 min to 6 h, compared to a vehicle. In addition, these EDCs induced cyclin D1, a cell cycle related gene, at 6 h after treatment. Taken together, these results suggest that BPA and phthalate can alter gene expressions of downstream targets associated with ras signaling and cell cycle related pathways in prostate cancer cells. A novel effect of EDCs may result in the cell growth of human prostate cancer cells. A further study warranties to determine the potential of EDCs in the carcinogenesis of prostate cancer. This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of Korea government (no. 2011-0015385).

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## P752

**Treatment with resveratrol resulted in an inhibition of cell proliferation induced by 17 β-estradiol or various endocrine disrupting chemicals via down-regulating the cell cycle progression in BG-1 ovarian cancer cells**

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Resveratrol (trans-3,4,5-trihydroxystilbene, RES), a phytoestrogen, exists in grape skin and red wine. Endocrine disrupting chemicals (EDCs) appear to promote the development and progression of the estrogen dependant cancers. In this study, we evaluated the inhibitory effect of RES on the cell growth and progression induced by various EDCs in BG-1 ovarian cancer cells expressing estrogen receptors (ERs). The various EDCs, i.e., bisphenol A (BPA), nonylphenol (NP), octylphenol (OP), methoxychlor (MXC), and hexabromocyclododecane (HBCD) were employed in this study. In the *in vitro* experiments, treatments of BG-1 cells with E2, BPA, NP, OP, MXC, or HBCD resulted in an increase of their growth. The treatment of BG-1 cells with ICI 182,780, a well known antagonist of ERs, reversed EDCs-induced cell growth in these cells, indicating that their growth-stimulatory effect is mediated through ERs. In addition, we evaluated the effect of RES in the presence of other EDCs by MTT assay. As a result, increased cell viability induced by these EDCs was reversed when co-cultured with RES. In addition, we further examined the regulation of cell cycle-dependent genes by RT-PCR. Concretely, the treatment with each EDC only decreased the gene expression of p21 and increased the expression of cell cycle-dependent kinase 2 (CDK2). However, co-treatment with RES and one of EDCs resulted in the increased gene expressions of p21 and the decreased expression of CDK2. Cyclin D1 was increased by down regulating p21 when only treated with each EDC in the absence of RES, while co-treatment with RES and each EDC decreased the gene expression of cyclin D1 by upregulating p21. Taken together, these results indicate that RES appears to be an inhibitor of Cyclin D1 and CDK2 and is responsible for the cell cycle arrest at G1 phase. In addition, when co-treated with each EDC, RES increased the expressions of p21 and resulted in the growth inhibition of BG-1 ovarian cancer cells. As a result, we confirmed the cell growth inhibitory effect of RES, a dietary phytoestrogen, on the estrogen-dependant ovarian cancer cells prompted by EDCs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P753

**Perinatal exposure to a commercial formulation of glyphosate reduces the mRNA expression and increases the protein content of beta TSH in the pituitary of male offspring.**

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Glyphosate is a broad spectrum herbicide that is effective against weeds, especially in association with transgenic glyphosate resistant crop systems, and represents approximately 30% of all herbicides used in agriculture. It was demonstrated that glyphosate has an activity of endocrine-chemical disruptor (ECD) in the reproductive axis, altering the production of testosterone and causing disturbances on the reproductive development of male offspring from dams treated during the gestational period. The hypothalamic-pituitary-thyroid axis is quite sensitive to be a target of many ECDs, but it is not known if glyphosate may affect it. To evaluate this possibility 90-day-old female Wistar rats were mated in a monogamous couples and the beginning of gestation (GD1 – gestational day) was confirmed by vaginal smear containing spermatozoa. Glyphosate Roundup Transorb was diluted in a watery suspension and administered to the mothers once a day, p.o. from GD18 to PND5 (post natal day) (50 mg/kg or 0 mg/kg for control group). The male offspring were decapitated at PND60 and the pituitaries submitted to analysis of beta TSH and alpha subunit mRNA by RT/qPCR and to protein content by Western Blotting. The genes RPL19 and GAPDH were used as internal control and the results were analyzed by Student's *t*-test ($P<0.05$). Perinatal exposure to glyphosate decreased the content of beta TSH mRNA and increased its protein content in the pituitary, without altering the expression of alpha subunit. These results show for the first time the effects of glyphosate in the pituitary-thyroid axis and demonstrated that the EDC effect of glyphosate is not restricted to the reproductive axis. It is not known whether the dose used in this study is in fact the levels of exposure of population to the glyphosate herbicide. However, it was shown that women occupationally exposed to this herbicide have reproductive disorders and may also need necessary to consider in the future the possibility of disturbances in the thyroid function.

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## P754

**Synergistic effects of bisphenol A and paraben on the induction of calbindin-D9k and progesterone receptors via an estrogen receptor pathway in rat pituitary GH3 cells**

E. Jung, K. Choi & E. Jeung
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Introduction
There are concerns about the combined estrogenic effects of endocrine disrupting chemicals (EDCs), since mixtures of these chemicals exist in our environment. This study investigated potential synergistic interactions between bisphenol A (BPA) and isobutylparaben (IBP), which are major xenoestrogens used in the manufacture of plastics, cosmetics, drugs, and other products. The combined effects of these two chemicals were analyzed by measuring expression of calbindin-D9k (CaBP-9k) in the rat pituitary GH3 cells.
Methods
GH3 cells were treated with single and combination doses of both chemicals (BPA single doses: $10^{-7}$, $10^{-6}$ and $10^{-5}$ M; IBP single doses: $10^{-7}$, $10^{-6}$ and $10^{-5}$ M, and each of BPA and IBP doses combined). Prior to treatment, the cells were temporarily transfected with a plasmid containing an estrogen response element (ERE). A luciferase activity was measured as an indicator of ERE activation by 17β-estradiol (E2), BPA, and IBP.
Results
In addition to E2, EDC induced a significant increase in luciferase activity. Twenty-four hours after treatment, dose-dependent effects were observed in both single and combined groups, and several combined doses induced a synergistic increase in the expression of CaBP-9k and progesterone receptor (PR) at both transcriptional and translational levels. Pre-treatment with fulvestrant, a pure estrogen antagonist, significantly reversed EDC-induced CaBP-9k and PR upregulation in GH3 cells.
Conclusions
Our results indicate that BPA and IBP may have synergistically increased estrogenic potency via an estrogen receptor-mediated pathway.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P755

**Benzophenone-1 promoted cell growth of BG-1 ovarian cancer cells with estrogen receptors via progression of cell cycle**

M. Park, H. Lee, E. Jeung & K. Choi
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Benzophenones are organic chemicals widely used as stabilizers to UV light irradiation in plastic surface coatings and food packaging. They have been known that their derivatives appear to have cytotoxic effects. 2-Hydroxy-4-methoxybenzophenone (Benzophenone-3, BP-3) and its metabolite, 2,4-dihydroxybenzophenone (Benzophenone-1, BP-1), are used mostly in the formulation of nail polishes, enamels, bath products, sunscreens and skin care products. In this study, the estrogenic effects of BP-1 were examined in an ovarian cancer BG-1 cells expressing high levels of estrogen receptors (ERs) by an cell viability assay, semi-quantitative reverse-transcription PCR (semi-quantitative RT-PCR) and Western blot analysis. The treatment of BG-1 cells with BP-1 ($10^{-8} \sim 10^{-5}$ M) resulted in an increase in their cell proliferation as 17-beta estradiol (E2) did. But, the cell growth stimulation by BP-1 was reversed by cotreatment with ICI 182,780, an ERs antagonist, suggesting that the proliferation of BG-1 cells is mediated by an ER-dependent pathway. In addition, BP-1 upregulated the expression levels of cell-cycle regulating genes, i.e., cyclin D1, which is a downstream target of ER, at 6 h after treatment. But, the expression of p21 gene was not altered by BP-1 at any time points. In translational levels, BP-1 also upregulated the expression level of cyclin D1 at 24 h after its treatment. Taken together, these results suggest that BP-1 is one of EDCs with have apparent estrogenic activities by stimulating cell proliferation of BG-1 cells and by inducing the expression of cyclin D1. Our results can support that BP-1 may have a potency to disrupt endocrine system and to stimulate cell growth in ER-positive cancer cells. [This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of Korea government (no. 2011-0015385).]
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## P756

**A phytoestrogen, genistein, reversed 17 β-estradiol or bisphenol A-induced cell growth via downregulation of the cell cycle progression in BG-1 ovarian cancer cells**

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One of estrogens, 17b-estradiol (E2), is a pleiotropic hormone that regulates the growth and differentiation of many tissues and also acts as a mitogen that promotes the development and proliferation of hormone-responsive cancers. Bisphenol A (BPA) is a widely used industrial compound and classified as one of endocrine disrupting chemicals (EDCs) and especially a xenoestrogen that imitates estrogen in living organisms. In this study, we examined the effect of a phytoestrogen, genistein, on the cell growth of BG-1 ovarian cancer cells expressing estrogen receptors (ERs) caused by E2 and BPA. In the cell proliferation test in vitro, E2 or BPA increased the growth of the BG-1 ovarian cancer cells expressing ERs. Their proliferation activity was reversed by the treatment of ICI 182,780, a well-known antagonist of ERs, which demonstrates that the cell proliferation by E2 or BPA is mediated by ERs and BPA certainly acts as a xenoestrogen in the BG-1 ovarian cancer cells. Genistein, an isoflavone, is one of phytoestrogens that are plant-derived, naturally occurring, and dietary xenoestrogens and influences multiple biochemical functions. In this study, genistein effectively suppressed the BG-1 cell proliferation induced by E2 or BPA by adversely down regulating the cell cycle progression that was upregulated by E2 or BPA. Concretely, E2 or BPA decreased the gene expression of p21, which is a potent cyclin-dependent kinase (Cdk) inhibitor and responsible for the cell cycle arrest at G1 phase, to proliferate the BG-1 cells. On the other hand, genistein upregulated the expression of p21 gene cultured in the presence of E2 or BPA, leading to the growth inhibition of the BG-1 cells. Also, the alteration of p21 gene expression by E2, BPA, or genistein affected the expression of its downstream genes of cell cycle, cyclin D1 and Cdk-4. Taken together from these results, we may suggest an anticancer effect of genistein, a dietary phytoestrogen, on the estrogen-dependant cancers like ovarian cancer prompted by E2 or BPA. [This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of Korea government (no. 2011-0015385)]
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## P757

**Proliferative and apoptotic effects of bisphenol A and its combination with estradiol in human breast carcinoma cells**

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Bisphenol A (BPA) is an endocrine disruptor (ED) used as plasticiser present in plastic products of daily use. The leakage of BPA monomer into food, drinks represents the ubiquitous exposure to the general population. BPA stimulatory effect on breast cancer cells growth *in vitro* and increased incidence of hormone dependent malignancies suspected BPA from carcinogenic effect.
The aim of this study was to investigate the effects of BPA alone and in the combination with estradiol (E2, 110-12M, COM) on cell proliferation, DNA synthesis and proteins of apoptosis. The time (24, 48, 72h) and dose ($1\times10^{-15}$–$1\times10^{-6}$ M) dependent effects were compared to E2 in breast carcinoma cells MCF7. Cells were maintained in RPMI 1640 medium containing 5% dextran-charcoal-treated FBS. MTT test was used in proliferation experiments, BrdU incorporation into newly synthesized DNA was analyzed. The expressions of proapoptotic Bax, p53 and antiapoptotic Bcl-2 proteins were determined by Western blot.

Higher BPA concentrations moderately (~130%), but significantly increased proliferation (vs COM) after 48h as the consequence of previous (24 h) huge (180–220%) increase of de novo DNA synthesis (vs E2, COM). The mixture E2 (10–12 M)+BPA induced 140–160% increase of DNA synthesis (vs BPA, E2) after 48 h, followed by enhanced proliferation (~140%, vs BPA, E2) after 72 h thus exceeding effects of individual compounds. The mixture had neither additive, nor synergic effect.
E2 (48 h) reduced p53 (by 20%) while BPA displayed a slight stimulation; E2+BPA inhibited p53 as E2. Similar tendencies of changes were observed in Bax protein. No modifications in Bcl-2 was present except moderate increase by BPA (vs E2 and COM) after 48 h.
BPA and mixture BPA+E2 stimulate MCF7 cells proliferation after preceding elevated DNA synthesis and by protein modification(s) of apoptosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P758

**Parabens inhibit ovarian follicle development of neonatal female rats**
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Introduction
Parabens, esters of para-hydroxybenzoic acid, are widely used as an antimicrobial agent in the cosmetic products and pharmaceutical industries. Recently, it has been reported that parabens including methyl-, ethyl-, propyl-, butyl-, isopropyl-, and isobutylparaben act as xenoestrogens, a class of endocrine disruptors. In this study, we hypothesized that parabens may disrupt ovarian follicle maturation in female rats during the neonatal period.
Methods
Fifty-five female neonatal rats were divided into eleven groups, and were given daily subcutaneous injection, with methyl-, propyl- and butylparaben (62.5, 250, or 1000 mg/kg/day) at neonatal 1–7 days. The total mRNA and protein were extracted from the ovaries excised at the postnatal day 8 of female rats, and the levels of AMH mRNA and protein were analyzed by real-time PCR and Western blot analysis. The ovaries were also fixed and stained with H&E for histological analysis.
Results
The regulation of AMH gene expression was modulated by propyl-, butyl-parabens and methyl-paraben. The mRNA and protein levels of AMH significantly were increased by treatment of butylparaben while they were decreased by methyl-paraben. The number of follicles was counted and classified into primordial, primary, or secondary stages after serial sections of the ovary. The histological results showed that total number of follicles and primordial follicles was increased by butylparaben compared to a negative control.
Conclusion
Butylparaben may have an estrogenic effect to disrupt ovarian follicle development in the neonatal female rat.
Declaration of interest
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## P759

**Bisphenol-A exposure from electronic gadgets: A risk assessment**
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We are living in the electronic era and day to day exposed by estrogenic disruptors such as Bisphenol-A (BPA). Electronic gadgets such as cellular phones, CDs, DVDs, RFIDs even baby feeding bottles are made with composition of BPA and their huge application are matter of debate in present. The chronic exposures of BPA have become a major health concern. In this study we assessed the neurobehavioral alterations caused by BPA in the rats. We examined the neurobehavioral changes in Albino Wister rats of (190±20) g body weight. Two groups of rats, control and BPA treated (4 mg/kg body weight in corn oil), were used to find the effects of BPA. Neuro-behavioural studies were carried out as per plan after the last dose of treatment. A set of five rats randomly selected from each treatment group was used to assess spontaneous locomotor activity 24 h after the last dose of treatment. The same set of rats was used to measure grip strength, 1 h after the spontaneous locomotor activity test. Locomotor activity parameters were assessed in control and BPA treated rats at age of 90 and 120 days. Exposure to BPA in rats caused a decrease in total distance travelled (42%), stereotypic time (52%), time moving (38%), rearing (44%) and an increase in resting time (17%) as compared to rats in the control group. Finally we observed that BPA exposure (4 mg/kg b.wt.) cause neurobehaviour alterations in rats. The chronic exposure of BPA may cause neurotoxicity and that can result mental problems like weakness, memory, cognitive and locomotor dysfunction etc.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P760

**Unconjugated bisphenol A cord blood levels in boys with descended or undescended testes**
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Background
Human toxicity of bisphenol-A (BPA), a weak estrogenic environmental endocrine disruptor widely used in plastics, babybottles, cans and dental sealants, is under investigation. Human pharmacokinetics involves a rapid inactivation by hepatic glucuronyl- or sulfoconjugation and renal clearance. Fetal or perinatal exposure in rodents is associated with programmed adult reproductive diseases. Human epidemiological studies remain scarce especially concerning testicular development.
Methods
Using a radioimmunoassay performed after extraction, validated by HPLC and mass spectrometry, active, unconjugated BPA (uBPA) cord blood (CB) levels were measured in 152 boys born after 34 weeks of amenorrhea with or without descended testes.
Results
Active uBPA was detectable in all CB samples with values in the control group (N=106) between 0.14 and 4.76 ng/ml, median: 0.9 ng/ml; mean±SD: 1.12 ng/ml±0.86 ng/ml. uBPA in controls correlated with CB inhibin B ($P<0.01$) and total testosterone ($P<0.05$) and maternal milk polychlorinated bisphenyl (PCB) 138 ($P<0.03$). uBPA did not correlate with clinical maternal or fetal parameters nor with other steroid or polypeptidic CB hormones assessed. uBPA levels were not significantly different in cryptorchid boys (N=46): 1.26±1.13 ng/ml ($P=0.38$).
Conclusions
uBPA was present in all cord blood samples assessed, illustrating placenta transfer and foetal exposure due to either placental hydrolysis of maternal conjugated BPA and/or immaturity of fetal detoxification capacity. uBPA correlation with testosterone CB levels could be in relation to androgen dependent regulation of glucuronyltransferase gene expression. Lack of difference of cord blood uBPA levels between control and cryptorchid groups in this prospective study, makes unlikely the participation of fetal exposure to uBPA in the physiopathology of undescended testes. However the observed uBPA concentrations (nM) similar to those able to induce relevant effects in vivo in rodents or *in vitro* in human cancer cell lines, invite to further assess epidemiological relationship between cord blood uBPA and other human reproductive or not reproductive diseases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P761

**Measurement of estrogen receptor alpha homodimerization caused by xenoestrogens using bimolecular fluorescence complementation**

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Introduction
After binding its ligand, a conformational change in estrogen receptor alpha (ERα) occurs, leading to subsequent homodimerization of two monomeric ERs. The dimer then binds to specific DNA-elements or to other transcription factors already bound on gene promoters to regulate target gene expression. Protein-fragment complementation assays in general are simple tools to monitor protein–protein interactions and are useful to visualize the subcellular sites of interactions in living cells. In case of bimolecular fluorescence complementation (BiFC), two fragments of a fluorescent protein (e.g. YFP) are genetically fused to proteins of interest. The single fragments show no fluorescence, but when the proteins mutually interact, the fluorescent protein refolds and gives a fluorescence signal. Here we aimed to use BiFC to monitor ERα homodimerization after stimulation with several estrogenic substances present in human environment.
Methods
To monitor ERα dimerization we fused full length ERα to the carboxyterminal and aminoterminal fragments of the citrine variant of YFP and cotransfected these constructs transiently into COS-7 cells. After stimulation with estrogens, fluorescence was measured in a photometer or visualized by confocal microscopy.
Results and Conclusions
Compared to mock transfected cells cotransfection caused a visible fluorescence signal in the nuclei of transfected cells. Stimulation of the cells with the ERα agonists 17β-estradiol (E2), bisphenol A, genistein and butylparabene but also with the antagonist fulvestrant (ICI) and the selective estrogen receptor modulator 4-hydroxytamoxifen (4-OHT) dose dependently increased fluorescence signals. Interestingly, ICI and 4-OHT stimulation caused higher fluorescence signals than E2. If this is caused by a higher amount of dimers or by conformational differences remains to be shown. Except stimulation with ICI, which translocated ERα-dimers into cytoplasma, fluorescence signals were only seen in the nuclei. The partial ERα agonist 4-bethylbenzylidene camphor as well as the heavy metal cadmium caused no increase of the fluorescence signal.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P762

**The effects of BPA exposure on fat mass and serum leptin concentrations have no impact on bone mineral densities in non-obese premenopausal women.**

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Objective
Bisphenol A (BPA) exposure may promote obesity, but its effect on bone mineral density (BMD) has not been reported in humans. In the present study, we aimed to examine the complex interplay between BPA exposure, fat mass, fat-free mass, serum estradiol, leptin, osteocalcin levels and BMDs in a group of premenopausal women.
Methods
A total of 246 Chinese premenopausal women aged 20 years and older with regular menstrual cycles were investigated. Body mass index (BMI), fat mass, fat-free mass and BMDs were measured by DXA. Serum estradiol, leptin, osteocalcin, urinary BPA and NTx levels were also tested.
Results
Urinary BPA levels were positively associated with fat mass ($r=0.193$, $P=0.006$) and leptin ($r=0.236$, $P=0.001$) but not with fat-free mass after adjusting for age and BMI. A multivariate stepwise regression analysis confirmed the positive association between BPA and leptin ($\beta=0.31$, $P<0.001$). Serum leptin levels were positively influenced by fat mass ($\beta=0.746$, $P<0.001$) and BPA ($\beta=0.127$, $P=0.01$) but negatively correlated with fat-free mass ($\beta=-0.196$, $P<0.001$). The changes of BMDs at the lumbar-spine ($\beta=0.298$, $P<0.001$) and femoral neck ($\beta=0.305$, $P<0.001$) were primarily explained by fat-free mass. BPA was not associated with either serum estradiol levels or BMDs. BPA was unrelated to the bone resorption marker, NTx, or the bone formation marker, osteocalcin.
Conclusions
BPA's effect on fat mass was mediated by leptin. The neutral effect of BPA on BMDs in premenopausal women is due to the exclusive role of fat-free mass, which is unrelated to BPA, rather than of fat mass or leptin in determining BMDs.
Declaration of interest
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## P763

**The effects of endocrine disruptor compounds on the hormone release of rat pituitary *in vitro***

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The endocrine disruptor compounds (EDC) (e.g. chlorobenzenes (ClB), phenurone (PU), monurone (MU), diurone (DU)) are accumulative, lipophilic, chemical pollutants that can disturb the homeostatic balance and exert tumorigenic effects. The aim of the present study was to investigate the effects of EDCs on the adrenocorticotropic hormone (ACTH) and prolactin (Prl) release in primary cell cultures derived from rat normal adenohypophysis and prolactinoma.
The prolactinoma formation was induced by estrone-acetate (2 mg/bw kg/day; subcutan for 6 months). The ten-day-old cell cultures, obtained from rat normal adenohypophysis and prolactinoma, were treated as follows: I. 0.1 μg/ml EDCs (ClB, MU, DU, PU) for 1 hour; II. 10 μg/ml arginine-vasopressine (AVP) for 1 hour in itself and subsequently 10 μg/ml corticosterone was also added; III. EDCs in combinations with AVP and corticosterone. ACTH and Prl levels were detected in supernatant media by radioimmunoassay. The protein content was measured by Protein Assay system.
The basal ACTH (1500 pg ACTH/mg protein) and Prl (7 ng Prl/mg protein) levels did not change significantly after the EDC treatment in the supernatant media of the adenohypophysis cell culture. The EDCs (ClB:14500; PU:11900; MU:13100; DU:13800 pg ACTH/mg protein) significantly increased the AVP induced ACTH release of normal adenohypophyseal cells (10200 pg ACTH/mg protein) but did not influence the effects of corticosterone. The prolactinoma cells showed increased secretions of ACTH (2200 pg ACTH/mg protein) and Prl (17,2 ng Prl/mg protein) compared with the normal adenohypophyseal cells. The basal ACTH and Prl levels in the supernatant media of the prolactinoma cells were significantly increased by the EDC treatments (ClB:2973; PU:2417; MU:2539; DU:2701 pg ACTH/mg protein; ClB:23.1; PU: 19.7; MU:21.5; DU:22.4 ng Prl/mg protein).
The ACTH and Prl synthesis and release capacity of normal adenohypophyseal and prolactinoma cells were altered by EDCs. This work was supported by: TAMOP 4.2.1/B-09/1/KONV-2010-0005; HURO/0901/037/2.2.2.
Declaration of interest
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## P764

**Activation of the enzymes of phase I (CYP1A1 and CYP2B1/2) and phase II (SULT1A and COMT) metabolism by BDE-47 in the ovary.**

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The aim of the current study was to determine whether the porcine ovary was capable of metabolizing polybrominated diphenyl ether – BDE-47, which is the most abundant congener found in wildlife and in humans. It have been showed by us that this congener disrupt ovarian function by direct action on steroidogenesis. In the current study we analyzed the activity and expression of enzymes involved in phase I (CYP1A1 and CYP2B1/2) and phase II (SULT1A and COMT) of BDE-47 metabolism. CYP1A1 and CYP2B1/2 activity was determinated by conversion of ethoxy- and pentoxyresorufin in resorufin respectively, using fluorescent method. Enzymes of II phase of metabolism were evaluated by colorimetric methods: SULT1A activity was based on sulfotransferase ability to release p-nitrophenol with the PAPS, and the activity of COMT, based on the ability of

catechol-O-methyltransferase to convert nitrocatechol (NC) for 3-metylnitrocatechol (2-methoxy-4-nitrophenol) with S-adenosylmethionine (SAM). Additionally, protein level was determined by immunoblot and ELISA. Basal CYP1A1 and CYP2B1/2 activity increased during culture. BDE-47 had no effect on CYP1A1 activity, however CYP2B1/2 activity increased after exposure for 1 and 6 h. Basal SULT1A activity was 2.5 fold lower than that of COMT, and both proteins were stable during culture. BDE-47 increased SULT1A activity after exposure for 6 and 12 h, and COMT activity increased after exposure for 24 and 48 h. For the first time we demonstrated SULT1A protein levels in pig ovary measured by immunoblot and ELISA analyses. BDE-47 had no effect on the expression of all investigated enzymes.
In conclusion, fast activation of CYP2B1/2 and late activation of COMT (with a very low basal SULT1A activity) indicates a possible action of locally produced hydroxylated metabolites prior to their detoxification.
Declaration of interest
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## P765

**Changes of hemostatic variables during cross-sex hormone treatment in transsexual people.**

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Introduction
Cross-sex hormone treatment (CHT) in male-to-female (M2F) transsexuals affects the hemostatic balance, with the emergence of a prothrombotic state with the use of certain estrogen (oral ethinyl estradiol vs 17β estradiol). Testosterone administration to F2M transsexuals had an antithrombotic effect. In female-to-male in (F2M) transsexuals administration of testosterone seems to carry a mild antithrombotic effect.
Objective
To quantify prevalence of alterations in the coagulation parameters in naive transsexuals people for CHT. To quantify changes of hemostatic variables after 12 months of physiology doses treatment: M2F (17βestradiol with cyproterone acetate/espyronolactone) and F2M (testosterone td, im).
Subjects and Methods
A prospective, observational study of 47 CHT-naive patients; available data of hemostatic balance in 15 patients (8 M2F, 7 F2M) baseline and after 12 months of treatment. Statistical analysis: descriptive statistics and prevalence; Wilcoxon test for paired data for changes in hemostatic state (SPSS 11.0 for windows).
Results
Prevalence of coagulation alterations 21%(IC95%12–35): 3 patients antiphospholipid antibody; 6 prothrombin gene G20210A mutations, 1 increased beta 2 glycoprotein. Antiplatelet treatment was initiated in two M2F (acetylsalicylic acid). Significant differences of hemostatic variables were demonstrated in M2F people on Trombin Time. There were not differences on F2M.
Conclusions
The prevalence of coagulopaties in transsexual people is moderated and similar to general population. The administration of 17βestradiol in combination with cyproterone acetate on M2F or testosterone on F2M it was not associated with changes of hemostatic balance.
Declaration of interest
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## P766

**The relation for normal fasting plasma glucose levels and metabolic syndrome in normoglycemic adults.**

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Background
Metabolic syndrome has been introduced to increase the incidence of cardiovascular diseases and type 2 diabetes mellitus. Our aim of this study was to investigate the relation for normal fasting plasma glucose levels and metabolic syndrome in normoglycemic adults.
Methods
The study subjects consisted of 636 individuals who visited the Center for Health Promotion in Pusan National University Hospital for a medical checkup in 2002 to 2005. Among 636 subjects, 469 subject who don't have metabolic syndrome and had normal fasting plasma glucose, were included and followed up for 3years. We measured fasting glucose, lipid profiles blood pressure and their metabolic components.
Results
As the quartile of plasma glucose, 3 year follow-up incidence of the metabolic syndrome was increased. Logistic regression analysis adjusting for sex, age, alcohol drinking status, smoking status, and physical activity showed that the odds ratio(95% confidence Interval, P-value) of each plasma glucose quartile was 1.127(0.549∼2.314, $P=0.744$), 2.131(1.121∼4.051, $P=0.021$), 3.235(1.682∼6.224, $P=0.000$), respectively
Conclusion
As shown above, These results showed that normal fasting plasma glucose level was closely related to the incidence of the metabolic syndrome.
Declaration of interest
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## P767

**Effects of neonatal exposure to thyroid hormones on gene expression profile of the pituitary-thyroid-liver axis in rats**

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Purpose
Disruption in thyroid hormone homeostasis by environmental chemicals during the prenatal and/or neonatal period might lead to developmental disorders since thyroid hormones (T3/T4) play a critical role in growth and the differentiation of many tissues including the central nervous system. Neonatally given T3 or T4 disrupts the thyroid hormone system resulting in decreased serum T4 level in the adulthood in rats. In the present study, changes in gene expression profile in the pituitary-thyroid-liver axis by neonatal hyperthyroidism were examined to identify possible neonatal effects of thyroid hormone disrupting chemicals.
Materials and Methods
Male F344 rats were exposed to T3, T4 or amiodarone (0.04, 4 and 0.4 mg/kg bw, respectively) at PND 1, 3 and 5. Animals were sacrificed at 8 weeks old and total RNAs were extracted from the pituitary, thyroid and liver. Expression of the genes related to the thyroid hormone homeostasis was measured by the quantitative RT-PCR method. Serum T3 and T4 were determined by ELISA.
Results and Conclusions
1) Neonatal hyperthyroidism resulted in lower serum T4 level in rats at 8 weeks old. 2) Expressions in thyroid hormone receptor alpha, T3 responsive genes (RLT3-2 and RLT3-24) and an enzyme related to T3/T4 metabolism, Sult1C3, increased in the thyroid and liver tissues in T3/T4 treated groups. 3) Amiodarone showed the similar effects on the gene expression profile with T3 albeit in a lesser extent. The present study demonstrated that neonatal exposure to thyroid hormones irreversibly altered the expression profile of the genes related to the thyroid hormone homeostasis. In addition, amiodarone, a chemical exhibiting thyroid hormone-like activity, showed the similar neonatal effects. The changes in gene expression profile in the pituitary-thyroid-liver axis could be markers for the neonatal effects by thyroid hormone disrupting chemicals.
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## P768

**Apoptosis induced by arsenic is partially reverted by antioxidants in anterior pituitary cells.**

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Exposure to arsenic (As) through consumption of contaminated water is considered one of the top environmental health threats worldwide. Human exposure to As has been associated with cancer of several organs, neurological disorders and reproductive problems. Nevertheless, there are no reports on the effect of As on anterior pituitary gland and little is known about the effect of As on hormone release. We have previously shown that 25 μM As (as sodium arsenite) reduces prolactin release and pituitary cell viability after 24 h. The aim of the present work was to study the mechanisms of arsenic citotoxicity in primary anterior pituitary cultures from male Wistar rats. Arsenic decreased cell viability from 9 h of treatment (MTT assay, Abs. 600 nm, % of control (C); 9 h: 83.6 2.8*, 18h: 82.2 5.4*, 24h: 70.3 2.6***; $*P<0.05$; $***P<0.001$ vs. C). Apoptotic morphology became evident after 18 and 24 h of As exposure (% apoptotic nuclei/total nuclei; C: 3.3 0.5, 9h: 4.9 1.2; 18h: 10.3 1.4**; 24h: 11.8 1.3**; $**P<0.01$ vs. C). Mitochondrial membrane potential (DiOC6 by flow citometry) was significantly reduced after 6 h of As exposure. In parallel, there was an early increase in reactive oxygen species levels during the first hour of As exposure (DHR 123 by flow citometry). Arsenic modified mRNA expression of stress response genes [relative units as % of C, heme oxygenase-1 (3h: 172; 6h: 128; 9h: 174); metallothionein-1 (3h: 174; 6h: 121; 9h: 160); nitric oxide synthase-1 (3h: 82; 6h: 71; 9h: 96)]. Antioxidant treatment with N-acetyl-cysteine (NAC) was able to partially reverse As-induced apoptosis (annexin V- propidium iodide staining, % of C, As: 243.6; As + NAC: 180.5). These results suggest that As citotoxic action on anterior pituitary cells is produced by an apoptotic process which can begin with an imbalance on cell redox status.

Declaration of interest

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## P769

**Nigella Sativa oil alleviating the reproductive toxicity of chlorpyrifos in male rat**

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Chlorpyrifos (CPF) is a worldwide used organophosphate insecticide for controlling many types of pests in the household, farm and residential settings. It has the potential to negatively affect the reproductive system and fertility. Nigella sativa has been used both as seeds or oil to promote health and fight disease. Hence, the present work was undertaken to evaluate the possible protective role of Nigella sativa oil (NSO) against chlorpyrifos induced reproductive toxicity in male rats. Thirty two adult male Wistar rats were randomly divided into 4 groups/8 of each and were daily treated for 30 consecutive days as follow: group I served as control, group II was orally administered Nigella sativa oil (NSO) (1 ml/kg/day); group III was orally given an emulsion solution of chlorpyrifos (CPF) at dose of 20 mg/kg/day, while group IV was pretreated with NSO (1 ml/kg/day) and after 30 min received CPF (20 mg/kg/day). In this experiment, body weight gain and relative weights of vital and reproductive organs, sperm characteristics, testosterone and free thyroxin (FT4) levels and histopathological changes in the testes were investigated. Results elicit that CPF induces a significant decrease in body weight gain (BWG), feed intake (FI), and the relative weight of testes (RTW) and epididymes (REW) whereas, an increase in the relative weight of liver (RLW), kidney (RKW) and adrenal gland (RAW) was noted. Besides, treatment with CPF affects markedly the semen characteristics that pronounced by a reduction in sperm count (SC) and spermatids number (SN), daily sperm production (DSP), sperm motility and viability, however abnormal sperm morphology was augmented. Serum testosterone and FT4 levels were significantly lowered in CPF-group. Histopathological examination of testes showed a decrease in the number of seminiferous tubules along by their shrinkage, enlargement of the connective tissue and gametogenic changes in germ cells. NSO co-administration with CPF reverses partly or completely the changes in the relative reproductive and vital organs weights, semen characteristics, hormones levels and the histopathological injuries of testes to the normal status and thereby improved semen quality. By the term of this research, we concluded that NSO can improve semen picture and moderate CPF-induced reproductive toxicity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P770

**The Interference Endocrinous – Metabolic in Thyro-gonadal Failure**

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It is known that the genetic similarity, embryonic and functional hypothalamic-pituitary-gonadal system has close correlation with hypothalamic-pituitary-thyroid axis. Recent research has suggested the existence of interactions between the gonad and the functional capacity of the thyroid.

The study was performed on 93 cases admitted to the Endocrinology Clinic during 2005–2011, whose age ranged from 16–44 years. Correlate with clinical features Thyro-gonadal function exploration objectified by hormonal and metabolic laboratory investigations performed in the context of specific clinical case. Note that all cases included in the study had an extra weight in varying degrees.

Hormonal assays results obtained by us show that overweight patients studied, ovary and thyroid functional capacity are significantly reduced. Reduced urinary excretion of estrogen, along with decreases in serum thyroid hormones, explaining increased serum cholesterol and β-lipoproteins and decreased progesterone involves a gradual increase serum lipids.

Conclusion

In view of relations between endocrinology and gynecology, comprehensive approach is required to study endocrino-metabolic correlation in thyro-ovarian failure, to adopt a differentiated therapeutic attitudes pathogens.

Declaration of interest

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## P771

**The Therapeutic Attitude Differentiated in Thyroadenitis**

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In the thyroid gland pathology, thyroadenitis prominently in their scope, whose etiologic diagnosis is often difficult to objectified and therapeutic attitudes are not yet codified today. mechanisms production in the thyroid thyroid lesions are still far from being elucidated, and concepts are still ongoing reshuffle. There is another explanation that can withstand a critical analysis of how the thyroid's self tolerance is repealed and the breaking of tolerance in a row followed by antithyroid antibodies and thyroid lesion.

Personal research were performed on 89 cases admitted to the Endocrinology Clinic in the period 2000–2009, whose age ranged from 15–68 years. The diagnosis was suggested by historical data and clinical and laboratory investigations confirmed the etiology of each case. Therapeutic attitudes were conditioned by the results of laboratory assays. He turned to antibiotics, corticosteroids, NSAIDs, antithyroid synthetic thyroid hormones or thyroidotomy.

The results show that the treatment of choice is thyroadenitis administration of glucocorticoid hormones. In subacute thyroiditis phase required a stunning combination of low doses of antithyroid synthetic beta-blockers. To prevent thyroid nodularisation phenomenon, it is desirable thyroid hormone replacement therapy when natural evolution of the disease remit to hypothyroidism. Surgical indication is considered appropriate only for cases resistant to other modes of therapeutic approach to cases abcedate.

Declaration of interest

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## P772

**Hypertension – Endocrinous Causes – Therapeutic Attitude**

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Hypertension is the most common cardiovascular disease in the mass population and one of the most important public health problems. Is recognized as a major risk factor for atherosclerosis, particularly coronary localized, brain, kidney and leading cause of cardiovascular morbidity and mortality in most industrialized countries and overall.
Our study was performed in 121 patients with endocrine disorders (hypercortisolism, acromegaly, hyperthyroidism, hypothyroidism), hospitalized in the Clinic of Endocrinology, which was assessed at the time of onset of hypertension and hypertension to monitor developments in relation to the evolutionary stage of endocrine disease. Objectivity endocrine disease diagnosis was based on correlative investigation: hormonal, imaging, electrophysiological and metabolic. It emerged following conclusion with practical implications: early diagnosis of endocrine disease and the establishment of adequate etiological therapy, is the prerequisite for prevention and monitoring of cardiovascular complications.
Declaration of interest
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# Endocrine tumours and neoplasia

## P773

**New insights into the molecular and cellular pathogenesis of human craniopharyngioma: do pituitary stem cells underlie the origin of these tumours?**

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Activating mutations in the gene encoding β-catenin have been identified in the paediatric form of human craniopharyngioma (adamantinomatous craniopharyngioma, ACP), an aggressive pituitary tumour accounting for up to 10% of paediatric intracranial tumours. Recently, we generated an ACP mouse model and revealed that, as in human ACP, nucleocytoplasmic accumulation of β-catenin (βcat-nc) and over-activation of the Wnt/β-catenin pathway occurs only in very few cells that form clusters. Here, combining mouse genetics, fluorescence labeling and flow-sorting techniques, we have isolated these cells from tumorigenic mouse pituitaries and show that the βcat-nc cells contain higher numbers of colony-forming cells when cultured in stem cell-promoting media, and longer telomeres, indicating shared properties with normal pituitary progenitors/stem cells (PSCs). Global gene profiling analysis has revealed that these βcat-nc cells express high levels of factors encoding secreted mitogenic signals, such as members of the SHH, BMP and FGF family, in addition to several chemokines and their receptors, suggesting an important non-cell autonomous role of these cells in the pathogenesis of ACP and a reciprocal communication with their environment. Through genetic approaches utilising inducible Cre mouse lines, we are targeting PSCs to exclusively sustain the activating mutation in β-catenin and assess if this leads to a pre-tumoral lesion. Alongside providing further support to the concept that PSCs may play an important role in the aetiology and/or pathogenesis of human ACP, our data reveal novel disease biomarkers and potential pharmacological targets for the treatment of these devastating childhood tumours.
Declaration of interest
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## P774

**Diagnostic and Prognostic Role of 68GA-Dotatate in Patients with Neuroendocrine Tumors**

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Octreoscan is currently the gold standard for diagnosis of somatostatin receptor positive NET but it is limited by a lower spatial resolution and physiological uptake noises. DOTATATE is a somatostatin analogue, radiolabelled with 68Ga and adapted for PET imaging.
Aim of this study was to evaluate the diagnostic performance of 68Ga-DOTATATE PET compared to Octreoscan in NET.
Fifty-one patients with NET (40 sporadic, 17 MEN1) of different origin were enrolled. 68Ga-DOTATATE PET was performed in all cases by acquiring whole body studies 40–60 min after the radioligand i.v. injection (74–111 MBq). Octreoscan was also performed in 27/51 patients using injection of Indium-111-DTPA-Phe1-Octreoscanreotide (120–200 MBq). Examinations results were rated considering histology, CT/MR examination and clinical follow-up.
The patient based sensitivity and specificity of PET was 77% and 100%,respectively; the lesion based sensitivity was 64% with a specificity of 85%. Positive and negative predictive values were 100% and 65%, respectively. The sensitivity was $\sim$100% in pancreas and gut, $\sim$75% in lung and thyroid, $<$50% in stomach. In the subgroup undergone both PET and Octreoscan, the result was concordant in 21 and discordant in 6 cases. The discordant cases were 5 PET positive/Octreoscan negative (3 pancreatic NET, 2 medullary thyroid cancer) and 1 PET negative/Octreoscan positive (atypical lung carcinoid). In PET-positive patients, a significant inverse correlation between SUVmax and Ki67 was recordered ($P<0.01$). At ROC analysis, SUVmax in patients with progressive disease was $\leq$17 while it was $>$17 in patients with stable or responsive disease ($P<0.01$). In patients with NET 68Ga-DOTATATE PET shows higher diagnostic performance than Octreoscan and is suggested to predict clinical behaviour of the tumor.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P775

**Meta-analysis of mRNA, microRNA expression and chromosome aberrations in phaeochromocytoma and neuroblastoma**

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Background
The pathogenesis of neural crest-derived tumours (phaeochromocytoma and neuroblastoma) is complex, and several studies applying functional genomics approaches have been performed in these tumours to date. However, the comparison of their genomic features has not been performed yet.
Objective
We have carried out an in silico meta-analysis of altogether 1784 neuroblastoma and 351 phaeochromocytoma samples to establish similarities and differences using analysis of mRNA and microRNA expression, chromosome aberrations and a novel bioinformatical analysis based on cooperative game theory.
Methods
Datasets obtained from Gene Expression Omnibus and Array Express have been subjected to a complex bioinformatics analysis using Gene Spring, Gene Set Enrichment Analysis, Ingenuity Pathway Analysis and own algorithms.
Results
Comparison of neuroblastoma and phaeochromocytoma with other tumours revealed the over expression of genes involved in development of noradrenergic cells. Among these, the significance of paired-like homeobox 2b (PHOX2B) in phaeochromocytoma has not been reported previously. The analysis of similar expression patterns in neuroblastoma and phaeochromocytoma revealed the same anti-apoptotic strategies in these tumours. Cancer regulation by stathmin turned out to be the major difference between phaeochromocytoma and neuroblastoma. Underexpression of genes involved in neuronal cell-cell interactions was observed in unfavourable neuroblastoma. By the comparison of hypoxia- and Ras-associated phaeochromocytoma, we have found that enhanced insulin like growth factor 1 signalling may be responsible for the activation of Src homology 2 domain containing transforming protein 1 (SHC1), the main co-factor of RET.

Hypoxia induced factor 1α (HIF1α) and vascular endothelial growth factor signalling included the most prominent gene expression changes between von Hippel-Lindau and multiple endocrine neoplasia type 2A-associated phaeochromocytoma and we have supplemented these previously described pathways with several new members.
Conclusions
These pathways include previously undescribed pathomechanisms of neuroblastoma and phaeochromocytoma and associated gene products may serve as diagnostic markers and therapeutic targets.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P776

**Metformin inhibits the androgen mediated up-regulation of Insulin Growth Factor-I Receptor in prostate cancer cells**
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Introduction
The insulin-like growth factor-I receptor (IGF-IR) plays a key role in regulating growth, survival and invasion of several human malignancies including prostate cancer. In prostate cancer cells, the IGF-IR expression is upregulated by androgens through a nongenotropic signaling unresponsive to antiandrogens and requiring AMP-response element-binding protein (CREB) activation. This mechanism sensitizes cells to the proliferative and protumor effects of IGF-I. Metformin, a widely used antidiabetic drug with pleiotropic activity, has been shown to be anti-mitogenic in several cancer cells, partially through the activation of AMP-activated protein kinase (AMPK).
Aim
We investigated whether metformin could affect androgen-induced IGF-IR upregulation in prostate cancer cells.
Methods and results
Using the androgen-sensitive LNCaP prostate cancer cells, we found that metformin specifically inhibits the androgen-mediated upregulation of IGF-IR mRNA and protein levels by blocking the mTOR/p70S6K pathway. These effects were only partially dependent on AMPK activation. Indeed, in the same cells, after knocking-down AMPK, metformin was still able to inhibit the IGF-IR upregulation induced by androgens. Furthermore, in 293HEK cells cotrasfected with constructs encoding for the androgen receptor and the full length IGF-IR promoter, metformin markedly reduced the androgen-stimulated IGF-IR promoter activity by inhibiting CRE activity and CREB phosphorylation at Ser133.
Conclusion
Metformin inhibits androgen-induced nongenotropic IGF-IR upregulation by acting through the inhibition of mTOR/p70S6K pathway, in a manner partially dependent on AMPK activation. These data open promising perspectives for prostate cancer treatment as they suggest that metformin may be a useful adjunct to the classic therapy with antiandrogens.
Declaration of interest
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## P777

**Investigations into the epigenetic mechanisms regulating the prostanoid receptor EP2 in estrogen dependent breast cancer**
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Estrogen excess is a major contributing factor to the development and progression of post-menopausal Estrogen Receptor positive (ER+) breast cancers. Increased activity of aromatase, the enzyme that converts androgens to estrogens, and upregulation of its encoding gene CYP19A1 is often observed in breast adipose fibroblasts (BAFs) surrounding ER+ tumours. Understanding the process by which CYP19A1 is regulated between normal and diseased breast stroma may help to improve outcomes for breast cancer sufferers.
Prostaglandin E2 (PGE2), secreted by breast tumours, stimulates CYP19A1 expression and estrogen production in surrounding BAFs. Whilst hormonal regulation of this process has been examined, there is increasing evidence suggesting that epigenetic mechanisms, such as DNA methylation, serve to influence the PGE2 pathway. These mechanisms are thought to lead to CYP19A1 transcription by silencing or activating upstream factors. The present study aims to determine whether an inverse correlation existed between expression of the prostanoid receptor EP2 and their promoter DNA methylation status in the context of breast cancer derived cell lines and clinical samples of cancer-associated stroma and matching normal stroma.
Sodium bisulfite sequencing of CpG methylation within the EP2 promoter revealed differential methylation showing inverse correlation with respective mRNA levels in cell lines MDA-MB-231 (ER-), MCF7 (ER+) and MCF10A (normal epithelial). Results were confirmed with inhibition of DNA methylation using 5-aza-2-deoxycytidine (5aza), which showed that expression of EP2 in MDA-MB-231 cells could be increased upon demethylation. Analysis of 9 clinical samples comprising ductal carcinoma in situ, invasive ductal carcinoma and triple negative tumours showed that differences in expression levels of EP2 between normal and cancer-associated stroma could not be attributed to differences in promoter methylation status. This suggests that upstream factors are responsible for the epigenetic regulation of CYP19A1 and EP2. Identification of these factors will further our understanding of epigenetic gene regulation in ER+ breast cancer.
Declaration of interest
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## P778

**GPR30, the non classical membrane G protein related estrogen receptor (GPER), is overexpressed in human seminoma and promotes seminoma cell proliferation.**
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Background
Testicular germ cell tumours are the most frequent cancer of young men. While pathogenesis and reasons of an increasing incidence all over the world remain unknown, epidemiological and clinical data have suggested that fetal exposure to environmental endocrine disruptors (EEDs), especially with estrogenic effects, could participate to testicular germ cell carcinogenesis. However, EEDs (like bisphenol A) are often weak ligands for classical nuclear estrogen receptors. In fact, this promoting effect could act through the non classical membrane G-protein coupled estrogen receptor (GPER/GPR30), which has been recently shown to mediate the effects of several xenoestrogens and also overexpressed in various estrogen dependent cancer cells (breast, ovary, endometrium).
Methods
The aim of this study was to demonstrate that GPER was also overexpressed in testicular tumours ($n=15$) compared to normal peritumoral testicular tissue and was able to trigger JKT-1 seminoma cell proliferation *in vitro*.
Results
In normal adult human testes, GPER was expressed by somatic (Sertoli cells) and germ cells (spermatogonia and spermatocytes). GPER was exclusively and significantly ($P < 0.05$) overexpressed in seminomas, the most frequent testicular germ cell cancer. In JKT-1 seminoma cells, GPER was localized at the cell membrane and triggered *in vitro* the proliferative effect induced by G1 (a GPER selective agonist) and by an impermeable E2-conjugate (E2-BSA). This effect was completely abolished by G15 (a GPER selective antagonist) and by GPER siRNA invalidation.
Conclusion
Our results confirm that human seminomas overexpressed functional GPER; moreover, agonist and antagonists demonstrate its ability to induce proliferative effect in seminoma cells. Thus, this non classical membrane G protein related estrogen receptor may represent a molecular basis for a possible effect of xenoestrogens during testicular carcinogenesis.

Declaration of interest
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## P779

**Sorafenib stops disease progression in the majority of patients with advanced differentiated thyroid cancer refractory to radioactive iodine.**

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Differentiated thyroid cancers (DTC) have an excellent prognosis with a 10 year disease-related survival of 85%. However, about 5% of DTC patients develop an aggressive disease with distant metastasis and loss of radioactive iodine (RAI) avidity. An effective treatment is not available for these patients and survival rates are less than 15%. The MAP-kinase pathway is strikingly involved in the pathogenesis of DTC. This is why compounds striking the MAP-Kinase pathway may be useful for treatment of advanced DTC refractory to RAI.
A phase II clinical trial was designed to assess efficacy and safety of the Tyrosine-Kinase inhibitor Sorafenib in 9 patients with progressive RAI-refractory DTC. Median follow-up period was 10 months. Sorafenib was administered at a starting dose of 400 mg b.d. Computed tomography scans were performed at baseline and at 12-weeks intervals to assess radiologic responses. Determination of Tg was performed at 4 weeks intervals to assess biochemical response. Patients were subjected to clinical and biochemical examinations at 4-weeks intervals to assess the occurrence of adverse events (AE).
In eight patients (89%) disease progression was arrested. According to RECIST criteria, 5 of them achieved a stable disease (56%) and 3 a parzial response (33%). Five out of 8 responding patients (62%) had a durable response. In all responding patients a dramatic drop in Tg levels was observed with a median time of nadir of 60 days. The most prevalent AE were hand-foot syndrome (87%), TSH increase (75%), asthenia and arthralgia (75%), hypertension (50%), anemia (37,5%), alopecia (25%). Only one patients stopped treatment because of toxicity. Toxicities were fully controlled by halving the dosage in 7 patients (77,7%).
Sorafenib is effective in controlling disease progression in the majority of patients with advanced DTC refractory to RAI, also resulting in objective response in one third of them.
Declaration of interest
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## P780

**The pilot study on clinical presentation of pituitary adenomas (Pa) in patients with multiple endocrine neoplasia type 1 (Men1) phenotype with and without *Men1* mutation**

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*MEN1* germline mutations are identified in 70% of the familial forms of MEN1 and about 10%of the sporadic cases. Little is known about clinical differences between MEN1 with and without identification of *MEN1* germline mutation particularly in terms of PA characteristics.
Aim
To compare the clinical features of PA in MEN1 cases with and without germline *MEN1* mutation and sporadic cases of PA. Patients and methods: Data were obtained in 39 patients: 22 with *MEN1* mutation (Group-I) and 17 sharing MEN1 phenotype but tested negatively for *MEN1* mutation (Group-II). All patients presented with PA and primary hyperparathyroidism (PHPT). The genetic diagnosis was performed by direct sequencing of *MEN1* exons and intronic boundaries. In 17 *MEN1*-negative patients large deletions of *MEN1* were excluded by MLPA and direct sequencing of *CDKN1B* gene did not reveal the presence of genetic mutations. Control group (Group-III) included 17 patients with sporadic PA and no evidence of other endocrine tumors (matched by type of secretion and follow-up period to those in Group-II).
Results
In Group-II PA as primary tumor site were more frequent than in Group-I (70% vs 51%, $P=0.04$). The distribution of the PA type was significantly different between Group-I and Group-II. In Group-II 52% had somatotropinomas, 17% prolactinomas, 17% nonfunctioning adenomas, 11% corticotropinomas. In contrast, in the Group-I 53% consisted prolactinomas and only one patient had acromegaly. The frequency of macroadenomas was not different in Group-I and Group-II (55% vs 59%; $P=0.8$), but was higher in Group-II patients than in Group-III (59% vs 44%, $P=0.041$). In secreting PA normalization of pituitary hypersecretion was not significantly different in Group-I and Group-II (50% vs 52%, $P=0.48$), whereas it was more frequent in Group-III than in Group-II (76% vs 52%, $P=0.001$). During the whole follow-up 81% patients in Group-I developed gastroenteropancreatic neuroendocrine tumors, whereas in the Group-II such tumors were identified in only 2 patients.
Conclusion
PA in MEN1-phenocopies have different clinical characteristics than those with germline *MEN1* mutations. The pituitary tumorogenesis in these patients, especially in those with GH-producing PA and PHPT, might involve mechanisms others than either *MEN1* or *CDKN1B* mutations.
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## P781

**Hepatocellular carcinoma (HCC) as a neuroendocrine tumor: a preliminar molecular study.**

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Hepatocellular carcinoma (HCC) is often diagnosed at late stage or metastatic condition. Surgical resection is considered treatment of choice and other treatment options include chemoembolization, radiofrequency ablation and photodynamic therapy. We evaluated the expression levels of the 5 somatostatin receptors (SSTRs) in 23 biopsies of HCC patients and also mTOR, p70S6K and 4eBP1 in HuH-7 and HepG2 and investigated the *in vitro* effect of somatostatin analogs (SA) and mTOR inhibitors on cell proliferation. The receptor expression was assessed by RT-qPCR and sstr2 and 5 localization by ICC. The proliferation was tested using DNA assay. Sstr2 > sstr1 > sstr5 were expressed in the patients. Cell lines showed the presence dell'sstr1, 2, and 5 as well as mTOR, p70S6K, 4eBP1. ICC showed a heterogeneous localization of sstr5 and sstr2 in Hep-G2 and Huh-7. Octreotide showed a no significant dose-dependent inhibitory effect in both cell lines. Bim23244 induced a significant inhibitory effect (18.2%, $P=0.02$) in HepG2 at $10^{-7}$ M and Bim23926 16.4% ($P=0.00$) at $10^{-7}$ M in Huh-7. Incubation with mTOR inhibitors resulted in a dose-dependent growth inhibition in both cell lines. Octreotide has not synergistic or additive effect with rapamycin at high concentration, but when it was used in combination with rapamycin at low concentration was able to revert the antiproliferative effect of rapamycin in HepG2 and HuH-7. This effect was also associated to an hyperexpression of sstr2 and 5 and upregulation of pERK1/2. Our data provide evidence of sstrs expression in HCC but other studies will be required to justify the clinical use of these compounds in the treatment of advanced HCC. mTOR inhibitors have an essential growth inhibitory effect in HCC cell line, even though *in vitro* the rapamycin effect can be reverted by octreotide, showing that HCCs do not act as a typical neuroendocrine tumor.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P782

**Urinary steroid profiling demonstrates induction of CYP3A4 and inhibition of 5alpha-reductase by mitotane treatment for adrenocortical carcinoma**

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Mitotane (o,p'DDD) is commonly used for the treatment of adrenocortical carcinoma (ACC), both for advanced disease and in the adjuvant setting. Mitotane induces adrenal insufficiency but specific effects on steroidogenic enzymes are unknown.

We investigated 24-h urinary steroid metabolite excretion in ACC patients on adjuvant mitotane (AD) or mitotane for metastatic disease (MET). We compared samples collected before mitotane treatment (BEFORE; MET $n=57$; AD $n=12$) to samples collected after 3–4 months of treatment when therapeutic range plasma mitotane levels had been achieved (AFTER; MET $n=47$; AD $n=11$). Steroid metabolite excretion was analysed by gas chromatography mass-spectrometry, facilitating the selective identification and quantification of 32 steroid metabolites. We also calculated steroid substrate over product ratios to diagnose impairment of distinct steroid converting enzymes including 5alpha-reductase (5alpha-tetrahydrocortisol/tetrahydrocortisol; 5αTHF/THF) and CYP3A4 (6beta-hydroxycortisol/cortisol; 6βOHF/F).

We found a strong inhibition of 5alpha-reductase activity by mitotane (5αTHF/THF: MET-before $3.6\pm6.3$ vs. MET-after $0.05\pm0.07$; AD-before $0.5\pm0.4$ vs. AD-after $0.02\pm0.01$; all $P<0.001$). The extent of this inhibition was comparable to patients treated with the known 5alpha-reductase type 2 inhibitor finasteride (5αTHF/THF $0.02\pm0.006$; $n=5$) and patients with inactivating 5alpha-reductase type 2 mutations (5αTHF/THF $0.04\pm0.03$ $n=23$). We also found evidence of a strong induction of the major drug and steroid-metabolising enzyme CYP3A4 (6βOHF/F: MET-before $2.1\pm3.6$ vs. MET-after $20.5\pm9.7$; AD-before $2.4\pm0.8$ vs. AD-after $30.2\pm5.1$; all $P<0.001$). 6βOHF represented only 3.4% (1–4%) of total cortisol metabolite excretion before mitotane, which increased hugely to 54% (39–70%) on mitotane ($P<0.001$) irrespective of tumour load.

Longitudinal data from patients receiving adjuvant mitotane ($n=4$) demonstrated lasting effects on CYP3A4 and 5alpha-reductase activities, with restoration to normal only after 12 months off treatment. CYP3A4 induction results in rapid inactivation of hydrocortisone replacement, explaining the increased hydrocortisone dose requirements in mitotane-induced adrenal insufficiency.

Declaration of interest

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## P783

**Bronchial carcinoid response to mTOR inhibitors depends on mTOR expression levels**

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Introduction

Bronchial carcinoids (BCs), originating from endocrine cells dispersed in the respiratory epithelium, can be divided into typical (TBC) and atypical (ABC). TBC are less aggressive, smaller, and much less likely to metastasize, while ABC are more aggressive and metastasize. mTOR has a central role in regulating cell growth, metabolism, and apoptosis. A differential mTOR activation in BCs has been previously demonstrated, suggesting that mTOR pathway might play a predictive role in patients eligible for mTOR-targeted therapies.

Aim

To evaluate the effects of mTOR inhibitors on human BCs cells lines.

Materials and methods

NCIH720 (ABC) and NCIH727 (TBC) cells were treated with Everolimus or with NVP-BEZ 235, a dual PI3K/mTOR inhibitor. Cell viability, caspase activity and cell cycle progression were evaluated after 72 h.

Results

Everolimus inhibits cell viability in both cell lines with a more pronounced effects on NCIH720, does not activate apoptosis but determined G1/G0 accumulation. NVP-BEZ235 had similar effects to Everolimus in terms of cell viability in NCIH727, but was more potent in NCIH720 cells. Caspase activity was not induced by NVP-BEZ 235 in NCIH727, but was enhanced in NCIH720. Western blot and Q-PCR were performed to assess whether this differential effect is due to variations in mTOR expression in BCs cell lines. mTOR expression is 1.5-fold higher in NCIH720 as compared with NCIH727.

Conclusion

These data demonstrate that mTOR inhibitors affect BCs cell lines differently depending on mTOR expression.

Declaration of interest

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## P784

**TNFα induced aromatase expression is mediated by the Early Growth Response transcription factors in breast adipose**

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Breast cancer remains the leading cause of cancer-related death in Australian women. Up to 70% of post-menopausal tumours are estrogen-receptor positive (ER+), dependent on estrogen for continued growth and proliferative advantage. Adjuvant anti-estrogen therapies are considered the cornerstone approach to the treatment of such tumours, and research is ongoing to maximize its effectiveness. The major source of estrogens for ER+ breast cancers is local conversion of androgen precursors by the enzyme P450 aromatase. Inflammatory factors such as Tumour Necrosis Factor-α (TNFα) stimulate transcription of CYP19A1, the gene that encodes aromatase, via its adipose-specific promoter I.4 (PI.4). The pathways by which this is achieved are not fully understood. We aim to identify the mechanisms underlying TNFα-dependent aromatase induction in the context of ER+ breast cancer.

TNFα treatment of human breast adipose fibroblasts (BAFs) increased mRNA levels of all four early growth response transcription factors, with a 7-fold EGR2 induction being the highest. Overexpression of EGR2 caused an increase in endogenous CYP19A1 expression in the human pre-adipocyte cell line SGBS, driven by increased PI.4-specific transcripts. PI.4 luciferase reporter activity is dose-dependently increased by EGR2, EGR3 and EGR4, with EGR2 causing a 70-fold luciferase response. Deletion analysis indicates this promoter activity was indirectly mediated by a short region of the promoter not containing any previously characterised binding sites, and we further show that EGR2 cannot bind directly to this promoter region. This suggests involvement of a secondary factor.

Our studies demonstrate that the Early Growth Response (Egr) transcription factors play an important role in the TNFα-induced signalling pathway resulting in elevated PI.4 transcription. This unveils a novel component of the aromatase gene regulatory network, and further enhances our understanding of estrogen production in the breast.

Declaration of interest

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## P785

**Mitochondrial respiratory chain defects: a novel molecular mechanism of Mitotane for treatment of Adrenocortical Carcinoma**

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Mitotane (o,p'DDD) represents the most effective treatment of adrenocortical carcinoma (ACC). However, molecular mechanisms of mitotane action remain poorly understood. Mitochondrial (mt) impact of mitotane has previously been suggested but not yet fully validated. We investigated functional consequences of mitotane exposure on mitochondrial steroidogenesis, respiratory chain activity and biogenesis, using human adrenocortical secreting H295R and non-secreting SW13 cells. We first confirmed that mitotane exposure inhibits cell proliferation in a dose- and time-dependent manner. We were however unable to detect mitotane metabolites, o,p'DDA and o'p'DDE, in cultured cells supernatant. We

demonstrated that mitotane drastically reduces cortisol and 17-hydroxyprogesterone secretions by 70%, at a 50 μM (14 mg/L) concentration, considered as the therapeutic plasma level threshold. This was accompanied by significantly decreased expression of genes encoding mitochondrial proteins involved in steroidogenesis (STAR, CYP11B1, CYP11B2). In both H295R and SW13 cells, 50 μM mitotane induced a significant and reproducible 50% decrease of the maximum velocity of the respiratory chain complex IV (cytochrome c oxidase, COX) activity. This was associated with significantly decreased expression of both the mtDNA-encoded COX2 and the nuclear DNA encoded COX4 COX subunits, and with an important reduction of the whole COX steady state expression, as revealed by Blue Native PAGE. Enhanced mitochondrial biogenesis was shown by an increased mt DNA content and enhanced expression of the transcriptional regulator PGC1α, consistent with an adaptive and compensatory mechanism against mitochondrial defect. Mitotane exposure also triggered a morphologic shift from filamentous to punctuate aspects of mitochondrial network, providing additional support for alteration in the mitochondrial compartment dynamics.

Collectively, our results provided first evidence that mitotane induces mitochondrial respiratory chain defect. Better understanding of mitotane pharmacology should enable us to identify predictive factors of efficacy and toxicity.

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## P786

### Screening of AIP mutations in young Romanian patients with sporadic pituitary adenomas

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Introduction

The pathogenesis of pituitary adenomas is incompletely understood. It was recently demonstrated that mutations in AIP, a novel tumor suppressor gene, are causing the familial isolated pituitary adenoma syndrome. Although initial data suggested that AIP mutations are rare in non-familial cases, a recent study demonstrated an increased prevalence in young sporadic macroadenoma patients.

Aim

To perform a systematic screening of AIP mutations in young Romanian sporadic pituitary adenoma patients.

Patients and methods

We studied 40 pituitary adenoma patients from a tertiary referral endocrinology center. Patients were evaluated clinically, biochemically and by pituitary imaging. After informed consent, blood DNA was extracted and all six AIP exons were PCR-amplified and sequenced (Beckman CEQ8000).

Results

The 40 patients included presented the following adenoma types: 9 prolactinomas, 24 GH-secreting, 2 mixed GH-PRL, 1 ACTH-secreting, 4 non-functioning pituitary adenomas (NFPA).

We found several AIP nucleotide single-base substitutions. Bioinformatic analysis was employed to distinguish mutations from known SNPs. One NFPA male patient associating mental retardation (38 yrs. old) presented a heterozygous R16H AIP exon 1 mutation. This has been previously described in familial and sporadic pituitary adenomas, 2 colon cancer patients and 1 control. One male acromegalic patient (18yrs. old at onset), with a large invasive macroadenoma resistant to somatostatin analog therapy, harboured a novel heterozygous exon 6 mutation – R314W. Another acromegalic male patient (29 yrs. old), presenting a macroadenoma resistant to chimeric dopamin-somatostatin analog therapy, harboured a novel heterozygous exon 2 mutation – W73R. Both substitutions affect conserved aminoacids.

Conclusion

We describe two novel putative AIP mutations. In vitro confirmation of their pathogenicity will be needed. Screening of a larger control sample will help exclude that these are rare polymorphisms. Our results suggest that current AIP mutation screening criteria may need to be extended, as evidence is accumulating for their significant prevalence in sporadic adenomas.

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## P787

### Expression of a novel isoform of thyroid peroxidase (TPO) gene in breast cancer (BC)

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Introduction

BC patients have a high prevalence of anti-TPO-autoantibodies (TPOAb), which seem to be protective in BC patients. We hypothesized the presence of T-lymphocytes citotoxicity against a common antigen between thyroid and BC cells.

Aim

To evaluate the possible expression of TPO gene in BC.

Materials

Frozen tissue specimens: 8 BC, 5 peri-tumoral breast tissues (PT), 3 pancreatic adenocarcinoma (P), 2 kidney cancers (K) and thyroid (T) as positive control. 3 BC cell-lines (C), MCF-7, T47-D and MDAMB-231, grown with and without β-estradiol and/or insulin-growth-factor-II (IGF-II).

Methods

TPO mRNA expression was evaluated by Reverse-Transcriptase-PCR using different primers pairs distinguishing among different known TPO isoforms. TPO protein expression was studied with Western Blot using monoclonal mouse antibody ab76935.

Results

Known TPO variants mRNA was expressed in all BC and PT, weakly in C independently from β-estradiol and/or IGF-II exposition, at the limit of detection in P and K and highly in T. In particular BC, PT and C presented a new TPO isoform without exons 14 and 16, weakly expressed in T and absent in P and K. TPO protein was found at the expected level (100–110 kDa) in T, C and many BC, PT, P, K; the signal was reduced or disappeared after ab76935 pre-absorption with recombinant TPO fragments, indicating a specific binding. All samples presented also other signals corresponding to lower molecular weight (MW) proteins, present also in T: they could represent corresponding proteins of smaller TPO isoforms.

Conclusions

TPO main isoforms mRNA and protein are weakly but clearly expressed in almost all samples. BC, PT and C present also a new variant without exons 14 and 16, absent in P and K. Further experiments are necessary to quantify TPO expression and to search for a lower MW protein corresponding to the new TPO isoform found.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P788

### Is combined radionuclide therapy with 90y-Dotatate and 177lu-Dotatate an effective treatment option for patients with metastasised neuroendocrine tumours? An ongoing study

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Introduction

Neuroendocrine tumors (NETs) over-express somatostatin receptors (SRs) and the efficacy of peptide receptor radionuclide therapy (PRRT) with somatostatin analogues labeled with high activities of β-emitting radioisotopes has been reported.

Aim

Ongoing study to evaluate efficacy and toxicity of combination treatment with 4 cycles of radiolabeled DOTATATE, alternating 177Lu and 90Yin patients with metastasised NETs expressing SRs refractory to conventional therapies.

Method
Since 2008 forty-four patients with disseminated NETs were included in the study prospectively to be treated with 4 cycles of PRRT, alternating 177Lu-DOTATATE (5.55 GBq) and 90Y-DOTATATE (2.6 GBq).Dosimetric evaluation on each patient was performed following administration of 177Lu-DOTATATE. Hematological and renal toxicities are graded according to WHO toxicity grading scales and treatment efficacy evaluated using RECIST criteria. Quality of life is assessed following each treatment cycle.
Results
So far twenty-six patients have completed all 4 cycles together with a 6 month treatment evaluation (SD = 38.5%, PR = 38.5%, CR = 8% and PD = 15%). A symptomatic improvement was reported by 90% of patients with pre-treatment carcinoid syndrome. The remaining eighteen patients are currently undergoing treatment. Side effects and blood toxicities were rare, mild and transient. To date no renal or hepatic toxicities have been observed. Average tumor absorbed doses were 13–63 Gy for 177-Lu and 20–93 Gy for 90Y.
Conclusions
Combined PRRT therapy represents an innovative treatment strategy for patients with metastatic NETs with the aim of improving therapy efficacy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P789

**The pathogenesis of non functioning pituitary adenoma with positive staining for glycoproteins is associated with MEG3/DLK1 under-expression/GSTP1 overexpression and modulated by microRNAs (miRNAs)**

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Context
Pathogenesis underlying non functioning pituitary adenoma (NFPA) formation and progression has not been well established. MEG3 is strongly expressed in the normal pituitary (NP) but not in NFPA derived from gonadotroph cells. High GSTP1 gene expression also seems to contribute to gonadotroph adenoma development. Recent reports indicate a role of miRNAs in the pituitary adenoma pathogenesis.
Objective
To evaluate the expression of MEG3, DLK1, GSTP1 genes and a panel of miRNAs and verify whether they correlate with clinical findings in NFPAs.
Material and Methods
MEG3, DLK1, GSTP1 genes and miRNAs were validated by real time PCR in 29NFPA and 15NP. GSTP1 protein was also evaluated by Western Blotting in 11NFPA with positive- (NFPA+), 11NFPA negative- (NFPA-) staining for glycoproteins, and 9NP.
Results
We observed underexpression of MEG3 ($-13.5$ fold; $P=0.0004$) and DLK1 ($-100$ fold; $P<0.0001$) genes and overexpression of GSTP1gene (4.8 fold; $P=0.01$) and its protein ($P=0.04$) in NFPAs. No expression of miR–21 and 145 and underexpression of miR-141, 16, let-7a, 133a, 150, 143 (ranging from $-14.3$ to $-4.0$–fold; $P<0.002$) were found in NFPAs. MEG3 underexpression and GSTP1 overexpression were associated with NFPA+ ($P=0.004$ and 0.05, respectively), which were also associated with bigger tumors ($P=0.02$). In addition, lower expression of miR–16 was associated with bigger tumors ($P=0.01$) and NFPA+ ($P=0.03$).
Conclusion
Our studies confirm that the loss of expression of DLK1 and MEG3 genes and overexpression of GSTP1 gene/protein are specifically important to the pathogenesis of NFPA+. Besides these genes, our data also show that miR143 underexpression seems to be involved in pathogenesis of NFPA+, while miR16 seems to regulate genes involved in controlling tumor size. In addition, we demonstrate that NFPA+ are bigger and have worse chance to achieve clinical control. These data shed lights on NFPA cytodifferentiation processes, which might improve future classification of its histotypes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P790

**The influence of surgery in the management of recurrent adrenocortical carcinoma.**

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Objective
The role of surgery for recurrent adrenocortical carcinomas (ACC) is not well defined. Therefore, we evaluated the outcome after surgery for tumor recurrence in patients from the German ACC Registry.
Methods
Only patients with first recurrence after initial R0 resection were investigated. Progression–free and overall survival (PFS, OS) after first recurrence were analyzed by Kaplan–Meier method. Cox proportional hazards regression models were used to identify prognostic factors.
Result
Of 154 patients with first recurrence, 101 underwent repeated surgery (R0 resection, $n=78$) and 99 received (additional) nonsurgical therapy. After a median interval of 6 (range 1–221) months, 144 patients (94%) experienced progressive disease. Multivariate analysis adjusted for age, sex, tumor burden, time to first recurrence (TTFR), resection status after surgery for recurrence and additional therapy indicated that only two factors were significantly associated with shorter PFS (hazard ratio for progression: TTFR $>12$ months 1.8 [95% CI 1.2–2.5] in comparison to TTFR $\leq 12$ months; R2 resection 3.4 [1.5–8.0] and no surgery 3.4 [1.7–7.1] in comparison to R0 resection) and OS (hazard ratio for death: TTFR $>12$ months 3.0 [2.0–4.6] in comparison to TTFR $\leq 12$ months; R2 resection 2.6 [1.0–6.7] and no surgery 4.2 [1.8–9.8] in comparison to R0 resection). Patients who had both TTFR $>12$ months and R0 resection of recurrent tumors ($n=22$) had the best prognosis (median PFS 24 months, median OS 58 months).
Conclusions
The best predictors of prolonged survival after first recurrence of ACC are TTFR $>12$ months and R0 resection. Patients with longer TTFR and tumors amenable to radical resection should therefore be operated, whereas patients with shorter TTFR or tumors not amenable to radical resection do most likely not benefit from incomplete surgery.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P791

**Over-expression of AIP protein in GH3 cells reduces cAMP signalling and Growth hormone secretion**

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Mutations in the AIP gene have been linked to familial cases of pituitary adenomas (Vierimaa et al, 2006). Analysis of the protein support its role as a tumour suppressor since mutations cause a loss-of-function with reduced protein interactions and over-expression of wild-type (WT) AIP reduces cell proliferation (Leontiou et al, 2008). AIP interacts with a number of interesting proteins, among them are the phosphodiesterases, PDE4A5 and PDE2A, the G proteins, Gαq and Gα13, survivin, RET, nuclear receptors and others (Trivellin & Korbonits, 2011). However, the mechanism by which AIP dysfunction causes increased susceptibility to pituitary adenomas remains unknown. Owing to AIP's interaction with the phosphodiesterases and G proteins, we investigated the effect of WT and mutant AIP proteins on cAMP signalling and its downstream effectors in cell cultures.
WT AIP, R304X-AIP mutant and empty vector (EV) were transfected into GH3 cells. Basal and forskolin – induced cAMP signalling was analyzed using cAMP assays, CRE-promoter luciferase assays, real-time PCR and finally growth hormone (GH) assays.
WT AIP was able to reduce forskolin-induced, but not basal, cAMP signaling. Total cAMP, the luciferase activity of cAMP-driven promoter and target gene expression were reduced when compared to EV and R304X mutant. Additionally,

analysis of GH secretion which occurs after cAMP cascade activation, was slightly but significantly reduced in WT over-expressing GH3 cells treated with forskolin. Addition of IBMX, a phosphodiesterase inhibitor, did not reverse the effect of AIP on cAMP signalling or GH secretion, indicating that this effect occurs independently of AIP-phosphodiesterase interaction.
AIP protein appears inhibit pituitary cells from proliferation by suppressing cAMP production, activation of which is known to cause tumour formation (Lania et al, 2003) and thereby also influencing GH secretion. However, this effect appears not to be mediated by the AIP-phosphodiesterase interaction, suggesting G protein involvement in mediating this outcome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P792

**Novel 45A > G & 46D > G mutation of SDHB Exon-1 in a Patient with Urinary Bladder & Posterior Mediastinum Paraganglioma.**

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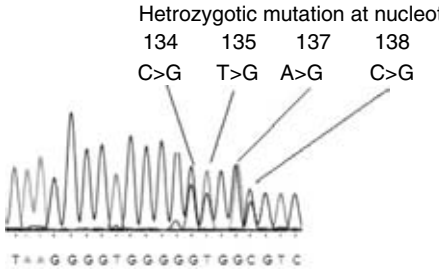
Introduction
Extra adrenal paraganglioma (PGL) account for approximately 15% of all pheochromocytoma. Urinary bladder & mediastinum are rarest form of PGL. We present a case of bladder &Mediastinal PGL with a novel mutation in exon 1 of SDHB gene.
Case Report
18 years old male patient was admitted with five years episodic symptoms of headache, sweating, and palpitations, especially after urination. He also complained of postural dizziness for last 6 months. He was admitted in some other hospital and was found to have labile blood pressure. His blood pressure readings varied from 210/120 mmHg to 80/50 mmHg. He was referred to our clinic for further investigations. His biochemical investigations showed elevated urinary 24 hour norepinephrine & metanephrines. MRI showed heterogeneously enhancing T2 hyperintense lesions of 7×4 cm in posterior mediastinum and 4.1×4.2 cm in urinary bladder, both of these lesions were confirmed in Ga68-DOTANOC imaging. He was operated with a collaborating team of urologist and thoracic surgeons. Post operatively, he developed hypotension and required ionotropic support for few days. Histopathological features were consistent with paraganglioma of both locations. Tumor cells were positive for synaptophysin & chromogranin & negative for cytokeratin. Patient is currently normotensive and asymptomatic after follow up of 10 months. The genetic analysis of the patient showed a novel 45A > G& 46D > G heterozygous germline mutation in SDHB exon-1.
Conclusion
Concurrence of urinary bladder PGL with mediastinum PGL has not been reported earlier to the best of our knowledge. We observed a novel 45A > G& 46D > G heterozygous germline mutation in SDHB gene in exon-1.




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P793

**Refractory Seizures as a Presenting Feature of Insulinoma**

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Introduction
Insulinomas are the commonest hormone-secreting tumor of gastrointestinal tract. Presentation is usually insidious with neuroglycopenia and fasting hypoglycemia. Many patients do not report adrenergic symptoms of hypoglycemia and present with neurological or psychiatric manifestations that often lead to misdiagnosis. The symptoms of insulinoma lack specificity, including various seizure disorders, personality change, bizarre behavior, amnesia, convulsions, and incidentally dystonia and polyneuropathy and cause delay in diagnosis. This case highlights the importance of considering hypoglycemia in atypical neurological or psychiatric episodes.
Case Report
A 29 yr man had 1yr history of multiple seizure episodes despite being on regular 3 antiepileptic drugs. Episodes occurred on awakening in early morning. The attacks were episodic stereotyped confusional spells characterized by abnormal posturing, perioral and eyelid twitching and unresponsiveness. Compliance was checked and metabolic causes ruled out. MRI Brain was normal. No interracial epileptic activity observed on video EEG monitoring. He presented with unconsciousness and hypoglycemia (48 mg/dl) which recovered immediately after treatment. It was apparent that his previous seizures tended to occur in early morning or several hours after the meal and post seizure confusion state could be shortened if he ate something. The patient gained 10 kg weight in last 6 months. Endocrine evaluation suggested insulinoma. CT revealed a solitary insulinoma in the head of pancreas. The patient had surgical removal and benign insulinoma was confirmed on histopathology.
Conclusion
1) Many patients with an insulinoma do not report the adrenergic symptoms of hypoglycemia and present with neurological or psychiatric manifestations that often lead to misdiagnosis. 2) Seizure disorder has been described in few contemporary cases of persistent hypoglycemia later on diagnosed as insulinoma and insulinoma presenting first time with refractory seizures is rare. 3) Benign insulin secreting adenomas are a potentially curable cause of seizures but may be fatal if unrecognized. 4) Our case reiterates the importance of evaluating the metabolic cause of refractory seizure disorders. 5) Early diagnosis and treatment can free many patients from burdensome multiple antiepileptic treatments.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P794

**Rare Case of Atypical Pituitary Adenoma: Intermediate form of Adenoma between the common Benign Adenoma & exceedingly rare Pituitary Carcinoma**

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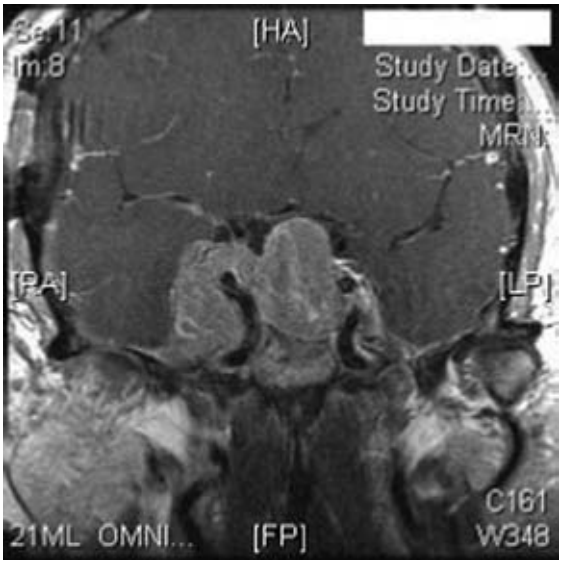
We describe a case of aggressive pituitary adenoma with atypical biological behavior treated with surgery radiation & chemotherapy. A 46 y/o F presented with nausea & acute vision loss R > L,with no symptoms of amenorrhea, hypothyroidism or hypoadrenalism. Brain MRI showed 4.7×3.6×3.6 cm pituitary macroadenoma with mass effect on optic chiasm, cavernous sinus extension, internal carotid artery encasement, extension into R foramen ovale, orbital apex, sphenoid & ethmoid sinus, prepontine cistern resulting in mass effect on midbrain & pons. Pituitary hormone profile was TSH 1.41 mcU/ml (0.4–5) FT4 0.53 ng/ml (0.8–1.8) ACTH 20pg/ml (6–46) Prolactin 20.6 ng/ml ( < 0.5–25) FSH 1.4 mU/ml (1–25) LH 0.2 mU/ml (0.1–77) GH 7.9 ng/ml cortisol 2.9 mcg/dl(5–25). She had tumor debulking & optic nerve decompression. Postop MRI showed residual tumor with extension into medial brain structures. Tumor pathology showed sparsely granular chromophobe staining pattern, chromogrannin immunohistochemistry +, CAM 5.2 & cytokeratin + fibrous bodies. Ki67 labelling index 10%. Pathological classification was nonfunctional chromophobe adenoma. Symptoms & vision improved but she had residual R visual loss. Postop pituitary hormone profile revealed TSH 0.088 mcU/ml, FT4 0.54 ng/dl, IGF 532 ng/ml (288–736), ACTH 13 pg.ml (5–27), am cortisol 10.6 ng/ml (4–22). She received DDAVP for transient diabetes insipidus, thyroxine for secondary hypothyroidism & hydrocortisone taper. 1 month CT showed marked increase in residual tumor, near doubling of tumor volume, complete encasement of optic

nerve, extension into orbit & cranial base pathways down to hypoglossal canal. She underwent gammaknife therapy, temozolamide chemotherapy & radiological surveillance. While there are no endocrinological, neuroradiological & histological criteria to distinguish pituitary adenoma & carcinoma MIB-1 index of 10%, invasive growth pattern & mitotic activity predicted aggressive biologic behavior of this atypical adenoma(1). As pituitary carcinomas are thought to arise from transformation of benign invasive macroadenomas, given the potential for malignant transformation of this invasive adenoma & due to higher risk of immediate revision surgery, temozolamide & radiation was initiated. We are monitoring response to radiation & chemotherapy with imaging.

1. J Neurosurg. 2011Feb;114(2):336–44.




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P795

**A novel mutation of the MEN1 gene in an Italian family with Multiple endocrine Neoplasia type 1**

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Introduction
MEN-1 is a rare autosomal dominant familial cancer syndrome characterized by involvement of parathyroid glands, enteropancreatic endocrine tissues and anterior pituitary gland. This disease is linked to germline mutations in the MEN1 gene (more than 460 described), whose identification allows the familial genetic counselling. Here we describe a novel germinal mutation in exon 10 of the MEN1 gene identified in an Italian family.
Case Report
A 61-yr-old woman presented with recurrent kidney stones (first manifestation at 25 years of age), osteoporosis, upper abdominal pain. She reported hysterectomy for uterus leyomiomas at 21 years of age, hyperprolactinemia since 30 years, surgical removal of multiple lipomas. Her sister had a diagnosis of prolactinoma at 20 years of age, primary hyperparathyroidism (PHPT), gastric tumor. She had a positive familial history for neoplastic diseases on both maternal (gastro-enteric tumors) and patern(pancreatic tumors and PHPT) sides of her pedigree.
Proband's laboratory examinations showed high values of PRL, gastrin and PHPT (high calcium and PTH with low phosphate). Neck US showed a voluminous parathyroid enlargement. MRI with gadolinium disclosed a pituitary micro-adenoma.
The proband's 40-yr-old son reported a history of recurrent kidney stones already treated by laser lithotripsy, sexual dysfunction, pseudo-gynecomastia, multiple lipomas. Laboratory data revealed severe hyperprolactinemia, hypogonadotropic hypogonadism, PHPT. The MRI showed a pituitary macroadenoma invading the right cavernous sinus. Neck US disclosed two enlarged parathyroid glands.
In all affected family members the genetic testing identified a novel MEN1 germline heterozygous deletion in exon 10, p.K564RfsX3(c.1691delA), resulting in a frameshift generating a premature termination codon consistent with a pathogenic effect.
Conclusion
We describe a new mutation of MEN1 gene with an apparent high degree of penetrance.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P796

**Pheochromocytoma releasing ACTH: two in one.**

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Introduction
Ectopic release of ACTH caused by pheochromocytoma is extremely rare. The clinical picture of hypercortisolism is overlapped by catecholamine excess that makes diagnosis more difficult.
Case report
A 74-year-old women was admitted to the hospital because of hypertension, recently diagnosed diabetes and weight loss (10 kg per six months). In physical examination: cachexia, dehydratation, pallor, hirsutism, proximal muscle weakness, elevated blood pressure, tachycardia and increased sweating. Laboratory studies revealed severe, resistant for treatment hypokalemia, elevated testosterone and 17-OH progesterone concentrations, severe hypercortisolism without circadian rhythm and not suppressed with 8 mg of dexamethasone; increased plasma ACTH concentration and elevated urinary cortisol and catecholamines levels. In CT tumoral mass in the right adrenal gland was found. The immunohistochemistry of the adrenal tumor confirmed diagnosis of both pheochromocytoma and ACTH.
Summary
Simultaneous secretion of catecholamines and ACTH by the same tumor is a very rare finding. Clinical implications of this co-secretion made diagnostics process more complicated and final diagnosis quite surprising.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P797

**Hypergastrinemia in a Patient with Lymphocytic Colitis and Chronic Gastritis**

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Serum gastrin (G) levels $>1000$ pg usually raise the suspicion for a neuroendocrine tumor (NET) secreting G. Rarely, such elevated G levels are seen in pts with pernicious anemia (PA) which most commonly is associated with autoimmune gastritis (AG) (1). AG can occur concomitantly with other autoimmune d/o incl. lymphocytic colitis (LC) (2). G stimulates enterochromaffin-like cells which increase histamine (H) secretion. H excess can cause diarrhea (D) as can bacterial overgrowth (BO) or LC.
A 57 yo WF presented with D, sporadic epigastric pain, and bloating. She also had a h/o interstitial cystitis and took pentosan, cetirizine, (pantoprazole in past). She had no h/o ulcers, renal impairment or carcinoid syndrome. PE was unremarkable. Serum G was 1846 pg. EGD revealed gastritis, a pH of 7 with low stomach acid. Bx of the antrum, body of the stomach, and CLO urease testing r/o H. pylori infection. Pathology: chronic gastritis without H. pylori. Serum G and plasma CgA were suggestive of a gastrinoma or NET (table). PA was unlikely b/o nl B12 (748 pg), methylmalonic acid, homocysteine, Hb and MCV.
CT abdomen/pelvis were noncontributory. Pancreatic protocol MRI with arterial, venous, and delayed phase imaging did not reveal a pancreatic lesion.

Random colon bx performed to assess for LC was compatible with LC which may explain her diarrhea, although we also considered excessive H from elevated G, BO, and pentoasan polysulfate which can cause D and be misleading in this setting, pointing to the dx of gastrinoma. She could not stop pentosan but started a PPI and rifaximin for 10 days which decreased bloating and D. Serum G levels decreased but remained elevated (686 pg on prednisone 20 mg and a PPI).
This case illustrates that D may be associated with very high serum G levels in the setting of chronic gastritis (possible BO), LC, and interstitial cystitis (pentosan use), without clear evidence for a gastrinoma or NET. If no h/o ulcers or liver metastases is present in such cases, watchful observation rather than an extensive/invasive and costly search for a NET may be justified.
1. Lewin KJ et al. Gut 1976;17:551
2. Pardi DS, et al. Lymphocytic colitis... Am J Gastroenterol 2002; 97: 2829
Declaration of interest
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Funding
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**Table 1** Main biochemical analyses of our case

| Celiac serology | Serum Gastrin | Chromo-granin A | VIP | Calcitonin |
|---|---|---|---|---|
| Anti-gliadin IgG,A; anti-endomysial IgA, anti-TTG IgA Negative | 1848<br>*N*<100 pg/mL | 262<br>*N*<225 ng/ml | 27<br>(*N*<50 pg/mL) | < 2<br>(*N*<5 pg/mL) |

## P798

**Primary hyperparathyroidism due to a parathyroid adenoma, a parathyroid carcinoma and a subsequent adenoma: A case report**
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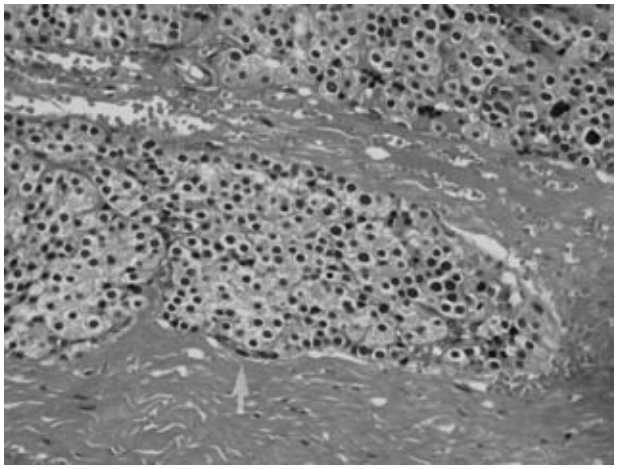
Objective
To describe a patient with an unusual course of primary hyperparathyroidism (PHPT) due to parathyroid adenoma in 2004, who developed recurrent hyperparathyroidism due to parathyroid carcinoma in 2010, and a subsequent adenoma in 2011.To our knowledge, this sequential occurrence has not been previously reported in literature.
Case
A 57-year old male was operated on for a parathyroid adenoma in the right inferior gland in 2004. He was asymptomatic until six years later (2010), he was hospitalized due to hypercalcemia with markedly elevated parathyroid hormone level (intact PTH 520 pmol/L). Parathyroid scan showed increased uptake in the inferior right thyroidal bed. Radio-guided parathyroidectomy with intra-operative PTH assay was performed (pre-excision PTH 520,10 min post-excision 8.7, 6 in post-excision 3.77). A 2.3 cm parathyroid carcinoma of the right inferior and superior glands was found. Post-operatively, he developed hungry bone syndrome and was managed with calcium and vitamin D supplements. Upon discharge, ionized calcium level normalized.
In 2011, he developed recurrent hypercalcemia with low phosphorus and elevated intact PTH levels. Repeat parathyroid imaging showed a new lesion in the suprasternal notch. Recurrent and/or metastatic parathyroid carcinoma was primarily considered. However, re-exploration of the neck revealed a 1.5 cm parathyroid adenoma with lymph node negative for metastasis. (Pre-excision PTH 22.7, 10 min post-excision 7.26, 30 min post-excision 5.12). Post-operative ionized calcium was normal.
Conclusion
It is very rare for three independent episodes of PHP to be caused by different pathology in the same patient within a 7-year time frame. Recurrent PHP after resection of a parathyroid carcinoma does not necessarily indicate persistent or recurrent parathyroid carcinoma but can also be caused by a parathyroid adenoma. This underscores the necessity of adequate long-term follow-up to ensure early detection and management of parathyroid tumors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1** Pertinent Laboratory Examinations

| Variable | 2004 | 2010 | 2011 | Normal Values (SI unit) |
|---|---|---|---|---|
| Ionized Ca | 1.54 | 1.38 | 1.23 | 1.00–1.20 mmol/L |
| Phosporus | – | 0.72 | 0.76 | 0.87–1.45 mmol/L |
| Intact PTH | 39.53 | 520.9 | 29.35 | 1.48–7.63 pmol/L |



Histopathological section showing parathyroid carcinoma with blood vessel invasion (arrow) in 2010. H&E staining.

## P799

**Neuroendocrine Tumor with Clinical Characteristics of Insulinoma**
J. Menezes, E. Rodrigues, C. Nogueira, C. Esteves, G. Jorge, M. Matos, L. Graça, J. Pinheiro, Loureiro, J. Lopes, J. Costa , Maia & D. Carvalho
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Introduction
Insulinomas are almost always located in the pancreas and are usually small. The peak incidence is from the third to fifth decade of life, with females being slightly more frequently affected. The average duration of neuroglycopenic symptoms prior to diagnosis is often prolonged, being more than 5 years in 20% of patients.
Case presentation
Female, 52 years old, medical history notable for hypertension, dislipidemia and Graves disease. She presented with a 4-month history of hypoglycemia with associated symptoms of weakness and tremor. The patient was admitted in the emergency room with garbled speech and disorientation. At that time, her finger-stick blood glucose level was 26 mg/dL. Because of the suspicion of insulinoma, a complete endocrine evaluation was performed specifically, the patient was observed while fasting. At 26 hours of fasting the blood glucose level was of 43 mg/dL, with response to glucagon injection, and the patient was found to have insulin level s $>3$ UI/mL and C-Peptide value $>=0.6$ ng/mL, with glycemia $<55$ mg/dL. Parathyroid hormone and calcium were normal. A high-resolution CT demonstrated a heterogeneous enhancing mass 2.9 cm. The patient was submitted to laparoscopic distal pancreatectomy. A a neuroendocrine well differentiated tumor ($2.8\times1.7\times0.9$ cm) – G1 (ENET) – pT2N0R0 was found in the tail of the pancreas. Immunohistochemical stains for chromogranin and synaptofisin were positive, however for insulin was negative. Less than 2% of the tumor cells were ki67+. Postoperativelty, the patient's symptoms resolved with no further hypoglycemic events.
Conclusion
We report this case because it took just 4 months for diagnosis, because of the dimensions of the tumor and also because, although the biochemical diagnosis of

insulinoma was established, there was no immunohistochemical evidence of insulin production. The patient glycemia however returned to normality with no further symptoms.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P800

**Effects of mTOR inhibitors in the control of non functioning pituitary adenomas cell growth**

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Introduction
The main therapeutic approach for non functioning pituitary adenomas (NFA) is surgery, since radiotherapy has several important side effects and medical therapy is rarely effective. Therefore, understanding the molecular pathways regulating NFA cell proliferation is crucial for future drug development. We here explore the possible role of mTOR inhibitors, Everolimus and BEZ235 (which also inhibits the PI3 K pathway) on the effects of Insulin-like Growth Factor-1 (IGF-1) in regulating NFA cell growth in primary culture.
Aims
To this aim 20 NFA primary cultures were incubated with or without IGF-1 in the presence or in the absence of Everolimus and BEZ235, which down-regulate IGF-1 signalling through the PI3 K/Akt pathway.
Materials and methods
Cell viability and apoptosis were evaluated after 48 h. AKT phosphorylation was evaluated by alpha-screen.
Results
Everolimus and BEZ235 significantly reduced NFA cell viability, by 30 and 40% respectively., while IGF-1 enhanced cell viability, an effect completely blocked by the presence of mTOR inhibitors. Co-incubation with an IGF-1 receptor blocking antibody enhanced the antiproliferative effects of Everolimus and BEZ235. Phosphorylation of p70S6K, a down-stream effector of mTOR in the PI3K/Akt pathway, was as well enhanced by IGF-1 and reduced by Everolimus and BEZ235, indicating that IGF-1 exerts its proliferative effects by inducing this pathway, which, in turn, can be effectively blocked by mTOR inhibitors.
We also observed that AKT phosphorylation is enhanced by IGF-1 treatment and significantly reduced by treatment with BEZ235.
Conclusion
In conclusion, our results indicate that IGF-I directly stimulates NFA cell viability through its own receptor. This effect is blocked by Everolimus and BEZ235 which may represent a new medical therapeutic approach for NFA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P801

**mTOR inhibitors hamper cell viability in selected human medullary thyroid carcinoma primary cultures**

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Introduction
It has been demonstrated that mTOR inhibitors have potent anti-proliferative effects in a human Medullary Thyroid Carcinoma (MTC) cell lines. We here explore the possible role of mTOR inhibitors, Everolimus and BEZ235 (which also inhibits the PI3K pathway) on the effects of Insulin-like Growth Factor-1 (IGF-1) in human MTC primary cultures.
Aims
To this purpose, 20 MTCs primary cultures, were treated without or with 1 uM Everolimus, 10 nM BEZ235, and/or 50 nM IGF-1.
Materials and methods
Cell viability and apoptosis were evaluated after 48 h. p70S6K phosphorylation was evaluated by ELISA.
Results
we observed that Everolimus and BEZ235 significantly reduced MTC cell viability, by 20 and 35% respectively, while IGF-1 enhanced cell viability, an effect completely blocked by mTOR inhibitors. Co-incubation with an IGF-1R blocking antibody enhanced the antiproliferative effects of. Everolimus and BEZ235. Caspase activity was enhanced by BEZ235 and reduced by IGF-1, an effect that was attenuated by co-treatment with mTOR inhibitors. Phosphorylation of p70S6K, a down-stream mTOR effector in the PI3K/Akt pathway, was evaluated to assess whether this effect is due to variations in mTOR activity. We observed that IGF-1 enhanced p70S6K phosphorylation, that is reduced by mTOR inhibitors, indicating that IGF-1 exerts its proliferative effects by inducing this pathway.
Conclusion
In conclusion, mTOR inhibitors reduced MTCs cell viability by inducing apoptosis, with a mechanism likely involving IGF-1 signalling, suggesting that these drugs might represent a possible medical treatment for persistent/recurrent MTCs.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P802

**Disturbed alternative splicing of growth hormone is associated with increased expression of SF2/ASF oncogene in pituitary adenoma**

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Introduction
Pituitary adenomas account for 10–25% of all intracranial tumours. Although rarely malignant, they are associated with high morbidity due to severe endocrine effects and difficult surgical access. Alternative splicing (AS) consists in selective removal of non-coding regions from pre-mRNA and joining of coding regions to produce mRNA variants that may be further translated. AS is regulated by splicing factors and is often disturbed in cancer. AS of growth hormone (GH) leads to synthesis of five transcript variants. Variant 3 encodes a 17.5 kDa protein which is a dominant negative and blocks secretion of biologically active GH proteins encoded by variants 1 and 2. AS of GH is regulated by splicing factors SF2/ASF and SC35. SF2/ASF is an oncogenic protein. In this study we hypothesized that AS of GH may be disturbed in pituitary tumours.
Methods
The study involved 28 human pituitary adenomas (21 gonadotroph adenomas and 7 other types adenomas) and five non-neoplastic pituitary tissue specimens obtained with the permission of local Bioethics Committee. The used methods were: RNA isolation, reverse transcription, PCR using primers designed to detect GH splice variants, cloning using pGEM-Teasy vector, sequencing, and real-time PCR.
Results
Using GH-specific PCR we found that while in control samples only transcript variant 1 was expressed, in adenoma samples variants 1, 2, and 3 were present. This was confirmed by cloning and sequencing of all three variants. Variant 3 was expressed in 20 (71%) of analyzed adenoma specimens. This was associated with statistically significantly increased expression of SF2/ASF and SC35 mRNAs in gonadotroph adenomas when compared with control samples.
Conclusion
Alternative splicing of GH is disturbed in pituitary adenomas, possibly due to impaired expression of splicing factors SF2/ASF and SC35. Altered splicing of GH may possibly serve as a neoplastic marker in pituitary adenomas.
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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P803

**Estradiol Potentiates the Inhibitory Effects of SOM230 on Prostate Cells *in vitro* Up-regulating Ligand Binding Domain Expression of SSTR2 and 5**

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The effects *in vitro* and *in vivo* of somatostatin (SS) analogues are linked to the presence of specific binding sites on target cells. Estradiol (E2) may modulate the expression of SS receptors (SSTR) in some cell models, including breast cancer and pituitary cells. Aim of this study was to evaluate the effects of E2 on SSTR1-5 ligand binding domains (LBD) expression levels in prostate epithelial cells (PEC).

Methods

We investigated the effects of E2 on the expression of SSTRs and its influences on SST-analogue SOM230 treatment on a PEC line (EPN) expressing both estrogen receptors (ER) alfa and beta. SSTR proteins were evaluated by Western-blot using specific MoAb recognizing their LBD epitopes (Y-SSTRs MoAb). Starved cells were treated with 20 mM E2 or $10^{-6}$ or $10^{-8}$ SOM230 or 20 mM E2+ SOM230 ($10^{-8}$ or $+10^{-6}$) for 48h. Cells were differently harvested for real time RT-PCR, Western blot or FACS assays.

Results

E2 up-regulated SSTR 1, 2 and 5 mRNAs and proteins. E2 or SOM $10^{-6}$ alone induced apoptosis and decreased slightly proliferation; 20 mM E2+ SOM230 ($10^{-8}$ or $10^{-6}$) combined treatment induced a stronger rate of apoptosis and a greater decrease of proliferation. The synergistic action was correlated to: a reduction in S-phase proliferation with an arrest in G0/G1 phase induced by SOM230 and increased by E2+SOM 230 co-treatment; a caspase-dependent apoptosis induced by SOM230; a reduction of bcl-2 levels induced after addition of E2 that amplified SOM230 effects at lower doses.

Conclusions

E2 induces an up-regulation of LBD of SSTR 1, 2 and 5 and potentiates the inhibitory effects of SOM230 in PEC *in vitro*. Thus estrogens may negatively control prostate tumorigenesis acting directly on cell growth and death and indirectly by up-regulating SSTRs.

Declaration of interest

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## P804

**β-Catenin signaling controls tumorigenesis in menin-deficient insulinoma**

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Multiple endocrine neoplasia type 1 (MEN1) is an inherited tumour syndrome characterized by the development of tumours of the parathyroid, anterior pituitary and pancreatic islets, etc. Heterozygous germ line mutations of MEN1 gene are responsible for the onset of MEN1. Here We reported totally 87 patients from 24 unrelated Chinese families associated with MEN1 and identified five novel mutations and several previously reported mutations. Furthermore, we detected a loss of heterozygosity (LOH) at chromosome11q in the removed tumours, including gastrinoma, insulinoma and parathyroid adenoma. After that, we try to explain how the mutated MEN1 genes induced the tumours in multiple endocrine neoplasia type 1 syndrome. Menin is able to interact with β-catenin, and regulates translocation and transcriptional activity of β-catenin via nuclear-cytoplasmic shuttling. To determine the role of β-catenin signaling in β-cells and menin-deficient insulinoma, we established pancreatic β-cell specific ctnnb1 knockout mice (BBKO) and Men1/ctnnb1 double-knockout mice (DBKO). BBKO mice were phenotypically normal compared to WT mice under a chow diet or challenged with a high fat diet, indicated that β-catenin is not crucial for β-cell development and physiological proliferation. Double-knockout of menin and β-catenin rescued the hypoglycemia phenotype and exhibit elevated survival rate compared with Men1 conditional knockout mice. Loss of β-catenin reduced the expression of a series of proproliferative genes, which were up-regulated in menin-deficient islets, including ccna2, pbk, mcm5, mcm6 and Mki67. These results indicate that β-catenin signaling is critical for tumorigenesis of menin-deficient β-cells.

Declaration of interest

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## P805

**Hypermethylated in cancer 1 (HIC1), a tumour suppressor gene epigenetically deregulated in hyperparathyroid tumours by histone modification**

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Primary hyperparathyroidism (pHPT) resulting from parathyroid tumours is a common endocrine disorder with incompletely understood etiology. In renal failure, secondary hyperparathyroidism (sHPT) occurs with multiple tumour development as a result of calcium and vitamin D regulatory disturbance.

The aim of the study was to investigate whether HIC1 may act as a tumour suppressor in the parathyroid glands and whether deregulated expression involves epigenetic mechanisms.

Parathyroid tumours from patients with pHPT included single adenomas, multiple tumours from the same patient, and cancer. Hyperplastic parathyroid glands from patients with sHPT and hypercalcemia, and normal parathyroid tissue specimens were included in the study. Quantitative RT-PCR, bisulfite pyrosequencing, colony formation assay, ChIP, and RNAi was used.

HIC1 was generally underexpressed regardless of the hyperparathyroid disease state including multiple parathyroid tumours from the same patient, and overexpression of HIC1 lead to a decrease in clonogenic survival of parathyroid tumour cells. Only the carcinomas showed high methylation level and reduced HIC1 expression. Cell culture experiments, including use of primary parathyroid tumour cells prepared directly after operation, the general histone methyltransferase inhibitor DZNep, ChIP, and RNAi, supported a role of repressive histone H3 modification rather than DNA methylation in repression of HIC1.

The results strongly support a tumour suppressor role of HIC1 in the parathyroid glands and suggest that perturbed expression of HIC1 may represent an early event during tumour development. Repressive histone modifications are involved in repression of HIC1 expression in hyperparathyroid tumours.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P806

**Stem cell genes are deregulated in parathyroid tumours**

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In our previous investigation on microRNAs expression pattern in parathyroid carcinomas (Ca), we detected the over-expression of microRNAs belonging to C19MC, the largest human cluster on chromosome 19q13.41. In the present study, the analysis of the expression of selected C19MC and the closely distal MIR371-3 clusters microRNAs (MIR512-3p, MIR517C, MIR520H and MIR372) was extended to 11 Ca, 24 adenomas (Ad) and 6 normal glands. The four microRNAs was expressed in 11% of parathyroid neoplasia. Almost all Ca showed elevated MIR517C levels compared to normal glands, while MIR517C over-expression occurred only in two Ad. MIR372 was detected in 45.5% of Ca and in 37.5% of Ad. By copy number variation analysis, C19MC amplification was identified in most Ca extending distal to the MIR371-3 cluster in almost all amplified samples. Conversely, C19MC amplicon was detected in a small subset of Ad extending distal to MIR371-3 in one sample. C19MC promoter was hypomethylated in 38% of the samples with no difference in frequency between Ad and Ca. Few tumours did not show C19MC amplicon nor hypomethylation. Since the C19MC as well as the MIR371-3 clusters have been linked with the signature characteristic for human embryonic stem cells, we investigated genes of the transcriptional regulatory network governing stem cells pluripotency and self-renewal. By immunohistochemistry, cells with a positive nuclear staining for NANOG were more abundant in Ca (50–70%) compared to normal glands (<10%) and to Ads (5–20%). In parathyroid tumours, NANOG expression was associated with the expression of OCT4 and the loss of the OCT4-inhibited gene DKK1, while SOX2 was undetectable evidencing an impairment of the core

regulatory circuit. Moreover, zfp42/REX1 gene expression was lost in some tumours, as described in other human cancers. These data identified a genetic stem cell signature in parathyroid tumours that seems to correlate with more aggressive tumoral features.
Declaration of interest
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## P807

**Long-acting somatostatin analogues are highly effective in men1 patients with early stage duodeno-pancreatic neuroendocrine tumors**

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Somatostatin analogues (SSA) represent a recognized therapeutic option in patients affected with functioning neuroendocrine tumors (NET). In non-functioning NET, SSA are reported to induce tumor stabilization in most of cases and objective response in $<5\%$. NET associated to Multiple Endocrine Neoplasia type 1 (MEN1) are inherited tumors, generally located in the duodeno-pancreatic trait, characterized by well differentiated histotype, high expression of somatostatin receptors and diagnosed at an early stage because of the genetic screening in all first-degree relatives of MEN1 patients. All these features make MEN1 NET susceptible to respond to SSA, however, this has never been specifically investigated.
To evaluate the efficacy of long-acting SSA in MEN1 patients affected with duodeno-pancreatic NET.
All first-degree relatives of MEN1 subjects who genetically diagnosed for MEN1 before the clinical diagnosis of NET and with evidence of one or more duodeno-pancreatic NET $<15$ mm in size were enrolled. Twenty-four patients with MEN1-related duodeno-pancreatic NET (age range 21–42 yrs) were treated with octreotide LAR or lanreotide autogel at standard doses. Treatment duration ranged 6–76 months. At the radiological evaluation (performed by multidetector-row computed tomography and endoscopic ultrasound), multiple duodeno-pancreatic NET (range 1–9), sized 3–14 mm, were detected.
An objective tumor response was observed in 17%, stable disease in 75% and progression of disease in 8% of cases. Due to the low number of progressions, the median time-to-progression could not be estimated. In seven patients with abnormally increased gastrin, insulin and/or chromogranin-A serum concentrations, a complete hormonal response was observed in 100% cases and was stable along the follow-up.
In conclusions, therapy with SSA is highly effective in patients with early stage MEN1 duodeno-pancreatic NET, resulting in long-time suppression of tumor and hormonal activity. Objective response is 17%, suggesting that MEN1 NET is a subgroup more sensitive to SSA than sporadic NET.
Declaration of interest
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## P808

**Insulin, IGF-II and a low-affinity insulin analog differentially regulate insulin receptor isoform A trafficking and induce a different balance of metabolic and mitogenic effects**

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Introduction
The isoform A of the human insulin receptor (IR-A) binds insulin with high affinity, and binds IGF-II with a 3–10 folds lower affinity. Cells lacking the insulin-like Growth Factor-I receptor (IGF-IR) and overexpressing the human IR-A (R-/IR-A cells) respond to IGF-II with reduced metabolic effects but unaltered or increased mitogenesis as compared to insulin stimulation. We hypothesized that this altered ratio of metabolic-to-mitogenic effects of IGF-II in R-/IR-A cells may depend on the differential regulation of IR-A trafficking. Moreover, we hypothesized that similar different biological effects are features of other low-affinity IR-A ligands.
Methods
We used R-/IR-A cells and evaluated IR-A phosphorylation and trafficking after stimulation with insulin, IGF-II and a synthetic insulin analog (NMeTyrB26-insulin) that binds IR-A with a similar affinity to IGF-II.
Results
We found that NMeTyrB26-insulin has a similar mitogenic activity than IGF-II. Insulin induced strong IR-A phosphorylation, which was instead reduced after IGF-II and NMeTyrB26-insulin stimulation. A clear reduction in cell surface IR-A was strongly induced by insulin, while IGF-II and NMeTyrB26-insulin promoted only modest IR-A internalization. Prolonged cell stimulation with insulin, but not with IGF-II or NMeTyrB26-insulin, targeted the IR-A for proteosomal and lysosomal degradation despite similar levels of IR-A ubiquitination. IRS-1 was also down-regulated by insulin but not by IGF-II or NMeTyrB26-insulin. Clathrin-dependent endocytosis was critical for IR-A-dependent Akt activation, while clathrin-independent IR-A endocytosis regulated ERKs activation. p70S6K activation was instead slightly increased by inhibiting IR-A internalization.
Conclusions
The lower IR-A phosphorylation elicited by IGF-II or NMeTyrB26-insulin, as compared to insulin, may protect IR-A and IRS-1 from negative feed-back down-regulation mechanisms, thus sustaining potent mitogenic stimuli in spite of decreased metabolic activity. These data may have profound implications in the design of insulin analogs and in our understanding of the role of IGF-II in cancer.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P809

**Immunohistochemical, ChIP, and microarray analysis reveals how stromal AR controls prostate cancer outcome through fibroblast specific action of androgen signalling on ECM production and DNA licensing**

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A breakdown in stromal-epithelial interactions mediated by androgens is emerging as a key factor in prostate cancer (PCa) aetiology and progression. Currently however, we have limited knowledge of the mechanics of androgen and androgen receptor (AR) action in prostate stroma, or how dysfunction in that compartment contributes to the disease process.
In this study, immunohistochemical analysis of 64 PCa samples revealed an inverse relationship between stromal AR content and both primary tumour burden and PCa-related death ($P<0.05$). In contrast, epithelial AR content was positively related to Gleson stage and tumour burden but not outcome ($P<0.03$). These relationships were not evident in patient-matched BPH samples. In order to understand how stromal AR might inhibit progression, we investigated androgen action in the AR positive prostate myofibroblast cell line, pshTERT-AR. By chromatin immunoprecipitation, we find lineage-specific chromatin occupancy of regulatory elements by the AR. These differences in DNA interactions leads to divergent enhancement of functional pathways and physiological response. Microarray profiling revealed that only 10% of the genes regulated by androgen in myofibroblasts were also regulated in PCa epithelial cells. This distinct set of genes regulated by androgens lead to regulation of adhesion and ECM production pathways specifically in fibroblasts, as well as opposing effects on apoptosis and cell cycle pathways in comparison to epithelial cells. A key outcome is divergent growth responses, with androgens inhibiting fibroblast proliferation (66% control at 5 days; $P=0.002$) potentially via DNA licensing protein, FBXO32, but stimulating epithelium (50%, $P=0.001$). Importantly, androgens were also found to alter the constituents of fibroblast-deposited extracellular matrix (ECM), resulting enhanced anchorage of both fibroblasts (40%; $P<0.05$) and PCa epithelia (35%, $P<0.05$). In summary, the loss of fibroblast/stromal AR is predicted to stimulate fibroblast proliferation, dysregulate stromal-epithelial interactions in the prostate microenvironment, decrease ECM adhesive characteristics and create an environment that predisposes to epithelial metastasis.
Declaration of interest
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## P810

**Looking for the molecular bridge between sex hormone-binding globulin (SHBG) and breast cancer cells**

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Sex Hormone-Binding Globulin (SHBG), the specific plasma carrier for sex steroids, interacts with cell membranes. In breast cancer cells, SHBG-cell interaction is closely related to estrogen-sensitivity and it is followed by a well-defined cascade of events. Soon after SHBG binding to cell membranes, cAMP accumulates in the breast cancer cells, activates PKA that in turn suppresses estradiol-induced ERK activation. ERK inhibition abolishes the anti-apoptotic effects of estradiol and reduces ERα transcriptional activity. The final result is a significant inhibition of estradiol-induced proliferation. The cell membrane molecule SHBG interacts with has never been certainly identified. A number of candidates, generally being part of the matrix-associated proteins, have been suggested, such as megalin and fibulin. The aim of the present study was to investigate the expression and potential function in SHBG-cell interaction of megalin and fibulin1 and 2, in estrogen-sensitive breast cancer cells (MCF-7 and T47D) where SHBG interaction and anti-estrogenic effect had been widely described. Megalin, fibulin 1 and 2 gene expression was evaluated in both cell lines in basal condition and after estradiol treatment with Real time PCR. Megalin and fibulin 2 were not expressed. Fibulin 1 expression, already detectable in basal condition, was increased by estradiol. The results were confirmed by Western blot, that allowed us to observe also the correct expression of the protein in cell membranes. Finally, immunofluorescence experiments in MCF-7 cells showed a colocalization of fibulin 1 and SHBG on membranes. We, therefore, suggest that fibulin 1 is likely to be the bridge between SHBG and breast cancer cells. SHBG, interacting with fibulin 1 communicates with breast cancer cell, triggers its molecular mediators, and, at the end, blocks estradiol-induced cell growth.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P811

**Parathyroid atypical adenomas: mutational screening of CDC73/HRPT2 gene**

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Atypical parathyroid adenomas represent a subset of tumors with histological features worrisome for carcinoma (PC), such as trabecular growth, fibrous bands and increased mitotic activity without unequivocal criteria of malignancy (local recurrence and/or metastasis). The question of whether these lesions might represent an anticipation of an aggressive clinical behavior that overtime may acquire the full blown features of malignancy remains to be established.

CDC73/HRPT2 tumor suppressor gene mutations have been reported in up to 80% of sporadic PC, mainly associated with reduced expression or loss of its encoded protein, parafibromin. With the exceptions of some studies on parafibromin expression, there are no genetic studies of HRPT2 gene on atypical adenomas.

We collected 15 parathyroid atypical adenomas from patients with sporadic primary hyperparathyroidism (PHPT), obtained at the time of surgery or retrieved from paraffin embedded sections. All but one patients, 8 males and 7 females, with a mean age at diagnosis of 47 years (range 16–68 yrs), were submitted to a single parathyroidectomy. Familial history of PHPT was negative in all patients. Follow-up after surgery ranged from 6 months to 9 years.Thirteen patients were cured by surgery, and two had persistent PHPT.

All specimens were screened for HRPT2 mutations. The entire coding region of the gene and splice sites were sequenced with a BigDye chemistry.

A heterozygous mutation in the donor splice site of intron 1 (c.131G>T) was identified in one adenoma. Unexpectedly, this mutation was a germline mutation, carried by the younger patient of our series. The mutation was also found in the patient's healthy father. The remaining tumors were negative.

Our results suggest that the involvement of HRPT2 gene in parathyroid atypical adenoma is rare. However, HRPT2 gene screening can be suggested in young patients with single atypical parathyroid adenoma.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P812

**Is biochemical screening for pheochromocytoma in adrenal incidentalomas expressing low unenhanced attenuation on computed tomography necessary?**

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Introduction

Pheochromocytomas are characterized by a high attenuation value on unenhanced computed tomography (CT). It is not known whether pheochromocytoma can be outruled as a cause of adrenal incidentalomas based on unenhanced attenuation values only. We evaluated the outcome of routine biochemical screening for pheochromocytoma in a series of patients with adrenal incidentalomas in relationship to the unenhanced attenuation values on CT.

Design and methods

An unenhanced CT was available in 174 of 184 patients with altogether 214 adrenal incidentalomas. All patients were screened for pheochromocytoma with 24hr urinary metanephrines and normetanephrines and for hypercortisolism (1 mg DXM-test and ACTH). Patients with hypertension were screened for aldosterone overproduction (aldosterone/renin ratio and 24hr urinary aldosterone).

Results

One hundred and forty-six incidentalomas in 115 patients had an unenhanced attenuation value $<$ 10 Hounsfield units (HU), but none of these patients had increased 24hr fractionated urinary metanephrines or normetanephrines indicating pheochromocytoma. Sixty-eight incidentalomas in 59 patients had an unenhanced attenuation value $\geqq$10 HU and nine (15.2%) of these patients had surgi-cally and histologically verified pheochromocytoma. Incidentalomas with a HU $\geqq$ 10 were characterized by significantly larger size (2.6$\pm$1.5 vs. 2.3$\pm$1.2 cm; $P<0.001$), were more often functional (27.9% vs. 8.9%, $P<0.001$) and more often operated (44.1% vs. 10.2%; $P<0.001$) than those with a HU $<$ 10.

Conclusion

Pheochromocytoma was outruled as a cause of adrenal incidentaloma if the attenuation value on unenhanced CT was $<$ 10 HU. The results challenge the need for routine biochemical screening of pheochromocytoma in adrenal incidentalomas discovered on unenhanced CT and characterized by a HU $<$10. However, if the incidentaloma is first discovered on contrast enhanced CT imaging, measurements of urinary or plasma fractionated metanephrines is mandatory in order to rapidly exclude the possibility of pheochromocytoma.

Declaration of interest

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## P813

**Clinical and biochemical characteristics of succinate dehydrogenase (SDH) mutation carriers**

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Background

Germline mutations in SDHB, SDHC, and SDHD cause hereditary phaeochromocytoma and paraganglioma (PGL) syndromes. The genotype-phenotype correlation of these mutations and relationship to penetrance is poor. Our objective was to assess characteristics of patients with SDH mutations seen in our dedicated multidisciplinary clinic.

Methods

A retrospective observational study of patients attending from May 2005 to May 2010, approved as an institutional case notes review. Clinical, genetic, biochemical and radiological characteristics were recorded. All patients underwent yearly biochemical screening with urinary catecholamines/metanephrines, and plasma metanephrines, and 2–3 yearly MRI of the sympathetic and parasympathetic chain using axial and coronal T1/T2 spin-echo sequences, with 18FDG-PET if needed for clinical decision making if abnormalities found

Results
From a total of forty-five mutation carriers, tumours were detected by MRI in eighteen patients (median age): 11/34 (26) SDHB (8 secreting), 2/4 SDHC (36) (1 secreting), 5/7 (48) SDHD (all non secreting). 4/11 SDHB patients belonged to one large family of 17 with frame-shift mutation c.379dupA(p.LLe127AsnfsX28) in exon 4 of SDHB Gene. Only one patient had a metastatic phaeochromocytoma at presentation. One patient with an aggressive glomus tumour (SDHC) secreted high levels of dopamine in isolation, treated effectively with Gamma knife. The SDHD associated glomus tumour was nonsecreting and SDHB glomus tumour secreted noradrenaline. Six SDHB patients had false positive biochemistry, most commonly urine metanephrine and normetanephrine, which subsequently normalized. Two new tumours were detected on screening (SDHB,SDHD).
Conclusion
MRI is effective to monitor these patients. False positive biochemistry is common in SDHB, but normal in SDHD despite the presence of tumours. SDHB present at a younger age with abdominal PGL. Dopamine can be the only biochemical abnormality in some aggressive tumors. Gamma knife radiosurgery can be considered for glomus tumours. No new tumours were missed by an interval of MRI imaging every 2–3 years.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P814

**Efficacy of Everolimus to prevent hypoglycemia in patients with metastatic insulinomas: for the French group of endocrine tumors (GTE-Renaten)**

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Background
Refractory hypoglycemia in patients with metastatic insulinomas (RHMI) is an important cause of morbidity and mortality and everolimus could be a new therapeutic option.
Methods
We conducted a restrospective multicentric study within the French group of endocrine tumors to evaluate the time to first symptomatic recurrence (TSR) in patients with RHMI after everolimus initiation. Ongoing hyperglycemic medical options and safety were recorded.
Results
Twelve patients with RHMI treated with everolimus (dose initiation: 10mg/d except one patient 5mg/d) between May 2007 and June 2011 were reviewed. Everolimus was given after a median of four previous therapeutic lines and median duration was 6.5 months (range, 1.5–32+ = ongoing months). Clinical benefits were observed in eleven patients. Median TSR was 6.5 months (range, 0–32+ months): 7 and 5 patients remained symptom free for more than 3 and 6 months respectively. 5 patients were still treated at 6, 7, 10, 12 and 32+months. Six patients discontinued additional hyperglycemic therapies including three glucose infusions. Three patients discontinued everolimus because of pulmonary side effects at 1.5, 3 and 7 months of initiation: two deaths occurred (one non-infectious pneumonitis and one pneumocistis pneumonia). Three patients discontinued everolimus because of disease progression at 2, 3 and 10 months of initiation, without hypoglycemia recurrence.
Conclusion
Everolimus appears as a new effective treatment modality for metastatic insulinomas complaining of refractory hypoglycemia. Pulmonary tolerance should be carefully monitored.
Declaration of interest
I fully declare a conflict of interest. Details below.
Funding
This work was supported, however funding details unavailable.

## P815

**Differential TNFα -synthesis and signaling in endocrine tumors after treatment with the Tumor-Vascular-Disrupting Agent ASA404 (vadimezan)**

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Recently, we investigated effects of the Tumor-VDA ASA404 in tumor models for neuroendocrine tumors of the gastroenteropancreatic system and adrenocortical carcinoma 24 hours after treatment of BON and NCIh295 tumor bearing mice with ASA404 (A), paclitaxel (P) or the combination (A+P). We detected for BON tumors extensive necrotic areas as well as a significant decrease in cell proliferation, increase of apoptotic cells and reduction of microvessels after A or A+P treatment while no comparable effects were detectable in NCIh295 tumors. As TNFα -signaling is assumed to mediate parts of A induced effects we thenceforth utilized these models with their different responsiveness for characterization regarding TNFα stimulation and signaling. We detected in both groups a significant increase of TNFα serum levels compared with controls ($P<0.05$), but no significant differences between both tumor entities upon A treatment (BON: 2818±999%; NCIh295: 1165±422%, $P=0.18$). However, intra-tumoral TNFα content was significantly increased for BON tumors after A treatment while no differences were detectable for NCIh295 (basal: 100%; BON: 1178±263%, $P=0.007$; NCIh295: 220±86%, $P=0.23$). *In vitro* we detected a TNFα dependent 4-fold higher induction of apoptosis (basal: 100%; BON: 823± 35% vs. NCIh295: 244±12%; $P=0.007$) and increase in IKK beta activity for BON but not for NCIh295 cells (basal: 100%; BON: 140±4%. $P<0.001$; NCIh295: 108±5%; $P=0.32$). Basal TNF receptor 1 expression was not significantly different, but we detected high levels of Toll-like-receptor (TLR)-4 in the BON tumor model *in vitro* and *in vivo* while receptor expression appeared to be abrogated for NCIh295. TLRs are widely expressed in cytokine producing cells and TNF alpha is an important downstream mediator of TLR-4 signaling. Thus, ASA404 treatment holds promise in the treatment of GEP-NETs. Furthermore, the utilized tumor models might help to delineate resistance mechanisms involved in VDA induced anti-tumor activity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P816

**Anti-proliferative effect of GHRH antagonist JMR-132 on ovarian cancer cell via EGFR-Akt pathway**

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Antagonists of growth hormone-releasing hormone (GHRH) are being developed for the treatment of various human cancers. In this study, we investigated the effectiveness of GHRH antagonist JMR-132 treatment in ovarian cancer cell lines (SKOV-3, CaOV-3 and OVCAR-3). Using MTT assay, we found that JMR-132 has a strong anti-proliferation effects on SKOV-3 and CaOV-3 cells in a time (0, 24 h, 48 h, 72 h, 96 h) and dose (0, 25 nM, 50 nM, 100 nM and 200 nM) dependent manner. Also, JMR132-induced the activation and increases cleaved caspase-3 in a time-dependent manner in both cell lines. In addition, flow cytometry assay showed that there is a G1 phase cell circle arrest after treatment of JMR-132 in these two cell lines. With 100 nM JMR-132 per day for 4 days, the phosphorylation of Akt (p-Akt) decreased significantly, suggesting that JMR-132 inhibit the PI3K-AKT pathways in ovarian cancer cells. Treatment of the SKOV-3 and CaOV-3 cell with specific PI3K inhibitor LY294002 (10 nM) in combination with the presence or absence of EGF (10 ug/ml) abolished JMR-132 attenuated EGF-induced proliferation in both cell lines. More importantly, continuous treatment with JMR-132 reduced the expression of total EGF receptor (EGFR) in a time dependent manner. Knockdown of the endogenous expression of EGFR by siRNA attenuated JMR-132 inhibited proliferation of SKOV-3 and CaOV-3 cells by EGF. In summary, the present study demonstrates that the antiproliferation effect exerted by GHRH antagonist JMR-132 is due in part by interference of the EGF receptor-Akt pathway in ovarian cancer cell.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P817

**Characterization of circulating tumor cells in prostate cancer patients**

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This work focuses on the characterization of circulating tumor cells(CTCs) captured from the blood of cancer patients using a microfluidic chip functionalized with an antibody against the epithelial cell adhesion molecule (EPCAM). We visualize CTCs captured from the blood of prostate cancer patients taking advantage of the unique tumor-associated marker, prostate specific antigen (PSA). Our data demonstrate that CTCs are detectable preoperatively in patients with early stage, resectable prostate cancer with an immediate dramatic postoperative decline ( < 24hrs). However, some patients had persistent CTCs for up to 3 months following prostate resection, suggestive of transient extraprostatic tumor deposits. Moreover, CTC counts in patients with metastatic disease declined following the initiation of androgen deprivation therapy or other effective treatments. Remarkably, dual staining of captured CTCs for PSA and Ki67 indicated a broad range in proliferative index of CTCs, reflective of the evolution of prostate cancer growth traits under castrate conditions. Thus, we believe that isolation and analysis of CTCs in both localized and metastatic prostate cancer will provide novel insight into early hematogenous dissemination of prostate cancer and enable molecular analyses of treatment-responsive and -resistant disease, supporting their application in long-term clinical studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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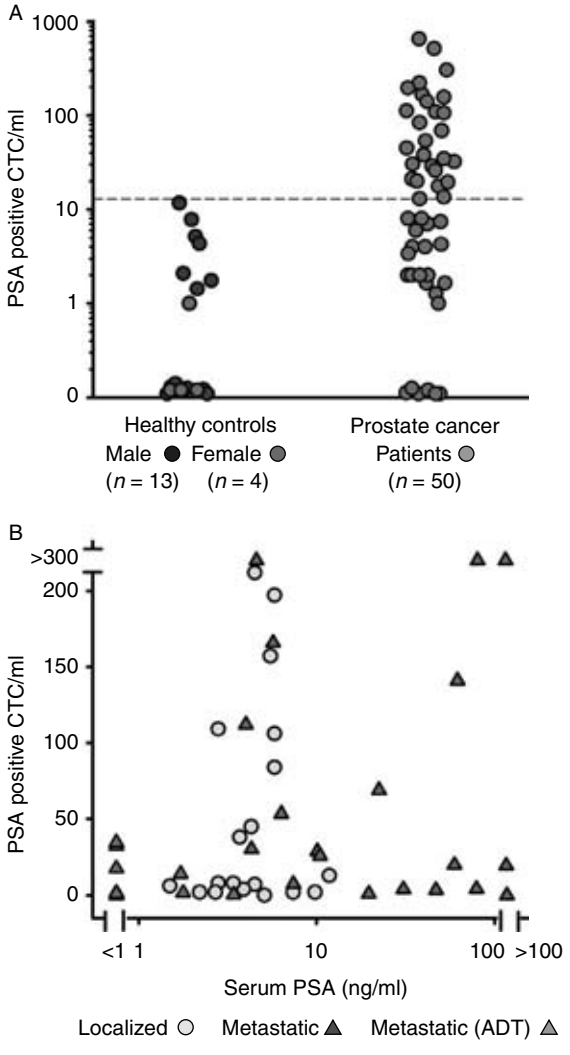


## P818

**Mitotane Inhibits Cell Growth Proliferation and Modulates MRP1 Protein Expression In Different Human Cancer Cell Lines**

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Background

Actually mitotane is an adrenal-specific agent employed in the treatment of adrenocortical carcinoma (ACC), and although it has been used for several decades, its exact mechanism of action, remain to be fully elucidated. Some authors reported that mitotane affected *in vitro* the multidrug resistance gene mdr-1/P-glycoprotein. MRP1, belonging a multidrug resistance protein family, exerts a chemo-resistance activity in many type of cancer leading to the failure of chemotherapy. Interestingly many aggressive tumours are often associated to MRP1 over-expression.

Aim

In this study we investigated the effect of mitotane in different human cancer cell lines. The aim of the present study was to investigate the hypothetical link between MRP1 protein expression level and response to mitotane treatment.

Materials and methods

Adrenocortical cancer, glioblastoma, medulloblastoma, ovarian cancer and breast cancer cell lines were grown in appropriate culture medium and treated with mitotane at different concentration. Cell cycle analysis was analyzed by flow cytometry (FCM). Cell cycle related protein and apoptotic molecules expression levels were detected by Western Blot analysis. Instead MRP1 protein was evaluated by immunofluorescence flow cytometric analysis.

Results

Mitotane treatment induced in all cell lines examined a cell growth inhibition characterized by an alteration of cell cycle. The expression level of MRP1 protein was modulated after mitotane exposure, indicating its possible role in response to treatment. In addition, we also observed a different expression of cell cycle molecules (cyclin B1 and E, cdk1) and apoptotic cell death protein (caspase 3) affected by mitotane treatment in human cancer cell lines examined.

Conclusions

Mitotane exposure shows an antiproliferative effect in several cancer cell lines, interfering with MRP1 expression. It could explain the pharmacological effect of the drug. These findings support the hypothetical role of mitotane in therapeutic approach in different type of human cancers.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P819

**Hormone combinations produce unique steroid receptor binding and regulatory profiles in breast cancer cells**

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Studies into estrogen receptor alpha (ERα), progesterone receptor (PGR) and the androgen receptor (AR) involvement in breast, uterine and prostate cancers has provided considerable insight into the molecular actions these receptors, but have thus far focused on each receptor individually and alone in different cell types. Here, whole genome expression microarray analysis and ChIP-sequencing in ZR-75-1 breast cancer cells, in response to combinations of 17β-estradiol (E2), progesterone (PROG) and 5α-dihydrotestosterone (DHT), was used to provide new information on action of steroid combinations. For each steroid/receptor alone, a remarkably small subset of high affinity binding sites and expression responses were found to be common amongst different cells and disease states ( < 10%). The larger proportion of lower affinity sites and responses were found to predominate, and likely govern cell specificity of hormone responses. Importantly, when we add more than one hormone, we identify distinct DNA binding patterns for each receptor, as well as unique gene expression profiles and important gene families critical in cellular function. For example, long term E2 treatment in combination with PROG results in a dramatic 10-fold increase in the number of PGR binding sites detected at an equivalent peak threshold in comparison to PROG alone. Furthermore, only E2 and PROG combination treatment resulted in PGR binding sequences enriched for hormone response elements and important coregulator binding sequences, such as FOXA1. These data suggest that the actions of steroid receptors in breast cancer cells are not additive or independent, but combine in a different way to produce a unique cellular response that is not evident by investigation of each receptor in isolation.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P820

### Medical and Peptide Receptor Radionuclide Therapy with Somatostatin Analogues in Well and Moderately Differentiated Neuroendocrine Tumours

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Introduction
Peptide receptor radionuclide therapy (PRRT) is a treatment choice for inoperable or metastasized neuroendocrine tumours and this therapy seems more effective in the biochemical and volume control of disease than somatostatin analogues (SSA).
Aims
To demonstrate the efficacy of medical therapy with SSA and PRRT in patients with well and moderately differentiated neuroendocrine tumours.
Materials and methods
We evaluated 60 patients (29 males, 31 females; mean age at diagnosis $53 \pm 17$ years) affected by well and moderately differentiated neuroendocrine tumours. 26 patients (43%) were affected by bronchial carcinoids, 16 patients (27%) by intestinal neuroendocrine tumours, 15 patients (25%) by pancreatic neuroendocrine tumours, 3 patients (5%) by metastases from neuroendocrine carcinoma of unknown origin. 25 patients (42%) with either metastatic or recurrent disease, unresectable primary tumours or symptoms of hormone excess were treated with SSA. Among these, 10 patients (17%) with metastatic disease or unresectable primary tumours with intense hyperuptake at the Octreoscan underwent 2 to 7 cycles of PRRT with 90Y-DOTATOC or 177Lu-DOTATATE. Mean follow up was $41 \pm 10$ months.
Results
Medical treatment with SSA allowed control of symptoms related to hormone excess in all patients but 1 with refractory carcinoid syndrome. Among the patients that underwent PRRT, 6 (60%) had minor or partial response, 3 (30%) had stable disease while 1 patient had progression of disease. Interestingly, 3 of the 6 patients with minor or partial response to PRRT had further progression with a mean PFS of 12.7 months.
Conclusions
Medical treatment with SSA allowed control of hormone secretion in the majority of patients. PRRT allowed minor or partial response of disease in a high proportion of treated patients. However, among these, a relatively high proportion of patients had further progression of disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P821

### Expression of ErbB2 in craniopharyngiomas: Possible therapeutic implications

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Background
Currently, there is no satisfying treatment option other than gross total or subtotal resection in combination with adjuvant radiotherapy for the treatment of craniopharyngiomas. However, recurrence could occur in a substantial proportion of craniopharyngiomas despite gross total resection and radiotherapy. Human epidermal growth factor receptor 2 (HER-2/ErbB2) overexpression was determined in various human carcinomas and is associated with aggressive clinical behavior. Therefore, ErbB2 has been the target for immunotherapy. To date, ErbB2 immunoexpression has not been determined in craniopharyngiomas.
Purpose
To determine the expression of ErbB2 in craniopharyngiomas to assess the rationale for targeting this receptor for the treatment of craniopharyngiomas.
Methods
The ErbB2 immunostaining was performed on 20 adamantinomatous craniofaryngiomas using avidin-biotin-peroxidase complex method. ErbB2 immunoexpression was evaluated using a light microscope and was interpretted according to the American Society of Clinical Oncology/College of American Pathologists criterions (score 0, no staining or membrane staining is observed in $<10\%$ of the tumor cells; score 1+, faint/barely perceivable membrane staining is detected in $>10\%$ of the tumor cells, and only part of the membrane is stained; score 2+, weak to moderate complete membrane staining is observed in $>10\%$ of the tumor cells; score 3+, strong complete membrane staining is observed in $>30\%$ of the tumor cells).
Results
Of the 20 cases evaluated, 2 (10%) were score 3+ for ErbB2. One of these patients was a 12 years old female child who was died soon after the first surgery and the other was a 30 years old male patient who underwent 3 surgeries and radiotherapy due to the repeated recurrences.
Conclusion
Results from this study suggests ErbB2 overexpression in a considerable number of craniopharyngiomas. Further studies are required to assess the efficacy of ErbB2 inhibitors in the treatment of conventional treatment resistant craniopharyngiomas with high ErbB2 expression.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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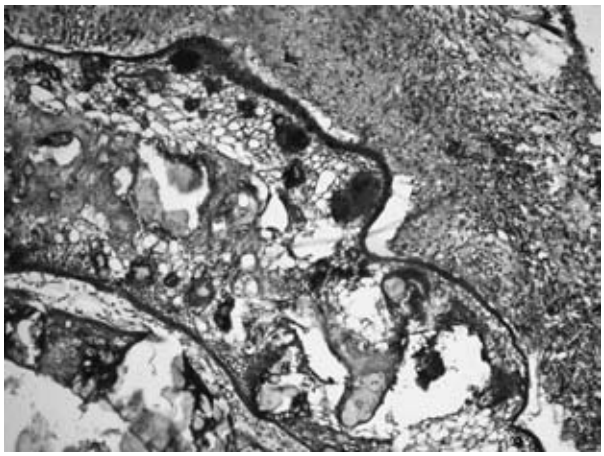

**Figure 1.** Score 3+ ErbB2 immunoexpression in adamantinomatous craniopharyngioma (case 1) (magnification × 40)

## P822

### Wnt-signalling in human insulinomas

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Objectives
The Wnt-signalling pathway is involved in tumor development in various tissues. Wnt-signalling molecules are expressed in pancreatic beta-cells. In addition, Wnt-signalling regulates insulin secretion and the proliferation of pancreatic beta-cells *in vitro* and in animal models. However, it is not clear whether Wnts play a role for the development of human insulinomas. Therefore, we investigated the expression pattern of Wnt-signalling molecules in benign and malign human insulinomas.
Methods
human insulinomas were analysed using tissues arrays. The insulinomas were classified as benign ($n=49$) or malign ($n=10$). Immunohistochemistry (IHC) for Wnt3a, Wnt4 and frizzled was performed. The expression of these molecules was assessed with a score model (expression level multiplied by number of positive cells, maximum score: 12). Healthy pancreas sections served as controls. Proliferation assays ([3H]-thymidine uptake) of INS-1 insulinoma cells were performed after stimulation with Wnt3a protein.

Results
1 Wnt3a, Wnt4 and frizzled are expressed in the normal human endocrine pancreas (control group). 2. The extracellular ligand Wnt3a is strongly over-expressed in human insulinomas (benign: $7.59 \pm 3.20$, $P < 0.05$; malign: $3.39 \pm 2.51$, *n.s.*) compared to controls ($2.50 \pm 0.50$). 3. The Wnt-receptor frizzled is over-expressed in insulinomas (benign: $6.21 \pm 3.44$, $P < 0.05$; malign: $5.47 \pm 3.34$, $P < 0.05$) compared to healthy control pancreas ($1.5 \pm 0.50$). Using *in vitro* assays we found Wnt3a to induce the proliferation of INS-1 insulinoma cells to 151.2% of untreated controls.
Conclusions
These data demonstrate that the expression of Wnt3a and frizzled is increased in human insulinomas compared to normal endocrine pancreatic tissue. On the functional level, Wnt3a stimulates the proliferation of insulinoma cells *in vitro*. This suggests that dysregulated Wnt-signalling is involved in the development of insulinomas. Wnt-signalling molecules are not useful as markers for malignancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P823

**How clinical presentation of insulinoma is changing**

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Introduction
In 2009 the Endocrine Society's clinical practice guidelines for management of hypoglycemic disorders, confirming previous suggestions, have stated that the new cut-off of insulin for the diagnosis of insulinoma should be lowed to 3 μU/mL in presence of hypoglycemia (serum glucose $\leq$55 mg/dL). Moreover hypoglycemia in post-prandial status has been reported in insulinoma, alone or associated with fasting hypoglycemia. Finally preexisting diabetes mellitus is increasingly recognized in these patients.
Aim
To evaluate clinical features and diagnostic criteria in a monocentric series of insulinomas including sporadic and multiple endocrine neoplasia type 1 (MEN-1) patients. In addition anamnestic glucose metabolic profile was investigated.
Patients and methods:
Clinical and biochemical data regarding 33 patients including 27 sporadic and 6 MEN1 were retrospectively analyzed.
Results
The 72 hour fasting test was positive in all cases. However in one case it was initially negative since hypoglycemia did not occur, but became positive after 2 year follow-up. Three patients showed a nadir insulin level $>3$ μU/ml but $<6$ μU/ml. In the remaining 30 cases lowest insulin level was $>6$ μU/ml. At presentation 27 patients (82%) reported only fasting symptoms, 3 (9%) only post-prandial symptoms and 3 (9%) both. Four cases (12%) had been previously affected by type 2 diabetes mellitus and 4 cases by impaired fasting glucose
Conclusion
The new cut-off of insulin has further increased the sensitivity of 72 h fasting test for the diagnosis of insulinoma from 87 to 100% in patients. However the absence of hypoglycemia during test does not rule out in definitive manner the diagnosis and the test should be repeated in any high suspicion case. Surprisingly previous glucose metabolism alterations are not infrequent and require further evaluation in wider series.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P824

**Effects of mitotane on gene expression in the NCI-H295R cell line**

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Background
The adrenolytic agent mitotane is widely used in the adjuvant treatment of adrenocortical cancer, but its mechanism of action including potential effects on mRNA expression is poorly elucidated.
Objective
To examine mitotane-induced mRNA expression changes in the H295R adrenocortical cancer cell line.
Methods
Various concentrations of mitotane in different treatment periods (24–120 h) have been tested in cell viability assays (MTT colorimetric assay and propidium iodide flow cytometry) and for hormone measurements (cortisol and androstenedione) to select the optimal mitotane concentration effectively inhibiting hormone secretion without affecting cell viability. Total RNA isolated from cultures treated for 48 and 72 hours have been subjected to Agilent $4 \times 44$ K microarray platforms. Significantly differentially expressed mRNAs have been validated by quantitative real-time polymerase chain reaction (qRT-PCR).
Results
The mitotane concentration $5 \times 10^{-6}$ M has been selected based on analysis of hormone secretion and cell viability. Altogether 227 genes with significant differences in expression have been found, and the underexpression of 3 genes involved in steroid hormone biosynthesis (3-beta-hydroxysteroid dehydrogenase types 1 and 2 (HSD3B1 and HSD3B2), 21-hydroxylase (CYP21A2)) and 3 significantly overexpressed genes (aldehyde dehydrogenase 1 (ALDH1L2), growth differentiation factor 15 (GDF15) and serpin peptidase inhibitor (SERPINE2)) have been validated relative to the ZNF625 housekeeping gene.
Conclusions
These results demonstrate that the mitotane-induced decrease in adrenocortical hormone secretion is in part mediated at the mRNA level, and mRNA expression changes might be involved in the adrenolytic action of mitotane.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P825

**Ouabain as an antiproliferative agent on adrenocortical cell models and on primary adrenocortical tumor cells**

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Ouabain is a cardiotonic steroid belonging to Strophanthus species. Recently its role in the treatment of tumors has been investigated, as ouabain seems to exert antiproliferative effects. Adrenocortical carcinoma (ACC) is a rare cancer, with poor prognosis, of which SW13 and H295R cells could be considered a cellular model.
Our aim was to evaluate if ouabain exerts anticancer property.
The effects of ouabain were assessed by MTT assay, by [3H] thymidine assay, by Wright's staining method, by flow cytometry analysis, by homogeneous caspase assay and by western blot.
Ouabain induced a reduction in cell viability at 24h and 72h in SW13, in H295R and 4 primary adrenocortical tumor cells. Proliferation rate decreased effectively in SW13 and H295R at higher doses. Marked morphological changes were observed both for SW13 and H295R, with an increase in necrotic process. Cell cycle distribution was altered by ouabain in SW13. Apoptosis analysis demonstrated an increase in caspase activity, clearly evident for SW13 at 72h. FACS analysis by Annexin V-FITC and propidium iodide confirmed an increase in necrotic process especially at higher concentrations. Western blot analysis showed Erk, Akt, p-Akt, P70s6k and p-P70s6k decreased in the treatments of SW13.
Our study demonstrated the antiproliferative effects of ouabain on SW13 and H295R cell models and on primary adrenocortical tumor cells. The results we obtained are promising, but more data are needed to better clarify the role of ouabain in these cellular models, before attempting any approach to animal models.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P826

**Profile of Patients with Pheochromocytoma & Paraganglioma**

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Introduction
There is scarcity of information regarding the profile of patients with Pheochromocytoma and paraganglioma from India.
Aim
To study clinical profile of patients with Pheochromocytoma (PHEO)/ Paraganglioma (PGL) attending the AIIMS hospital.
Methodology:
Study protocol was approved by the AIIMS ethics committee. Written informed consent was taken from patients (parents in case of children). Patients of PHEO & PGL attending the AIIMS hospital from March 2009 were enrolled for this study. The patients with the features of VHL & MEN-1 &2 syndromes were excluded from the study.
PHEO/PGL were diagnosed on the basis of clinical profile, elevation of metabolites Epinephrine, Norepinephrine, Dopamine, Vinylmandelic acid, Normetanephrine, Metanephrine, MRI, MIBG uptake, Ga-DOTANOC uptake etc. Diagnosis was confirmed with histopathologically.
Results
Forty three new patients were diagnosed to have PHEO/PGL during 22 month period starting from March 2009. Age range from 14–62 years (mean 35.09 y $\pm$ 14.07), 18M & 25F.Twenty two (51.1%) *P* were PHEO, 2(4.6%) had PHEO along with extra-adrenal PGL, 1 (2.3%) had PHEO along with parathyroid adenoma. 18 (41.6%) patients had PGL. Out of these 18 patients, 2(11%) had 2 PGL, (one with chest & urinary bladder (UB) PGL & one with Carotid body & thyroid), 1 (5.5%) with UB PGL, 2(11%) with chest PGL, 1(5.5%) with pulmonary, and 1 (5.5%) with abdominal PGL. 11 (61.1%) had head & neck PGL. 10 (23.2%) patients with PHEO/PGL had positive family history. 9 (90%) of these patients with positive family history were below 45 years of age, whereas only 1 (10%) patient with age above 45 year had positive family history. One patient had malignant PHEO. Twenty three (53%) patients had elevated blood glucose. Tumor size range from 2$\times$2$\times$2.1 cm to16.2$\times$9$\times$9 cm.
Conclusion
Patients with PHEO/ PGL with positive family history are diagnosed at early age.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P827

**The role of circulating and tumor infiltrating T-cells on clinical outcome in adrenocortical carcinoma**

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In recent years, it was demonstrated that tumor infiltrating and circulating regulatory and cytotoxic T-cells are associated with clinical outcome in several solid tumors. However, their role in adrenocortical carcinoma (ACC) was never studied, although 60% of these tumors secrete autonomously cortisol, a well-established potent immunosuppressant that most likely influences the balance between different T-cells.
Firstly, we analyzed the circulating regulatory T cells (T(reg); CD4+ CD25highFOXP3+) in 173 blood samples of ACC patients (44 tumor-free, 86 with tumor but no endogenous cortisol excess and 43 with present cortisol excess) compared to 20 healthy blood donors. Secondly, we performed immunofluorescence analyses on paraffin embedded primary tumor tissue from 58 ACC patients for T(reg)(CD3+FOXP3+), cytotoxic T lymphocytes (CTL; CD3+CD8+) and T-helper cells (CD3+CD4+). Furthermore, we performed uni- and multivariate survival analyses.
The percentage of circulating T(reg) in ACC patients with cortisol oversecretion was significantly higher than in the other ACC groups (7.7$\pm$4.5 vs. 5.7$\pm$3 and 5.5$\pm$2.5%; $P<0.05$) and much higher than in healthy donors (3.6$\pm$0.9%; $P<0.001$). While univariate analysis showed a significantly negative association of T(reg) with survival (HR=1.78; $P=0.02$), this influence disappeared in a multivariate analysis adjusting for tumor burden and cortisol excess.
In 41(70%) of ACC samples CTL tumor infiltrates were present (mean 17.8$\pm$ 46.1 cells/HPF) and 31 samples (64.5%) showed T(reg) infiltrates. However, no significant correlation with survival could be demonstrated in a multivariate analysis. Surprisingly, infiltrating CD4+T-cells, present in 17(35.4%) of cases, were independently associated with a better survival (HR=0.39(95%CI 0.16–0.91); $P=0.03$).
In conclusion, neither circulating T(reg) cells nor tumor infiltrating T(reg) nor CTL are associated with clinical outcome in patients with ACC after adjusting for tumor burden and cortisol secretion. Interestingly, CD4+T-cell infiltrates were significantly associated with a better prognosis suggesting a potentially relevant role of these cells in ACC.
Declaration of interest
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## P828

**Surgical management of primary hyperparathyroidism in a unit where Intra-operative PTH (IOPTH) monitoring is not routinely performed**

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Introduction
Parathyroidectomy is performed in primary hyperparathyroidism using either focussed approach or bilateral neck exploration depending upon the pre-operative localisation results. The type of localising investigations and utility of intra-operative adjuncts like IOPTH vary in individual units. In our unit, parathyroid sestamibi scan and neck ultrasound (USS) are done routinely for all cases, and SPECT/CT and CT or MRI are reserved for selective patients. This study looks into our practice to evaluate our first-time success rate and eventual cure rate.
Methods
All consecutive patients who underwent parathyroidectomy for primary hyperparathyroidism between January 2009 and October 2011 were reviewed. 10 patients who had intra-operative sestamibi injection with gamma probe guidance and IOPTH were excluded.
Results
Out of 150 (Male/Female: 42/108) patients, 67 had focussed parathyroidectomy and 83 had bilateral neck exploration. Median age was 64 years (range 22–86). All 150 had sestamibi scan and 147 had USS (one had CT neck, one had SPECT/CT and one had none) with concordance in 69 cases (46%). The median duration of operation was 34 minutes (range 8–126).
The serum calcium level was normal in 142 patients at follow-up (first time success rate 94.7%). Out of the remaining 8 patients (2 focussed and 6 bilateral exploration), 5 had re-exploration and were cured, 3 did not have further operation (2 are awaiting further investigations for MEN1, 1 patient has normal calcium and is being actively monitored). Histology showed single adenoma in 137 cases, 2 double adenomas, 6 multi-glandular hyperplasia, 3 normal parathyroid gland, 1 thyroid colloid nodule and 1 thymic tissue. The eventual cure rate was 98% pending three patients who have not had re-operation yet.
Conclusion
Parathyroidectomy for primary hyperparathyroidism can be safely performed with acceptable cure rates reserving the costly and time-consuming intra-operative adjuncts for cases with difficult preoperative localisation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P829

**Enhanced antitumor efficacy of fructose conjugated-gefitinib on lung cancer cell lines *in vitro* and *in vivo***

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Cancer cells have shown an overexpressed fructose transporter GLUT5 and preferential utilization of fructose as compared to glucose, implicating that a

fructose-based analogue would be a useful target for diagnosis and treatment of cancer. We have successfully synthesized the fructose conjugated-gefitinib compounds {N-[4-(3-chloro-4-(3-fluorobenzyloxy)phenylamino)-7-methoxy-quinazolin-6-yl]-4- [N-methyl-N-(1-deoxy-1-fructosyl)]aminobutanamide}, and examined its antitumor efficacy on cancer cell lines *in vitro* and *in vivo*. Conjugation of fuctose with gefitinib, a sparingly water-soluble drug, dramatically improved its aqueous solubility. Dose-and time-dependently anti-proliferation and apoptosis effect of fructose-gefitinib compound 1 have been observed with MTT assay and FACS in both human colon adenocarcinoma cell line SW480 and human lung adenocarcinoma epithelial cell line A549, the median IC50 values of compound 1 for SW480 and A549 cell lines at exposure durations of 72 h were 2.96 μM and 10.69μM, considerably lower than 8.26 μM and 21.4 μM of gefitinib on SW480 and A549 cell lines, respectively. Furthermore, its attenuated efficacy have been observed in cells trasfected with Glut5 SiRNA. In addition, the enhanced antitumor effects of the compound I was confirmed in A549 tumor xenograft models. Our data demonstrate, for the first time, that conjugation of fuctose and gefitinib imporved the solobility and ehnhanced antitumor effect of gefitinib on lung cancer *in vitro* and *in vivo*, at least, via increased fructose-medicated gefitinib uptake, and suggest that metabolic characterization of cancer could be a potential target for diagnosis and treatment.

Declaration of interest

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## P830

### Specific transcriptional response of four blockers of estrogen receptors on estradiol-modulated genes in the mouse mammary gland

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Introduction

The efficacy and exceptionally good tolerance of estrogen blockade in the treatment of breast cancer is well recognized. Acolbifene (ACOL) is a novel and unique completely free of estrogen-like activity in both the mammary gland and uterus. In theory, this new antiestrogen represents a unique opportunity for a highly potent and specific blockade of estrogen action in the mammary gland and uterus while exerting estrogen-like beneficial effects in other tissues (selective estrogen receptor modulator or SERM activity).

Description of methods/design

In order to better understand the specificity of action of acolbifene, we have used Affymetrix GeneChips containing 45,000 probe sets to analyze 34,000 genes to determine the specificity of this compound compared to the pure antiestrogen fulvestrant, as well as the mixed antagonists/agonists tamoxifen and raloxifene, in their ability to block the effect of estradiol (E2) and to induce effects of their own on gene expression in the mouse mammary gland. The genes modulated by E2 were those identified in two separate experiments and validated by quantitative real-time PCR (Q_RT-PCR).

Results

Three hours after the single subcutaneous injection of E2 (0.05 μg), the simultaneous administration of acolbifene, fulvestrant, tamoxifen and raloxifene blocked by 98%, 62%, 43% and 92% the number of E2 upregulated genes, respectively. On the other hand, 70%, 10%, 25% and 55% of the genes down-regulated by E2 were blocked by the same compounds. Acolbifene was also the compound which, when used alone, modulated the smallest number of genes also influenced by E2, namely 4%.

Conclusion

The present data offer a possible explanation for the potent tumoricidal action of acolbifene in human breast cancer xenografts where 61% of tumors disappeared, thus bringing a new paradigm in the hormonal therapy of breast cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P831

### Non pituitary effects of GnRH analogues in prostate cancer cells: revertion of maligant phenotype and synergism with bicalutamide and radiotherapy

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Background

Gonadotropin-releasing hormone (GnRH) receptor is a G protein-coupled receptor involved in the synthesis and release of pituitary gonadotropins. GnRH analogs constitute the most widely employed medical treatment for prostatic cancer. The predominant mechanism of action is presumed to be via the inhibition of gonadotropins and resultant decrease in androgen. Although pituitary and extra-pituitary GnRH-R transcripts appear identical, their functional characteristics may differ and most extra-pituitary GnRH-Rs may not discriminate between agonists and antagonists in the same way as do pituitary GnRH-Rs. However, GnRH analogs have also been shown to directly inhibit prostate cancer cells both *in vitro* and *in vivo*. GnRH analogues influence the *in vitro* proliferation of cultured human cells by complex interactions that are still partially understood.

Aims

Our hypothesis is that GnRH analogues modulate prostate cancer progression acting on invasive capacity and androgen responsiveness of tumor cells. So, GnRH triggering may revert maligancy of advanced castrated refractory prostate cancer models.

Results

We demonstrated that: GnRH analogues show non pituitary effects both in hormone sensitive and castrated refractory prostate cancer cell models. GnRH analogues induce antiproliferative and pro-apoptotic effects altering the p75NTR:TrkA and p75NTR/TrkB ratios, activating extrinsic apoptotic pathways and partecipating to reduce anoikis and survival in the blood stream and in metastatic sites. In particular, GnRH analogues induce PTEN expression and reduce Akt actity in PTEN+/AR+ 22rv1 cells and induce extrinsic apoptosis pathways triggering TRAIL-Receptor signaling. GnRH analogues revert malignant phenotype by induction of p75 NTR and AR expression both in AR positive (22rv1 and LAPC-4) and negative (PC3) cell models resulting synergistic with bicalutamide and Radiotherapy both *in vitro* and *in vivo*.

Conclusions

Our results support a potential use of these agents for the treatment of locally aggressive as well as advanced prostate cancer.

Declaration of interest

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## P832

### Histone deacetylase inhibitor belinostat (PXD-101) represses androgen receptor expression and acts synergistically with castration and bicalutamide treatment to inhibit prostate cancer growth in hormone refractory models

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Rationale and aims

Aberrant activation or "reactivation" of androgen receptor in the course of androgen-ablation therapy shows a potential cause for the development of castration-resistant prostate cancer. This study tested the hypothesis that belinostat (PXD101), a potent pan histone deacetylase inhibitor, may potentiate hormonal therapy and prevent onset of castration resistant phenotype Material and Methods. A panel of human prostate cancer (Pca) cells with graded castration resistant phenotype and *in vivo* models were used to verify this hypothesis.

Results

In this report we demonstrated that hormonal manipulation favors the onset of castration-resistant phenotype by the increase of HDAC expression and activity as well as by the increased expression and activity of AR, EGFR, HER2 and Akt. Consistent with these observations, the functional knockdown of HDACs by Belinostat prevented the onset of castration resistant phenotype with a significant down-regulation of AR, EGFR, HER2 and Akt expression/activity. The deregulation of functional cooperation between HDAC-6 with hsp90, on one hand, and between GSK-3beta with CRM1, on the other hand, may explain the biological effects of Belinostat. In this regard, the HDAC-6 silencing or the

functional knockdown of hsp90 by 17AAG resulted in the selective down-regulation of AR, EGFR, HER2 and Akt expression/activity, while the decreased p-GSK-3beta expression after Belinostat treatment increased the nuclear expression of CRM1 which in turn modified the AR and survivin recycling with increased caspase-3 activity.
Conclusions
HDAC inhibitors retain the ability to prevent the onset of castration resistant phenotype and, therefore, merit clinical investigation in this setting. However, further information are necessary to develop clinical treatment strategies for this disease stages.
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## P833

**Confirmation of pathogenicity of the MEN1 missense mutations by analysis of protein instability and aberrant splicing**

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Multiple endocrine neoplasia type 1 (MEN1) is a relatively rare autosomal dominantly inherited condition characterized by hyperplastic and neoplastic disorders of endocrine organs such as the parathyroid, anterior pituitary and gastroenteropancreatic endocrine tissues. Germline mutation of the causative gene, MEN1, which localizes to human chromosome 11q13 and encodes the 610 amino-acid nuclear protein menin, can be identified in most affected subjects. MEN1 gene mutation analysis is a powerful tool for the early diagnosis of MEN1, but the clinical significance of MEN1 gene mutations is not always obvious. In the case of frameshift mutations, nonsense mutations or large deletions, it is relatively straightforward to consider those lesions as pathogenic. However, when identified mutations are missense mutation or in-frame deletions, molecular diagnosis of MEN1 is not so simple, since the pathogenicity of these mutations is not clear per se. We recently identified previously unreported missense MEN1 gene mutations, c.824G>T and c.1118C>T, from patients with primary hyperparathyroidism, and evaluated for their pathogenicity.
These mutations were predicted to generate putative missense menin proteins, R275M and P373L, respectively. A stability test using a quantitative fluorescent immunohistochemical method (Yaguchi 2004, Shimazu 2011) demonstrated that the P373L mutant is highly unstable, suggesting that this mutation is likely causative for typical MEN1, whereas the stability of the R275M mutant was reduced only slightly. Given that the c.824G>T mutation occurred at an exon-intron junction, this mutation was suspected to act as a splicing mutation rather than a simple missense mutation. Analysis of leukocyte mRNA and minigene experiments indicated that the mutant c.824G>T allele gives rise to abnormally spliced menin mRNA.
We conclude that both mutations are likely causative for typical MEN1. The menin stability test and leukocyte mRNA analysis may be valuable for the initial molecular diagnosis and subsequent management of patients with germline MEN1 mutations, especially in the case of putative missense mutations of ambiguous pathogenicity.
Declaration of interest
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## P834

**Expression of MAPK7 and CCNL1 in corticotrophinomas and functional analysis of miR-143 and miR-145 in corticotrophic tumoral lineage AtT20**

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Introduction
miR143 and 145 seem to be involved in the pathogenesis of cortitotrophinomas by modulating the expression of MAPK7 and CCNL1 genes, which protein products are involved in cell proliferation, differentiation, and cell cycle progression. Objective: To analyze the modulation of miR-143 and -145 in the expression of MAPK7 and CCNL1 genes in corticotrophinomas and corticotrophic tumoral lineage (AtT20).
Material and Methods
The expression of MAPK7 and CCNL1 was evaluated by Real Time PCR in 25 corticotrophinomas obtained from patients with Cushing's disease and 6 normal anterior pituitaries and in primary culture of anterior pituitary cells and in AtT20 culture cells from mouse. Functional analyses of the interaction of miR-mRNAs were performed by the transfeccion of miR precursors in AtT20 cell lineage (siPORT, Applied Biosystems).
Results
We observed no differential expression of MAPK7 ($0.94\pm0.36$ vs $1.16\pm1.30$) and CCNL1 ($0.90\pm0.29$ vs $0.64\pm0.51$) genes between normal pituitaries and corticotrophinomas. We observed an overexpression of MAPK7 ($3.61\pm1.84$ vs $0.93\pm0.17$, $P=0.002$) and CCNL1 ($2.6\pm0.7$ vs $0.99\pm0.08$, $P=0.002$) in AtT20 cells compared to primary pituitary culture cells. The transfections were confirmed by overexpression of miR143 ($1.25\pm0.79$ vs $1552\pm730$, $P<0.0001$) and miR145 ($1.43\pm1.42$ vs $4567.6\pm1235$, $P=0.0003$). We observed no differential expression of MAPK7/ERK5 gene neither with miR-143 ($1.04\pm0.2$ vs $1.07\pm0.02$) nor with miR-145 ($1.04\pm0.2$ vs $1.13\pm0.2$) in AtT20 transfected cells. There was also no differential expression in CCNL1gene in AtT20 cell transfected with miR143 ($1.05\pm0.3$ vs $1.05\pm0.3$); however, there was CCNL1 overexpression ($1.05\pm0.3$ vs $1.17\pm0.2$;$P=0.003$) in AtT-20 transfected cells with miR145.
Conclusion
The differential expression between corticotrophinomas and AtT-20 tumoral culture cells may be due to culture immortalization. The interaction of miR-143 and MAPK7 gene does not occur by mRNA degradation, suggesting a posttranscriptional event. miR-145 may up regulate CCNL1 gene, which might lead to deregulation of the cell cycle and contribute to the pathogenesis of corticotrophinomas.
Declaration of interest
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## P835

**Tc- 99m Sestamibi parathyroid scan in primary hyperparathyroidism and surgical outcomes**

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Tc- 99m Sestamibi parathyroid scan (SP scan) is performed to identify location of either hyperplastic or adenomatous gland(s) in primary hyperparathyroidism (HPT). In this study we evaluated diagnostic outcome in 55 consecutive patients with clinical diagnosis of HPT who were referred for SP scans and subsequently had parathyroid surgery. These patients with HPT were of $66.9+12.46$ (mean + SD) age, and had serum calcium $10.9+0.93$ mg/dl, phosphorus $3.0+0.51$ mg/dl, chloride $107+3$ mEq/L, chloride/phosphate ratio $36.9+5.4$, creatinine $1.16+0.32$ mg/dl, and intact parathyroid hormone level $132+83$ pg/ml. SP scans were performed early (at 0 to 30 min) or delayed (90 to 210 min) following injection of 20 mCi of Tc- 99m Sestamibi. Of 55 patients, SP scan finding were concordant with surgical findings in 36 (66%) patients. In 19 patients (34%) SP scan findings were discordant with surgical finding. All 19 discordant scans were reevaluated. Of 19 discordant SP scans, four were read as adenoma. An adenoma and one large hyperplastic parathyroid gland were found in one of these four, and in the other three the adenoma was actually found contralateral to the side indicated on the scan. The scan findings were confirmed at reevaluation. In the reevaluation of remaining 15 discordant SP scans read as hyperplasia, the previous SP scans reading was confirmed in three patients. However, in 12 patients a focally increased radioisotopic uptake was present during the early images of SP scan but not in the delayed images. The early increased focal radioisotopic uptake in these SP scans coincided with surgical findings of parathyroid adenomas in all 12 patients thus increasing SP scan concordance to surgical outcomes to 87% (48 of 55 SP scans). These findings suggest that in HPT careful evaluation of early increased radioisotopic uptake during SP scan can be important in identifying parathyroid adenomas when delayed images show no focal retention of radiotracer.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P836

**Vitamin D from genetics to the clinical in prostate cancer**

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Background
The prostate cancer (CaP) is among the most commonly diagnosed cancers, accounting for about 20% of all newly diagnosed cancers. Subject of recent studies is the role of vitamin D in the pathogenesis of CaP. Literature data speculated about the role of vitamin D in the progression of Cap. In addition, several studies have shown an association between VDR gene polymorphism FokI and CaP especially in the Asian population.
Objective
The aim of this study is to compare two populations: CaP vs benign prostatic hyperplasia (BPH), to study both the potential role of serum 25-OH vitamin D (25 (OH) D3) and assess the relationship between serum 25 (OH) D3 and VDR gene polymorphisms FokI and TaqI and between polymorphic sites and the risk of developing cancer.
Material and method
Two hundred patients (aged 41 to 83 years) in whom biopsies revealed CaP or BHP were enrolled in the present study. The concentration of 25 (OH) D3 was measured by chemiluminescence immunoassay and polymorphisms were detected by PCR-restriction fragment length polymorphism (RFLP).
Results and conclusion
The results showed a significant association ($P=0.002$) between the genotype FF FokI polymorphism, responsible for the translation of a shorter protein and therefore more functional than that normal one, in the BPH population compared to CaP, as well as the genotypes of the TaqI polymorphism ($P=0.009$). Serum 25 (OH) D3 did not showed significant correlation respect to polymorphisms and compared to disease, although the concentration was low ($<30$ ng/mL) in 97% of the analyzed samples of the two populations. Concluding the CaP is associated with a less functional VDR variation due to a lower frequency of the genotype FF and for the first time there was evidence that the tt genotype of TaqI polymorphism (synonymous polymorphism) is associated negatively to the disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P837

**Progesterone receptor B induces autophagy antagonizing the phosphatidylinositol-3 kinase PI3K/AKT pathway in breast cancer cell lines**

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Progesterone (PRG) deficiency has been linked to an increased risk of cancer, while normal levels of PRG in the body actually help protect you against some forms of cancer. The tumor suppressor role of this steroid was envisaged, however the mechanism through which this occurs was not clearly established. The effects of progesterone act via the progesterone receptor (PR) of which two isoforms, called A (PR-A) and B (PR-B) were discovered. Herein, we present a novel finding since PRG through PR-B induces of cell death by autophagy in breast cancer cells. The link between autophagy and cancer appears to be complex and multifaceted. Of importance is the understanding of the circumstances under which autophagy promotes either cell death or cell survival in the context of tumorigenesis, as this may have implications in cancer therapy. Analysis of different autophagy-related markers such as AMPK, AKT, mTOR, PI3III, Beclin 1, AMBRA and UVRAG indicated for the first time the ability of OHPg/PR-B to induce their expression. The monitoring of autophagic process, assessed by either biochemical (MDC) and ultrastructural methods (TEM), confirmed the molecular data. In the present study we report that PRG, through the PR-B isoform, produces a significant inhibition of MCF-7 breast cancer cell survival by autophagy. These data underscore a novel mechanism through which PRG/PR-B exerts its beneficial effects on breast cancer, sustaining that PRG/PR signaling may be considered as useful pharmacological tool to be exploited for innovative adjuvant strategies in breast cancer treatment.
Declaration of interest
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## P838

**Insulinoma in patients with multiple endocrine neoplasia type 1**

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More than half of patients with multiple endocrine neoplasia type 1 (MEN1) develop gastroenteropancreatic neuroendocrine tumors (GEPNETs). Among GEPNETs in MEN1, insulinoma is the second common functioning tumor and often diagnosed by its pathognomonic clinical feature, i.e. hypoglycemia. Compared to other functioning and nonfunctioning GEPNETs in MEN1, insulinoma is known to develop in younger age. We have recently constructed a database of Japanese patients with MEN1 and here report clinical features of patients with MEN1 who developed insulinoma. Among 582 registered patients, 560 cases were considered to be eligible for analysis. GEPNETs were seen in 314 patients and 69 patients had insulinoma. Tumors predominantly occurred in body and tail of pancreas. Among 54 patients whose age at diagnosis of insulinoma was recorded, only 11 patients (20.4%) had a single tumor at the diagnosis; that complicates surgical decision making. Surgery was performed for 51 patients and 6 patients needed multiple surgery. Age at diagnosis of insulinoma was $38.9\pm18.1$ yrs for proband (including sporadic cases) and $30.9\pm13.9$ yrs for family members. It is to be noted that 13 patients (24.1%) were diagnosed of insulinoma before 20 yrs of age. Five were probands and their parents were diagnosed as having MEN1 after diagnosis of their offsprings. Such young onset was not seen in other GEPNETs. According to recent epidemiological study which analyzed more than 3,000 cases with pancreas NET in Japan, only 1% of patients developed pancreas NETs at younger than 20 yrs. It also revealed that 10% of patients with pancreas NETs had MEN1. These results indicate that insulinoma diagnosed before 20 yrs strongly suggests the presence of MEN1 and warrants further investigation including MEN1 genetic testing.
Declaration of interest
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## P839

**Multiple endocrine neoplasia syndrome type 1 (MEN-1) in Sardinian population: low prevalence of Men-1 mutations and detection of a new inactivating mutation of the CDKI gene p27**

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Introduction
The genetic basis of multiple endocrine neoplasia type 1 (MEN-1) syndrome is often represented by inactivating mutations of Men-1 gene, found in 50–80% of different series. Recently, other mutations of genes encoding for the CDKI complex (p15, p18, p21, and p27) and of the AIP gene have been described in a small number of Men-1-negative patients.
Methods and results
Since 2002 we tested for Men-1 mutations 16 patients born and living in Sardinia, an island with approximately 1,500,000 inhabitants and peculiar genetic background due to long-lasting isolation. The series included 13 patients with a clear MEN-1 phenotype (7 with familial history, for a total of 5 families, and 6 apparently sporadic) and 3 with apparent MEN-1 phenotype (1 familial and 2 apparently sporadic). Men-1 mutations were detected in only 3 cases (2 familial, 1 sporadic), corresponding to 27.2% of patients with clear MEN-1 phenotype. Identified mutations were Arg229His in exon 4 and splicing 894-9 G > A (familial cases) and frameshift 1284-of G in the exon 8 (sporadic case). We then started to look for the presence of other mutations in Men-1-negative patients and to date

we completed the analysis for p27. A new p27 mutation (c.372_373delCT in exon 1) was found in a patient with an apparently sporadic typical MEN-1 syndrome (neuroendocrine tumors of the pancreas and distal duodenum and primary hyperparathyroidism due to multiple parathyroid adenomas).
Conclusion
These results suggest that in the particular context of the Sardinian population, the genetic basis of MEN-1 could be different from that commonly observed, with a relatively low prevalence of Men-1 as compared to non-Men-1 mutations. The detection in this small series of a new p27 mutation confirms that a complete screening for rare mutations is advisable in all Men-1 negative patients with clear MEN-1 phenotype, irrespective of positive family history.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P840

**Atypical paragangliomas responsible for adrenaline-dominant catecholamine secretion due to ectopic expression of phenylethanolamine-N-methyltransferase**

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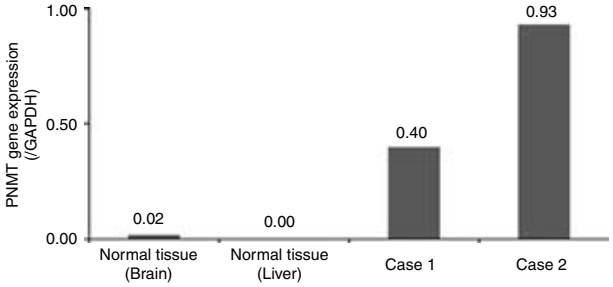
Introduction
Pheochromocytoma (PHEO) is a rare tumor of chromaffin cells with variable clinical presentation. Adrenal PHEOs secrete excessive amounts of noradrenaline or of noradrenaline plus adrenaline, whereas extra-adrenal PHEOs (paragangliomas) mostly secrete noradrenaline alone due to lack of expression of phenylethanolamine-N-methyltransferase (PNMT), the key enzyme converting noradrenaline into adrenaline. We report two exceptional cases of paragangliomas responsible for adrenaline-dominant catecholamine secretion.
Case Report
[Case 1] A 58-year-old man presented hypertension, hot flush, and dizziness. Computed tomography (CT) of the abdomen revealed right retroperitoneal tumor (25 mm in diameter) 1 year ago. He had been treated with nifedipine. CT also revealed gradual enlargement of the tumor (41 mm in diameter) during 2-year follow up. Endocrinological evaluation showed PHEO due to very high levels of 24-hour urine adrenaline (877.5 μg/day), noradrenaline (307.5 μg/day), metanephrine (4.6 mg/day), and normetanephrine (1.0 mg/day). [Case 2] A 74-year-old woman had presented resistant hypertension for 2 years and nocturnal transient hypertension attack for a few weeks. Endocrinological evaluation showed PHEO due to high levels of 24-hour urine adrenaline (109.6 μg/day), noradrenaline (214.7 μg/day), metanephrine (0.92 mg/day), and normetanephrine (0.56 mg/day). Magnetic resonance imaging (MRI) revealed right retroperitoneal mass (19 mm in diameter) beside inferior vena cava. The tumors were confirmed by 131I-MIBG scintigraphy in both cases. Laparoscopic tumorectomy was performed, and pathological findings indicated paragangliomas in both cases. The symptoms and the catecholamine excess diminished after surgery. Interestingly, quantitative RT-PCR analysis showed significant PNMT gene expression in both tumors.
Discussion
Atypical phenotypes of adrenaline-dominant catecholamine excess in two paraganglioma cases may be due to aberrant PNMT gene expression supposed to be induced by high local glucocorticoids levels adjacent to the adrenal glands and by other transcription factors.

**Aberrant PNMT gene expression in paragangliomas compared with normal tissues.**




Conclusion
We present 2 atypical cases of paragangliomas responsible for adrenaline-dominant catecholamine secretion.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P841

**Evaluation of patients with adrenal diseases operated laparoscopically between 1997 and 2011**

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Aim of the study
Presenting the changes in surgical management and patients' characteristics based on a single-center data.
Material and method
From 29/10/1997 to 31/04/2011 824 adrenalectomies were carried out, 641 (78%) laparoroscopic and 183 (22%) open. In all cases of laparoscopic adrenalectomy (LA) lateral transabdominal approach was used. Bilateral simultaneous adrenalectomy with the changing patient position was performed for bilateral pheochromocytoma, while in hypercortisolemia two-stage adrenalectomy with time interval was preffered. Indication for LA in 349 (54%) were hormonal active lesions, in 292 (46%) non-active lesions. In this study the characteristics of patients, size of the tumor and technical aspects of laparoscopy were compared between two periods: 1997–2005 and 2006–2011.
Results
The number of patients operated due to hormonal active lesions increased (53.4 vs 55.2%), mainly due to hypercortisolemia (34.6 vs 39.5%) and pheochromocytoma (33.6 vs 39.5%). The higher incidence of patients with hypercortisolemia is a result of rise of cases of Cushing's disease (2.5 vs 4.5%) and pre-Cushing's syndrome (16.1 vs 18%). The amount of cases with Cushing's syndrome didn't change significantly (16.8 vs 17%). In the same time the number of patients with Conn's syndrome has decreased (30.9 vs 19%). Among non-active tumors: the amount of adenomas inreased (63.8 vs 72.2%), while the remaining number of cases lowered (46.6 vs 44.8%). Distribution of tumors size was comparable. The number of tumors with diameter: $< 4$ cm was 46%, $\geqq 4 < 6$ cm - 33%, $\geqq 6 <$ 8 cm - 16% and $\geqq 8$ cm - 5%.
Conclusion
Laparoscopic operations became recommended method of surgical treatment of adrenal pathology. Despite greater availability of imaging exams which increase detection of adrenal diseases the proportion between the active and non-active lesions qualified for laparoscopy hasn't changed significantly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P842

**Immunohistochemical detection of FSH receptors in endocrine tumors**

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Introduction
Follicle stimulating hormone receptors (FSHR) are physiologically expressed in gonads. FSHR are also expressed in gonadal cancers, but nothing is known on FSHR appearance in non-gonadal endocrine tumors. The present paper reports on the immunohistochemical detection of FSHR in pituitary, thyroid, adrenal and neuroendocrine tumors (NET).
Materials and methods
The study included samples of 80 endocrine tumors (28 pituitary adenomas, 7 thyroid tumors including 3 cancers, 34 adrenal tumors including 8.pheochromocytomas and 2 adrenocortical cancers and 11 NET).
FSHR immunostaining was performed using the antibody raised against 1–190 amino acid sequence from the human FSH-R (sc-13935).
Results
In the investigated tumors, positive immunostaining with anti-FSHR antibody occurs in the tumoral cells cytoplasm and/or endothelia of the intra- and

peritumoral blood vessels. The cytoplasmic immunostaining was found in 7/8 of pituitary adenomas, 7/8 of pheochromocytomas, 22/22 of adrenal adenomas, 2/2 adrenal cancers, 1/.4 of benign thyroid tumors, 3/3 of thyroid cancers and 11/11 NET. FSHR immunostaining in blood vessels was present 28/28 of pituitary adenomas, 5/8 of pheochromocytomas, 12/22 of adrenal adenomas, 2/2 adrenal cancers, 1/.4 of benign thyroid tumors, 2/3 of thyroid cancers and 6/11 of NET.
Conclusions
FSHR are often detectable in tumoral cells and in the endothelium of intra- and/or peritumoral blood vessels of benign and malignant non -gonadal endocrine tumors. It is hypothesized that FSHR expression in these tumors (like in gonads and gonadal tumors) mediates stimulation of cell growth and angiogenesis. This presumption needs further studies to be proved.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P843

**Tumour-asssociated myofibroblasts in parathyroid tumours**

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Tumour-associated myofibroblasts are activated fibroblasts known to influence many aspects of tumour development. Parathyroid tumours show increased microvessels and atypical/malignant tumours are characterized by fibrous bands suggesting activation of the stromal cells. The aim of the present study was to investigate the role of myofibroblasts in parathyroid tumorigenesis. Human parathyroid tissues were analysed by specific immunostaining for the myofibroblast marker alpha-smooth muscle actin (alpha-SMA). Alpha-SMA+ cells were well represented in the parenchyma of normal parathyroid glands ($n=3$) lining the acinar cellular structures. In typical adenomas (PA, $n=5$) intraparenchymal alpha-SMA+ cells were variably reduced, though they surrounded new microvessels suggesting a role in neoangiogenesis. In atypical adenomas ($n=3$) and carcinomas ($n=3$), the parathyroid chief cells proliferating in sheets were not sustained by myofibroblasts, which were highly represented in the stroma of fibrous bands and capsula. Interestingly, in human fetal parathyroids ($n=2$; 19 and 24 weeks of gestation), myofibroblasts were exclusively found lining blood vessels. By RT-PCR activated fibroblasts markers such as vimentin, stromal derived factor-1 (SDF-1) and fibroblast activated protein (FAP) were detected in PA samples. To characterize alpha-SMA+ cells, tumor explants ($n=5$) were cultured on fibronectin in 10% FBS for 9 days. Large spindle-shaped alpha-SMA+ cells outgrew from explants. By immunofluorescence (IF), a subset of alpha-SMA+ cells co-expressed the specific parathyroid marker GCM2, and TBX1, an embryonic nuclear transcription factor involved in endodermic pharynx development. Moreover, by FACS and IF, cells expressing the SDF-1 receptor CXCR4 were detected in PAs. In conclusions, we identified cells showing features of tumour-associated myofibroblasts in parathyroid tumours. Data suggested that: 1) they might be involved in fetal as well as in tumoral neoangiogenesis; 2) they might contribute to matrix deposition in fibrous bands; 3) they might cross-talk with parathyroid cells by the SDF-1/CXCR4 signalling; 4) they might derived their origin from epithelial-to-mesenchymal transition.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P844

**Establishment of a Multidisciplinary Tumor Board for Patients with Neuroendocrine Neoplasms**

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Introduction
Neuroendocrine neoplasms are rare and multiform requiring a multidisciplinary approach. We report the experience of a Neuroendocrine tumor board (TB) established at our University Hospital.
Aims
The aim is to share clinical and diagnostic data for best decision-making according to the existing ENETS guidelines.
Materials and methods
According to the indications for ENETS Centers of Excellence, meetings with experts in Endocrinology, Endoscopy, Nuclear Medicine, Oncology, Pathology, Radiology and Surgery are held; interactive computer-assisted presentation of 3 or 4 cases each time presented by one representative of the caring equipe; electronic reports written, circulated and stored; descriptive statistics for data analysis.
Results
TB started in December 2010, 21 biweekly meetings were completed, 50 cases discussed, 18 more than once; the diagnostic-therapeutic decision was tailored for each patient, in approximately 70% of cases according to the ENETS guidelines; the gallium PET/CT was identified as a special need and introduced as diagnostic standard in the second half of 2011; electronic reports constitute a shared database.
Conclusions
Our data indicate a good correlation between ENETS guidelines and clinical practice but underline the need for improvement for selected areas. The TB is a valid tool to develop suitable programs of care according to the best clinical evidence and guidelines.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P845

**Low detection Rate of Primary Aldosteronism when Screening Among Hypertensive Patients with Ongoing Medication**

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Objective
Primary aldosteronism (PA) is a common cause of secondary hypertension. Antihypertensive agents interfere with the renin-angiotensin-aldosterone axis, and screening with the aldosterone to renin ratio (ARR) is preferably made without medication. Withdrawal may however be harmful. We examined the detection rate of PA in hypertensive patients taking their prescribed medication, apart from amiloride and spironolactone.
Design and setting
Hypertensive patients recruited from a primary care unit were investigated in a university hospital setting. The cut-off level for a positive ARR, indicative of PA, was set at $>50$ pmol/ng with aldosterone $>350$ nmol/l which is lower than practise to minimize false negatives. A positive ARR was followed by a confirmatory evaluation.
Subjects
233 hypertensive patients aged 25 to 70 were recruited and 78% agreed to participate. The majority had at least two antihypertensive agents.
Results
The frequency of confirmed PA was 1.6% and including cases with positive ARR who refused further investigation it would be 3.3% at most. In primary hypertension ARR decreased with the number of antihypertensive agents used due to increasing renin. Compared to untreated patients, median ARR was reduced by approximately 70% when taking 3 or 4 agents. Angiotensin receptor blockers, ACE inhibitors and thiazide diuretics markedly suppressed the ARR.
Conclusion
The low detection rate of PA does not support screening in the general hypertensive population. The marked suppression of ARR by antihypertensive drugs indicates that other cut-off levels should be defined for patients on treatment. At present, screening for PA should be carried out as far as possible without medication.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P846

**B-Lymphocyte Stimulator in neuroendocrine tumors: a new prognostic marker?**

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Aim of the study
To test B-Lymphocyte Stimulator (BLyS) as a new serological marker in the follow-up of patients with neuroendocrine tumors (NET).
Methods
Eighty-one consecutive patients with NET and 56 age and sex-matched controls were enrolled in the study. According to the clinical course, patients were classified in 2 subgroups: evidence of persistent but stable disease or in remission ($n=45$) and patients with evidence of recurrent disease (progressive patients, $n=36$). BLyS and Chromogranin A (CgA) serum levels were analyzed by ELISA.
Results
BLyS levels were more elevated in NET patients than in controls ($1153\pm529$ pg/ml vs $655\pm158$ pg/ml; $P<0.0001$) and correlated with tumor differentiation ($1058\pm398$ pg/ml in gastroenteric G1 - typical lung vs $1325\pm640$ pg/ml in gastroenteric G2 - atypical lung; $P=0.026$). Stable/remission patients displayed significant lower BLyS levels than patients with recurrent disease ($889\pm251$ pg/ml versus $1461\pm623$ pg/ml; $P<0.0001$). BLyS levels did not change in patients who remained stable after $6.6\pm2.8$ months (from $864\pm283$ pg/ml to $809\pm235$ pg/ml; $P=$ns), while further increased in patients with disease progression (from $1575\pm810$ pg/ml to $1887\pm1163$ pg/ml; $P=0.045$). CgA, instead, showed inconsistent changes. Metastatic patients displayed higher BLyS levels than non metastatic ($1391\pm724$ pg/ml vs $1079\pm422$ pg/ml; $P=0.022$).
Conclusion
Elevated BLyS levels characterize more aggressive NET patients. BLyS appears as a new potential prognostic marker in the follow-up.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P847

**Clinical and morphological characterization of C cell hyperplastic and neoplastic lesions of thyroid**

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Introduction
Medullary thyroid carcinoma (MTC) and C cell hyperplasia (CCH) have a variable clinical presentation and prognosis and few data are available on the correlation between immunohistochemical characterization (IIC) and clinical behavior.
Aim
We studied the clinical and morphological characterization of CCH and MTC and we evaluated IIC expression of PTTG-1, SSTR2A, VEGFR-1, VEGFR-2 and VEGFR-3 in 23 cases of CCH and/or MTC, correlating it with clinical features
Material and methods
23 patients, affected by CCH and/or MTC, were included from 1990 to 2010. All patients underwent total thyroidectomy, pre and post operative serum calcitonin assay, staging, histological examination with immunohistochemical evaluation of PTTG, SSTR2A, VEGFR-1, VEGFR-2 and VEGFR-3 expression and 18 of them were tested for RET mutations.
Results
After surgery 3 cases presented only CCH and 10 cases had lymph node metastases (N+) and during follow-up 7 patients (all N+) were considered uncured. PTTG and VEGFR-1 were significantly overexpressed in lympho node metastatic and uncured patients. VEGFR-2 was not much expressed in all of patients, while VEGFR-3 and STTR2A were strongly expressed in about all of patients. Four cases were familiar (2 MEN2B, 1 MEN2A and 1 FMTC).
Conclusions
Our data suggest that PTTG-1 and VEGFR-1 seem related with a poor prognosis, while the expression of VEGFR-3 and SSTR2 in all patients confirms the key role of IIC evaluation for the therapeutic approach of these patients. Finally, a more complete IIC evaluation with new markers can be useful for diagnosis, prognosis and therapy of MTC.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P848

**Hormonal Therapy Promotes Hormone-Resistant Phenotype by Increasing DNMT Activity and Expression in Prostate Cancer Models**

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We hypothesized that hormonal therapy favors the development of the hormone-resistant (HR) phenotype through epigenetic mechanisms. Human prostate cancer (Pca) tissues and *in vitro* and *in vivo* models were used to verify this hypothesis. We demonstrated that tumor cells continuously treated with BCLT or cultured in androgen depleted medium progressively acquire higher DNMT activity and expression. Increased DNMT expression and activity also paralleled the up-regulation of truncated AR isoforms which favors the development of the HR phenotype. Following DHT stimulation, DNMT activity was significantly reduced in comparison with hormonal therapy. Consistent with these observations, the silencing of DNMT3-a and DNMT3-b significantly decreased the DNMT activity levels. These findings were also directly correlated with PTEN down-regulation and activation of ERK and PI3K/Akt pathways. The use of a pan-DNMT inhibitor (5-Azaciticine) greatly reduced the development of the HR phenotype induced by long-term BCLT treatment and this finding correlated with DNMT activity decrease. The regulation of DNMT activity was, in some measure, dependent on the AR, as siRNA treatment targeting the AR greatly decreased the modulation of DNMT activity under androgenic and antiandrogenic stimulation. These observations were correlated *in vivo* in patients, as demonstrated by immunohistochemistry. Patients treated by BCLT prior to surgery had higher DNMT3a and DNMT3b expression than patients who had not undergone this treatment. Our findings provide evidence of a relationship between the HR phenotype and DNMT in human Pca.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P849

**Gene expression profile of LNCaP human prostate cancer cells after treatment with an antagonist of growth hormone-releasing hormone**

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Antagonists of growth hormone-releasing hormone (GHRH-ant) may exert their antiproliferative effects directly on cancer cells, which are mediated by the tumoral GHRH receptors identified by us. Furthermore we showed that GHRH-ant are able to induce apoptosis in LNCaP human prostate cancer cells through a Ca2+-dependent pathway. However, the molecular features involved in the antiproliferative effect of GHRH-ant have not yet been elucidated. To study this, gene expression profile of LNCaP cancer cells treated with a GHRH-ant MZ-J-138 (10 μM) for 1, 5 or 18 hours was analyzed and compared to that of untreated control LNCaP cells. The genome-wide cDNA microarray chip type in the experiment was Illumina Human HT-12 v.4 Expression BeadChip. Expression profiling showed 181, 152, 16 up-regulated genes and 98, 43, 8 down-regulated genes in LNCaP cells treated with GHRH-ant for 1, 5, 18 hours, respectively (average fold difference $>1.5$; $P<0.0001$). We observed overexpression of PMEPA1, BRCA1, KLF6, FANCG, RBL1, E2F2 and down-regulation of SLC45A3, TMPRSS2, MYC in LNCaP cells after treatment with GHRH-ant. In addition, many cell cycle genes are constituently activated in treated LNCaP cells. The up-regulated negative regulators of cell proliferation and the down-regulation of some factors expressed in high concentration in prostate cancer cells may

explain why GHRH-ant can reduce cell proliferation of LNCaP cells. Our data suggest also the involvement of p53-dependent and independent mechanisms in the antiproliferative effect of GHRH-ant. Treatment with GHRH-ant may offer a new approach to the therapy of prostate and other hormone-sensitive cancers (supported by OTKA K 068452).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P850

**Evaluation of beta-cell function and insulin sensitivity in a large series of insulinomas in one single tertiary centre**

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Background
Patients with an insulinoma are at variable risk of severe hypoglycemia due to wide ranges of both tumoral insulin secretion and peripheral tissue sensitivity to chronic hyperinsulinaemia.
Objectives
In this study we investigated the clinical, biological and tumoral characteristics of a large series of patients with immunohistochemically confirmed insulinoma and we assessed both insulin sensitivity and beta-cell function in fasting steady-state conditions.
Methods
This was a retrospective analysis of 48 patients with an insulinoma, in whom insulin sensitivity and beta-cell function were evaluated by a fasting HOMA test.
Results
The median age at the time of diagnosis was 51 years (range: 17–83) and mean BMI was $26.6 \pm 10.6$ kg/m$^2$. In 8 patients (17%), the diagnosis of malignant insulinoma was considered. Pre-operative localization was possible in 94% of the patients. The most sensitive imaging methods were echo-endoscopy (sensitivity 92%) and intra-operative ultrasonography (sensitivity 97%). The average diameter of the tumors was 20.3 mm and the most frequent localizations were the head (28%) and body (57%) of the pancreas. Successful post-surgical remission was finally obtained in 80% of the cases.
The fasting glucose level was variable, ranging from 25 to 116 mg/dl. Insulin sensitivity (assessed by HOMA-S) ranged from 9.7% to 336% (median value 75%) and beta-cell function (assessed by HOMA-B) varied from 47% to 2544% (median value 563%). No difference of insulin sensitivity was observed as a function of gender or age. A significant negative correlation ($r = -0.30$, $P < 0.05$) was observed between insulin sensitivity and body mass index (BMI) and a negative correlation ($r = -0.18$, $P < 0.05$) was found between insulin sensitivity and tumor size in women but not in men.
Conclusions‘
Patients with an insulinoma usually show slightly decreased but very variable insulin sensitivity which may help to explain their differential susceptibility to undergo severe hypoglycemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P851

**Can proteomic approach help us in diagnosis of Riedel's thyroiditis? A case report**

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Background
Riedel's thyroiditis (RT) is a rare thyroid disease. Clinical and citological differential diagnosis with thyroid malignancy is difficult pre-operatively and often only pathological report may confirm the diagnosis.
Methods
We report a case of a 72-year-old Italian woman with a known history of goiter, which showed a rapid increase in size at ultrasound check, suggesting malignancy. Based on non-diagnostic cytology (Thy 1), a total thyroidectomy was performed. Fine needle aspiration (FNA) of the removed thyroid was processed by two dimensional electrophoresis (2-DE) and the proteome compared both with anaplastic cancer and control samples (normal thyroid tissue). Protein spots found to be significantly diffenrently expressed were identified by western blot (WB) analysis using specific antibodies.
Results
The protein pattern of Riedel's FNA revealed a superimposition with that of the control samples (Fig. 1). The comparison of RT FNA protein pattern with anaplastic thyroid cancer evidenced differentially expression of ferritin heavy chains, ferritin light chains and haptoglobins, as previously reported over-expressed in thyroid cancers. Therefore, we performed WB analysis of these proteins and we found that their expression were low or absent in RT and control samples despite the high concentrations present in FNA anaplastic samples.
Conclusions
The concurrent absence or low expression levels of haptoglobin, FLC and FHC in RT FNA sample strongly indicates the benign nature of the thyroid lesion. These results suggest the potential applicability of FNA proteome analysis for RT diagnosis and more generally to differentiate thyroid malignancy into thyroid nodule with non-diagnostic citology.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

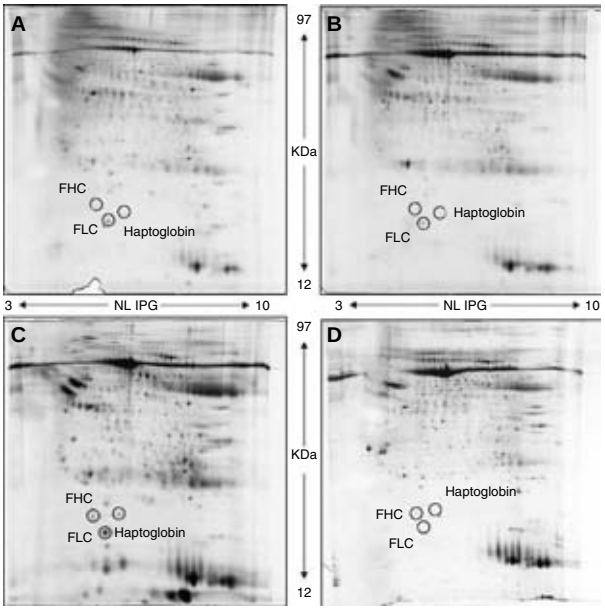


**Figure 1** Proteomic analysis. 2-DE images. (A) Ridel's thyroiditis (RT). (B) Control tissue (normal thyroid tissue). (C) Anaplastic thyroid cancer. (D) Control tissue.

## P852

**Effect of dopamine agonists on the tumor size of prolactinomas: are suppressive doses different from those that normalize prolactin serum levels?**

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Prolactin-secreting (PRL) adenomas are about 40% of all pituitary adenomas. The most important clinical symptoms of PRL excess are gonadal and sexual dysfunction as a result of tumor expansion in patients with macroadenomas. Medical therapy of prolactinomas relies on the use of dopamine agonists which induce normalization of PRL levels and shrinkage of the tumor mass in the majority of patients. The present study evaluated whether doses of dopamine agonist inducing normalization ($2 \leq$ PRL $\leq 20$ ng/ml) or suppression ($<2$ ng/ml) of serum PRL levels may have a different effect on the tumor size.

A total of 145 patients with prolactinomas treated with cabergoline were retrospectively evaluated. Seventy patients had microprolactinomas, 68 had macroprolactinomas and seven had giant invasive prolactinoma. The therapeutic dose of cabergoline was 1 mg/week in 48% of patients, 2 mg/week in 38% and between 2 and 5 mg/week in only 1%. The mean time of the treatment was about 70 month and the overall dosage of cabergoline was $<1000$ mg in 75% of patients. Tumor shrinkage occurred more in patients with macroprolactinomas than in those with microprolactinomas (38 vs 18%, $P=0.016$); particularly, 17.6% of the macroprolactinomas were reduced to microprolactinomas whereas tumor regression was observed in 20.6% of cases. No correlation between shrinking tumor and PRL levels (suppressed, normal or high ($>20$ ng/ml) was found. In conclusion, suppressive doses of cabergoline do not seem to be associated with an increased rate of tumor shrinkage.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P853

### Expressions of estrogen receptor-β splicing variants in papillary thyroid cancer

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The purpose of the present study was to elucidate expressions of ERβ1 and ERβ2 expression in papillary thyroid cancer (PTC). ER β splicing variants expressions were examined on formalin-fixed, paraffin-embedded thyroid tissues from 106 PTCs and 30 nodular thyroid goiters (NTGs) by immunehistochemical methods using Elivision plus two-step system. There was significant difference in the subcellular localization of ERβ1 expression ($P<0.001$), but not ERβ2, between PTCs and NTGs. ERβ1 expression decreased, but ERβ2 expression in PTCs increased in PTCs, comparing with those in NTGs. ERβ1 expression in reproductive-aged female patients with PTC was lower than that in reproductive-aged male patients ($P=0.017$) while ERβ2 expression is just opposite ($P=0.032$). The reproductive-aged female PTC patients (18–45 years) with lower ERβ2 expression were more likely to have lymph node metastases ($P=0.035$). The patients with nuclear ERβ1 expression were less likely to have lymph node metastases and extrathyroidal extension ($P=0.018$ and $P=0.0495$, respectively). ERβ2 expression was positively correlated with Ki-67 expression in female patients ($>45$ years) with PTC ($P=0.030$). ERβ1 expression was negatively correlated with MtP53 expression in reproductive-aged female patients with PTC ($P=0.028$).In reproductive-aged male patients with PTC, ERβ1 expression was negatively correlated with VEGF expression ($P=0.036$), but ERβ2 was just opposite ($P=0.044$). VEGF expression was associated with the subcellular distribution of ERβ1 in PTCs ($P=0.036$). This is the first study to determine the expression patterns of ERβ splicing variants in various types of thyroid lesions and elucidate the importance of ERβ splicing variants in the development and progression of PTC.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P854

### The high-mobility group A1-oestrogen receptor β nuclear interaction impairs in human testicular seminomas

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It is well established that oestrogens participate in the control of normal spermatogenesis and endogenous or environmental oestrogens are involved in pathological germ cell proliferation including testicular germ cell tumours. The effects of oestrogen are now known to be mediated by oestrogen receptor-α (ERα) and ERβ subtypes, but only ERβ has been found in human germ cells of normal testis. However, its expression was markedly diminished in seminomas, embryonal cell carcinomas, and in mixed germ cell tumours but remains high in teratomas.

The interaction and the expression of ERβ and HMGA1 were studied by using 32 cases of cryopreserved seminomas and the GC1 and TCam-2 germ cell lines.

Here, we shown that ERβ interacts with HMGA1 in normal germ cells, while down regulation of ERβ associates with transcriptional coregulator HMGA1 over-expression and cytoplasmic localization in human testicular seminomas. In addition, we show that 17β-oestradiol induces an HMGA1 increased cytoplasmic expression associated to an ERβ down-regulation in TCam-2 cell line derived from human testicular seminoma.

Taken togheter, our results suggest to hypothesize that exposure to oestrogens or oestrogen-mimics, in some as of yet undefined manner, diminishes the ERβ-mediated growth restraint in human testicular seminoma, due to the HMGA1 cytoplasmic delocalization associated with ERβ down-regulation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P855

### Long-term outcome of primary endocrine non-Hodgkin lymphomas

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Introduction

Primary lymphomas of the endocrine glands are extremely rare entities. We retrospectively evaluated the clinical profile and the patterns of outcome among patients who were treated in our center with the diagnosis of high-grade B-cell primary endocrine lymphomas.

Methods

Between May 1980 and August 2010, 450 patients were diagnosed as extranodal non Hodgkin Lymphomas (NHLs). Among them, 18 cases (4%) were primary testicular lymphomas (PTLs), eight cases (1.78%) were primary thyroid lymphomas (PTHLs), whereas four cases (0.89%) were primary adrenal lymphomas (PALs). The therapeutic approaches were variable, including chemotherapy, surgery and radiotherapy.

Results

Twelve of the PTLs patients (66.67%) achieved complete response (CR), 2 (11.11%) had partial response (PR) and 3 (16.67%) did not respond (NR). However, five of them (28%) relapsed within a median time of 21 months (range 4–52). On the other hand, all patients with PTHL obtained CR after front-line therapy. However, one patient relapsed 35 months after CR. Regarding the PAL patients one achieved CR, two had PR, whereas one did not respond. The responders relapsed shortly after treatment in a median time of 2.5 months.

The PTHLs had better outcome (median OS 43 months) in comparison to the PTLs (median OS 18.50 months) and the PALs (median OS 7 months), which had the worst prognosis.

Conclusions

We conclude that the adrenal lymphomas had the worse outcome. Given the rarity of these entities and the lack of standard therapy the existing treatment strategies for PTLs and PALs fail to provide long-term survival. Therefore, we suggest that new treatment protocols should be applied.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P856

### Performances of a new automated chromogranin-A assay

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Background

Chromogranin A (CgA) is essential for the formation of secretory granules and sequestration of hormones in neuroendocrine cells. Measurement of CgA levels is

included in the diagnostic procedure of neuroendocrine tumors and pheochromocytoma. The aim of this study was to assess the reliability of the Kryptor assay for CgA measurement.
Methods
Imprecision of the Kryptor CgA assay was determined with two levels of CgA concentration. Reference values for the Kryptor CgA (B.R.A.H.M.S GmbH, Thermo Scientific, Germany) assay were obtained from thirty five healthy subjects. Method comparison was performed with our routine CgA assay (Dako, Glostrup, Denmark) with eighty five patients' samples.
Results
Between run imprecision ($n=6$) performed with quality controls materials for the CgA assay were 4.0% and 3.4% for mean concentrations of 98 ng/ml and 481 ng/ml, respectively. CgA levels measured with the Kryptor were significantly correlated with CgA levels obtained with our routine assay ($r=0.96$, $P<0.0001$) .The agreement between the two methods was very good (weighted $\kappa$ coefficient: 0.84). However, seven cases were discrepant between the two methods. For low concentrations (below 23 UI/l with the routine assay), Passing and Bablok regression analysis showed a slope of 5.36 and an intercept of 37.38 ($n=40$). Bland and Altman plot evidenced a positive bias (mean difference of 22.1) with higher values for Kryptor assay. For high concentrations (above 23 UI/l with the routine assay), Passing and Bablok regression analysis showed a slope of 3.49 and an intercept of 10.76 ($n=36$). Bland and Altman plot evidenced a positive bias (mean difference of 526.4) with higher values for Kryptor assay.
Conclusions
Our study showed that the Kryptor assay is reliable for chromogranin-A measurement. However, CgA assays are not commutable and laboratories must inform the physicians of the characteristics of potential new routine assay.
Declaration of interest
I fully declare a conflict of interest. Details below.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P857

**Virilization due to adrenocortical carcinoma in a 7 years-old female: a case report**

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Adrenal cortical carcinoma (ACC) is a rare malignancy, especially in children, and the annual worldwide incidence has been calculated as 0.3 cases per million children younger than 15 years old. We present a case of functioning ACC in a 7 years-old female child who manifested with virilization. She presented with low-pitched voice, excessive pubic hair growth, growth acceleration, clitoral enlargement and increased muscle strength at the age of six. Her blood pressure was normal, and her breast was Tanner stage 1, but her bone age was advanced. Hormonal findings were characterized as an increase of androgens, especially serum testsosterone, DHEAS and urinary 17-ketosteroid (17-KS). The abdominal magnetic resonance imaging revealed a large right suprarenal mass sized 55×50×40 mm. The brain, lung and liver were free from metastases in MRI and CT. She had a right adrenalectomy without any complications. The histological diagnosis was ACC according to the criteria of Weiss: nuclear atypia, atypical architecture and atypical mitosis; and Weiss criteria three out of nine. The labeling index of Ki67 staining was 10–15%, and DNA analysis of her peripheral leukocytes for the p53 tumor suppressor gene was negative. After histopathological confirmation of the diagnosis she received mitotane treatment (2 g/day) and radiation therapy. Two weeks after adrenalectomy, her serum testosterone and DHEAS and urinary 17-KS rapidly decreased to the normal level. We must pay attention to recurrences because little is known of ACC's long-term natural history.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P858

**Diagnosis and treatment of the surgical pathology of the suprarrenales glands. Bibliographical revision**

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Introduction
The adrenal glands are paired organs neuroendocrine, formed by the adrenal cortex and medulla, two distinct structures for histology, function, and embryological origin. The processes that most frequently affect the adrenals are adenomas 81.8%, 11.8% cysts, metastases, 11.8%, 5.9% carcinoma, pheochromocytoma 2.9%, 2.9% adrenalitis. 20–40% of adrenal masses are afuncionantes and/or discovered incidentally (incidentalomas) scans during abdominal imaging. The objetive of this study is to present a bibliographical update and our experience in the diagnosis and treatment of the surgical pathology of the suprarenal glands.
Methods/design
Retrospective study of 48 patients diagnosed and treaties of suprarenal masses (2000–2011). Clinical history has been reviewed: signs and symptoms of adrenal pathology, radiological, hormonal study, stay average post-operating and anatomo-pathological diagnosis.
Results
Of 48 cases, 48% are men and 52% women. In 4 patients bilateral disease was detected. Average stays post-operating, 12 days. Anatomo-pathological diagnoses: pheochromocytoma 15 cases; functional adenoma 13 cases (Cushing 7; Conn 5; diffuse virilizante adrenogenital syndrome 1), hyperplasia two cases (syndrome of Cushing), hyperplasia to nodular two cases (hyperaldosteronism), suprarenal carcinoma three cases, non-functional masses 13 cases.
Conclusions
Before the hypercortisolims suspicion free cortisol is determined in tinkles of 24 h plasmatic ACTH, and test of weak suppression of dexametasona. In hyperaldosteronism, seric potassium concentration, aldosterone and ratio aldosterone-activity of plasmatic renina. Before pheochromocytoma we determined plasmatic catecholamine's and tinkles of 24 h. If the hormonal study is suspected to a suprarenal carcinoma it will depend on the type of suspected endocrine syndrome. The location of the suprarenal masses is made with computerized tomography, magnetic resonance nuclear, ultrasounds or gammagraphy. At the moment the surgery laparoscopic and 'Da Vinci robotic system' are considered 'gold standard" for the surgical treatment of the suprarenal pathology.
Declaration of interest
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## P859

**1,25-Dihydroxyvitamin D inhibits cell proliferation by promoting cell-cycle arrest in the human adrenocortical cancer cell line nci-h295r**

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Vitamin D receptor (VDR) and its ligand 1,25-Dihydroxyvitamin D3 (1,25OHD3) play, in general, an inhibitory role on the growth of normal and malignant cells. However, the mechanisms for this anti-proliferative action remain not completely understood. Recently, a 1,25OHD3-mediated effect on hormone production and steroidogenic genes has been reported in the human steroid-secreting adrenocortical cancer cell line NCI-H295R (Lundqvist J *et al.* 2010). The aim of this study was twofold: i) to test expression of vitamin D metabolism genes in normal adrenals and in a series of adrenal tumors as well as in the NCI-H295R cells; ii) to investigate the potential anti-proliferative action of 1,25OHD3 on NCI-H295R cells underlying the molecular mechanisms behind this effect. mRNA levels for CYP2R1 (i.e. the enzyme converting vitamin D3 to 25OHD3), CYP27B1 (i.e. the enzyme converting 25OHD3 to 1,25OHD3) and VDR were measured by RT-PCR and/or western blotting in both NCI-H29R cells and adrenal tissues. DNA synthesis was evaluated according to (3H)TdR cell incorporation after 96 h treatment of NCI-H295R cells with 1,25OHD3 at increasing doses, in comparison to untreated control cells. The effect of 1,25OHD3 on cell apoptosis and cell cycle was analyzed with a flow cytometer. CYP2R1, CYP27B1 and VDR were expressed in NCI-H295R cells and in all adrenal tissues analyzed (non-functioning adenomas, $n=5$; cortisol/aldosterone-

secreting adenomas, $n=9$; cortisol-producing carcinoma, $n=1$). 1,25OHD3 inhibited cell proliferation by 20% at a dose of $10^{-8}$ M and induced a concomitant decrease in aldosterone and DHEA-S production. 1,25OHD3 induced cell cycle arrest, promoting accumulation of cells in G0/G1 phase without inducing apoptosis.
Conclusions
1,25OHD3 has cytotoxic effects on the NCI-H295R cells by promoting cell cycle arrest. Expression of vitamin D metabolism genes in NCI-H295R cells as well as in normal and tumor adrenals suggests a potential paracrine role of vitamin D in adrenal growth and function. Because of its anti-proliferative action 1,25OHD3 could be considered for the treatment of patients with adrenocortical cancer.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P860

**A case of multiple endocrine neoplasia type 2a associated with rectal adenocarcinoma**

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Multiple endocrine neoplasia type 2A (MEN2A) is an inherited syndrome caused by the mutation of RET gene. The classic variant of this syndrome includes the presence of medullary thyroid carcinoma (MTC), in association with pheochromocytoma and parathyroid neoplasia. MTC is usually the first presentation, with palpable neck mass and hypercalcitonemia, metastatic spread to local lymph nodes or distant sites.
We report the case of a 41 year old Caucasian man who underwent colorectal surgery for stage IV rectal adenocarcinoma with liver metastases, confirmed by the anatomopathological report. The postoperative evolution was complicated with recurrent hypertensive episodes controlled with medication. An abdominal CT was performed which described multiple bilateral adrenal masses. Hormonal assessment revealed significantly high levels of urinary metanephrines (12.52 µmol/24 h, normal range $<1.62$), normetanephrines (11.72 µmol/24 h, normal range $<2.13$) and calcitonin ($>2000$ pg/ml, normal range $<18.2$). Serum calcium was increased (5.36 mEq/l, normal range 3.5–4.5) and iPTH was to the upper limit (73.97 pg/ml, normal range 14–73). Thyroid ultrasound showed hypodense, irregular macronodules (2.3/1.8 cm, respective 1.34/1.45 cm) in both lobes, with microcalcifications and intranodular intense vascularization on Doppler screen, and laterocervical limfonodules with the same characteristics. After bilateral adrenalectomy (with the removal of left adrenal gland and kidney in block for macroscopic capsule penetration and extension to the surrounding adipose tissue), the patient underwent total thyroidectomy with neck dissection and bilateral inferior parathyroidectomy. The histopathological exam confirmed bilateral pheocromocytoma with extracapsular extension with possible malignancy (PASS score $>4$), MTC with limph node metastasis and parathyroid hyperplasia. Genetic testing revealed the heterozygote mutation of codone 634 (Cys634Trp). Actually the simptomatology of his non-endocrine tumor led to the diagnosis of the second severe condition - a multiglandular endocrine tumor syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P861

**Leptin affects gene expression in OVCAR-3 ovarian cancer cells. Effects on cell proliferation and apoptosis**

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Several studies have addressed the possible role of leptin, the product of the obesity gene (Ob), in ovarian cancer development and progression. Herein, we used the OVCAR-3 cell line expressed both the long (ObRb) and short (ObRt) isoforms of the mRNAs of the leptin receptor, to analyse the effect of leptin on the expression of selected genes involved in the cell cycle and apoptosis. OVCAR-3 cells were exposed to 2 and 20 ng/ml of leptin (noted in non-obese women) or 40 and 100 ng/ml of leptin (noted in obese women). Cell proliferation was determined by measuring BrdU incorporation and propidium iodine DNA staining (flow cytometry). Apoptosis was measured by DNA fragmentation using the Cellular DNA Fragmentation ELISA kit and caspase-3 activity by fluorometric assay. Expression of selected genes involved in the cell cycle and apoptosis were evaluated by real-time PCR. At a physiologically relevant concentration, leptin has no effects on cell proliferation, why in concentration noted in an obese women leptin stimulated cell proliferation. 1.2- to 1.9-fold induction of genes responsible for inducing cell proliferation and a 1.2- to 1.9-fold suppression of genes responsible for inhibition of proliferation was noted. A statistically significant inhibition of caspase-3 activity and DNA fragmentation was observed under the influence of leptin at 2, 20, 40 and 100 ng/ml respectively. 1.4- to 1.8-fold suppression of genes involved in the extrinsic apoptotic pathway and a 1.2- to 2.2-fold suppression of pro-apoptotic genes involved in the intrinsic apoptotic pathway were observed. In conclusion, leptin by up-regulating genes responsible for inducing cell proliferation and down-regulating pro-apoptotic genes in extrinsic and intrinsic pathways could contribute to ovarian cancer progression. However, further research with ovarian cancer explants are needed to confirm the relationships between ovarian cancer risk and obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P862

**Malignant adrenal incidentaloma**

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With the development of medical imaging, many malignant lesions of the adrenal lodge are incidentally discovered. In this prospective monocentric study we are looking for factors of presumption of malignancy in the preoperative stage on a non-oncological series of 91 IS.
Monocentric prospective study, AI revealed by TDM $\pm$ echo supplemented by a clinic/hormonal adrenal exploration $\pm$ isotopic. In the absence of against indications, the indication of the adrenalectomy has been systematic for histological typing. The diagnosis of malignancy is retained for 26 IS on Weiss criteria $\geq 3$ for 17 unilateral AI removed /22 (10 adrenal cortical carcinoma ACC, 2 malignant PH and 5 non-glandular MAI) and 9 MAI having benefited from palliative treatments: 4 bilateral metastatic AI and 5 secretant expansive ACC.
Results
Prevalence of MAI is 28.5%, 10F/16 M, median 45.6 years. The average axis $=$ $10.2 \pm 5$ cm, median 10 cm (3.4–20 cm). Clinically rare co-morbidities, toxic habits 34.6%, rapid deterioration of general condition 53.8%, painful abdominal syndrome 80.8%, prescription of major analgesics 19.2%, severe general signs 42.3%, weight loose 23.1%, HTA 7.7%, an endocrine syndrome is noted in 19.3% (virilization two cases, moderate Cushing one case, atypical adrenergic syndrome two cases). AI were apparently clinically benign in 34.6%. Biologically: high prevalence of night hypercortisolism (83.3%) in the MAI glandular, freinable in 28.6%. Secretion of androgen precursors or metanephrines is noted only in glandular MAI. Isotopic profile of nine MAI shows lack of fixation in five MIBG and discordant fixation in four norcholesterol scinti scans.
Conclusion
In addition of classical suspicious TDM signs, male sex, age $<45$ans, rapid physical deterioration, mixed hypercortisolism and the lack or discordant scintigraphic fixation are suggestive of MAI; lesions $>3$ cm must be removed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P863

**Maxillary gland could harbor a neuroendocrine metastasis**

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Introduction
Pancreatic neuroendocrine tumors (PNET) are rare with an incidence of 3–4/1000,000. These tumors are non functional in 70–80% of the cases. The vast majority of them are differentiated neuroendocrine carcinoma. Distant metastasis detected frequently at the onset of diagnosis. Liver and lymph node metastases are the most frequent localizations. Cerebral, skeletal and pulmonary are not infrequent. Primary and metastatic small cell carcinomas with NE differentiation in the parotid gland are also documented.
Case report
A 54 years old patient presented with cerebral metastatic lesions in 2005. Post surgical histopathological examination confirmed the neuroendocrine nature of these lesions. Her primary lesion, a PNET, was discovered in 2008 only after discovery of ovarian metastases. One year later, a maxillary nodule was extirpated. This was followed by normalization of CgA levels. Histopathological study of her pancreatic, ovarian, cerebral and maxillary lesions confirmed the same type of neuroendocrine tumour.
Discussion and conclusion
Salivary glands usually harbor metastatic melanomas and other skin cancers. Few cases of metastatic NETs in the parotid glands were documented in the literature. Neuroendocrine differentiation of salivary carcinomas is also reported. We describe a metastatic PNET in the maxillary gland which we treated with surgery followed by radio-chemotherapy. It is wise to include NET in the differential diagnosis of salivary gland tumours and to do immunohistochemical staining by NE markers on a salivary tumour in patients with history of NET.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P864

**Circadian rhythm of prolactin release in patients with breast cancer**

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Background
A number of studies have evaluated the association between prolactin (PRL) and breast cancer risk, but some results have been inconsistent.
The aim of this study was to investigate, if the circadian rhythm of PRL was maintained in pre- and post-menopausal patients with breast cancer and if there is a correlation between serum PRL level, serum concentration of insulin-like growth factor 1 (IGF1), tumor staging and expression of estrogen, progesterone and HER2 receptors in tumor tissue.
Patients and methods
Sixty-four women with the breast cancer aged 36–78 years and 20 healthy controls matched for age were studied. Serum PRL was determined using immunoradiometric method. Blood samples for PRL determination were taken four times daily at fixed hours of the 24 h cycle and obtained results were analysed using the Cosinor test.
Results
Sixteen patients with breast cancer (25%) demonstrated high serum PRL level, and 29 (45%) showed high serum IGF1 level. Circadian rhythm of PRL was maintained in all patients with breast cancer. Both in patients and in healthy subjects the circadian rhythm indicated the highest serum concentration of PRL in night hours.
Both in pre-menopausal as well as in post-menopausal patients there was no statistically significant correlation between serum PRL, IGF1 levels, the degree of tumor staging and expression of estrogen, progesterone and HER2 receptors in the tumor.
Conclusions
Patients with breast cancer show a circadian rhythm of prolactin release, not significantly different from rhythm in healthy subjects.
Some patients with breast cancer demonstrated hyperprolactinaemia and/or high serum IGF1 level. These data suggest that higher plasma PRL and IGF1 levels could be associated with an increased risk of breast cancer.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P865

**Non-medullary neuroendocrine carcinoma in the thyroid gland: a case-report**

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Introduction
Neuroendocrine lesions in the thyroid gland are uncommon. The differential diagnosis usually includes medullary thyroid carcinoma, paraganglioma and metastases. There are few non-conclusive reports of primary neuroendocrine carcinomas of the thyroid.
Clinical case
A 49-year-old woman with a past medical history for familial hypercholesterolemia and right oophorectomy for mature teratoma with struma ovarii, was evaluated for a rapidly growing cervical mass. A firm, hard, enlarged right thyroid lobe was detected on physical examination. Neck ultrasound, full-body-computerized-tomography (CT) and PET-CT were performed. The latter showed a $49 \times 32 \times 59$ mm mass with high metabolic activity in the right thyroid lobe infiltrating the trachea, esophagus and sternohyoid and platisma colli muscles as well as ipsilateral cervical adenopathies and an unspecific milimetric nodule in the upper left pulmonary lobe. Full blood count and biochemistry were normal apart from a total cholesterol of 461 mg/dl; she was euthyroid with negative thyroid autoimmunity; undetectable calcitonin, normal catecholamines, 5-HIIA, β2-microglobulin and CEA. Neuron specific enolase was slightly increased (17.32 ng/ml, normal 0–16 ng/ml). Cervical lymph node biopsy consistent with metastases from large cell neuroendocrine carcinoma (LCNC). Total thyroidectomy, total laryngectomy and right cervical lymphadenectomy were performed, revealing a LCNC invading blood vessels, soft tissue and laryngeal adventitia; negative immunostaining for calcitonin and tiroglobulin, positive for TTF-1 and CD56; Ki-67 $>90\%$. Postsurgical $^{111}$In-pentetreotide scintigraphy was negative. She received six cycles of cisplatin-etoposide. One year after diagnosis there is no biochemical or radiological evidence of persistent disease.
Conclusions
We cannot fully exclude the possibility of a metastatic primary lung tumor, but the clinical course, the size of the thyroid mass, the absence of any other clear lesion outside the neck and the immnunostaining pattern suggest a primary thyroid LCNC. To the best of our knowledge, this could be the first case report of these tumors arising in this location.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P866

**Adrenal myelolipomas in CAH: is there role for ultrasound screening of the adrenal glands?**

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Background
Persons with CAH may be at increased risk of developing adrenal myelolipomas, particularly if their CAH is poorly controlled. Adrenal masses can be detected by ultrasound with high sensitivity and specificity. Contrast-enhanced ultrasound (CEUS) may be a useful method in the diagnostic work-up of adrenal mass with excellent sensitivity for the diagnosis of malignancy. However, ultrasound is not included in the diagnostic algorithm of patients with CAH.
Case Report
A 50-year-old man with CAH due to 21-OH-deficiency presented with increasing abdominal girth and abdominal pain. An abdominal ultrasound showed hyperechoic adrenal masses ($3.6 \times 9.5$ cm in the right, $10.4 \times 10.4$ cm in the left

gland), in B-mode and CEUS consistend with myelolipomas. On MRI the diagnosis of mesenchymal tumor or sarcoma was suspected. Because of suspected malignancy a bilateral adrenalectomy was performed, histopathology revealed the diagnosis of benign bilateral myelolipoma.
Discussion
Adrenal myelolipoma has been described in CAH and is a rare benign tumor formed by mature fat tissue admixed with hematopoetic elements. Ultrasound becomes more important in diagnosis of adrenal masses, especially in combination with CEUS. So far, there is no screening for adrenal masses in patients with CAH. In B-mode and Doppler mode myelolipomas are described with homogenous mostly hyperechoic tissue lacking signs of hypervascularisation or irregular tumor vessels, contrast-enhanced sonography shows an early arterial contrast enhancement without any significant wash-out.
With a very high sensitivity in diagnosing malignant lesions and an increasing availability of B-mode and CEUS ultrasound should be used as screening methods in patients with CAH. But there are further data needed on the incidence and prevalence of adrenal masses, especially myelolipomas. In addition, careful follow-up of suspected myelolipomas is needed as it is yet unclear whether there is an increased risk for malignant transformation of this entity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P867

**An unusual association of hyperparathyroidism, ectopic GHRH secretion and bronchial carcinoid in a MEN1 family**

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Introduction
MEN1 is characterized by tumors of parathyroid glands, pituitary and pancreas. Pituitary tumors frequently produce PRL and GH, but acromegaly due to ectopic GHRH secretion has been reported in $<1\%$ of cases. Here we present a case of two patients belonging to a MEN1 family (c.207delC; p.P69PfsX118 mutation (ENST00000377313), affected by primary hyperparathyroidism, in association with acromegaly due to ectopic GHRH secretion and bronchial carcinoid, respectively.
Case report
A 18-year-old boy came to our observation with clinical, biochemical findings and scintigraphic image diagnostic of primary hyperparathyroidism. He successfully underwent subtotal parathyroidectomy. Given the young age and the presence of multiple parathyroid hyperplasia, the patient underwent genetic analysis that revealed the presence of a MEN1 gene mutation. Despite the absence of signs and symptoms, acromegaly due to ectopic GHRH secretion was diagnosed (RMN negative for pituitary adenoma and GHRH level: 60.14 ng/ml, normal $9.4 \pm 5.5$). Search for an ectopic tumor through chest-abdominal TC scan, total-body PET/CT and 111 In-Pentreotide (Octreoscan) was unsuccessful, and treatment with somatostatin analogs was started. Recently, scintigraphy with somatostatin analogs (68-Ga-DOTATOC-PET) showed three focal areas in the pancreatic tail. Distal pancreasectomy revealed a multiple pancreatic neuroendocrine tumors. The evaluation of the patient's mother, revealed the presence of primary hyperparathyroidism, elevated gastrin, chromogranin A, pancreatic polypeptide and a 4 cm-lung mass radiologically evident. The patient underwent subtotal right pneumonectomy and the histological analysis was consistent with the diagnosis of a typical bronchial carcinoid secreting the above polypeptides.
Conclusions: In this report we emphasize the uncommon presence of acromegaly due to pancreatic ectopic GHRH secretion and bronchial carcinoid in two patients belonging to a MEN1 family. An early and full diagnostic procedure appears to be important in order to recognize this rare syndrome that may be lethal as happened to the proband aunt who died for a malignant gastrinoma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P868

**Medullary thyroid carcinoma with liver metastasis presenting as watery diarrhea for 1 year**

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A 66-year-old woman had no history of systemic diseases. She had developed watery diarrhea for 4 months initially, without abdominal pain, fever, any aggravating or relieving factor. She visited gastroenterologist, and colonoscopy showed only mixed hemorrhoid. Irritable bowel syndrome was impressed then. Thus, anti-spasmodics and anti-diarrheals were prescribed. However, diarrhea persisted for 1 year, and body weight loss of 7 kg occurred during this period of time. She later visited a general practitioner, and abdominal ultrasonography revealed a 6.9 cm non-cirrhotic hepatic tumor. Her virological markers of HBsAg and anti-HCV were negative, and the serum alpha-fetoprotein level was within normal limits. She was referred to our hospital for further investigation. Tumor markers revealed high CEA (208.5 ng/ml) and normal CA19-9 (35.6 U/ml) levels. Tri-phase abdominal CT scan displayed three non-cirrhotic hypervascular lesions. After admission, core-needle biopsy for the largest hepatic tumor was arranged. Pathology revealed metastatic carcinoma, and immunohistochemical stains showed positive TTF-1, Calcitonin, and Synaptophysin. Thyroid ultrasonogrphy and fine needle aspiration were carried out, by which medullary thyroid carcinoma was impressed. Her serum calcitonin level was more than 10 000 pg/ml. Radical thyroidectomy and liver bi-segmentectomy were done. Amyloid accumulation was noted in the pathological specimens of thyroid and liver. The post-operative serum calcitonin level decreased to 3736 pg/ml, and diarrhea partially improved. Adjuvant chemotherapy was not suggested owing to futile outcome, and further target therapy is under evaluation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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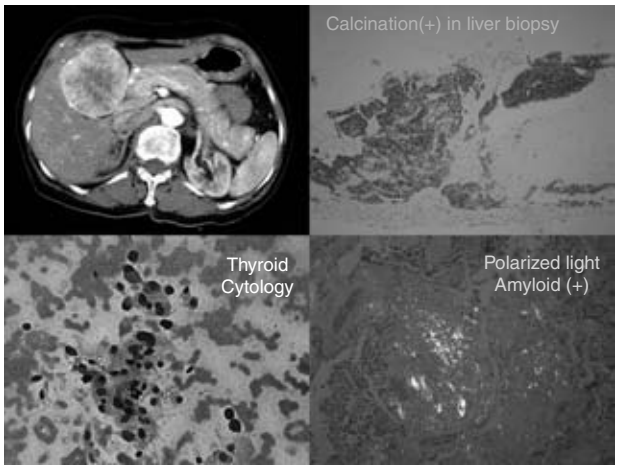


## P869

**Antiproliferative effect and control of catecholamine release with octreotide in a patient with giant bilateral pheochromocytoma**

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Introduction
Pheochromocytomas are rare but treacherous catecholamine-producing tumors of the medulla of the adrenal glands (originating in the chromaffin cells), or extra-adrenal chromaffin tissue. We refer a case with stabilization of tumor growth and hormonal improvements following repeated s.c. injections of octreotide.
Case report
We report a female patient aged 35 with a finding of bilateral adrenal mass with sever cardiovascular symptomatology (frequent paroxysms of headache and

palpitations accompanied with hypertensive episodes). Biochemical screening showed 20-times higher level of adrenalin, 13-times higher level of noradrenalin, 27-times higher level of chromogranin A and 30-times higher level of vanillylmandelic acid in 24 h urine. Enhanced MR imaging (Fig. 1A) confirmed the findings of adrenal gland tumors (the left adrenal mass was 11×9 cm, and right adrenal mass was 4×3 cm in size). I-131-MIBG scintigraphy showed uptake in both adrenal glands. As a member of the Jehovah's Witnesses, the patient refused surgery definitively not accepting a possibly needed blood transfusion, but was successfully treated with octreotide at 50 μg s.c. injection twice daily during 2.5 years with relief of symptoms and tumor shrinkage on enhanced MSCT scan (Fig. 1B). We also observed a significant reduction in catecholamines and chromogranin A levels.

Conclusion

We stress the importance of complete hormonal screening in all cases of adrenal masses, especially in patients with cluster of heart symptoms. Pheochromocytomas are best treated surgically to obviate the associated dangerous cardiovascular catecholamine-mediated paroxysmal events. However, successful treatment with somatostatin analogues such as octreotide could be potentially considered as the treatment of choice for patients with pheochromocytoma especially those considered unsuitable for surgery or in cases where the operation is not possible.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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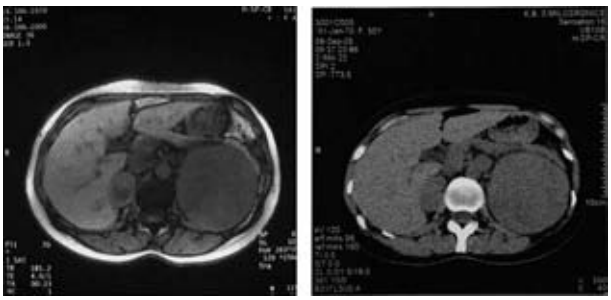

A B

**Figure 1** (A) Abdominal MR imaging: giant bilateral adrenal pheochromocytoma (left adrenal tumor: 11×9 cm; right adrenal tumor: 4×3 cm in size). (B) Abdominal MSCT: bilateral pheochromocytoma with a partially necrotic zones during octreotide treatment.

## P870

### Survival of patients with adrenocortical carcinoma: experience of one center

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Adrenocortical carcinoma (ACC) is rare and aggressive malignancy with poor prognosis. Patients present with hormone excess or a local mass effect, or incidentally. ACC has undergone metastatic spread in 40–70% of patients at the time of diagnosis. Surgical removal is treatment of choice. Standard chemotherapy with mitotane has limited efficacy.

The aim of this paper is to present the results of single tertiary center for diagnosis and treatment of ACC in Serbia. A retrospective review was performed of all patients who were hospitalized from 2005 to 2011. Kaplan–Meier curve was used as the univariate version of survival analysis.

We analyzed 46 patient with ACC (28 females, 18 males), median age 47 years at diagnosis. Ten patients were lost during the follow-up. The mean size of the tumor was 11.6 cm. Fifty-two percent of patients were stage IV (ENSAT staging), 44% stage II, and one patient was stage I. Patients with hormone secreting ACC (40%) were presented with Cushing's alone (20%), or combined with androgen excess (17%), 54% were asymptomatic. Primary tumor was operated in 88% of patients. Sixty-five percent of patients were treated with mitotane and 26.5% received chemotherapy. At the time of diagnosis 43% of patients had distant metastasis. Mean overall survival was 68 months (95%CI 54–83) with 5 year overall survival 66.7 and 52% in stage IV, and 80% in stage II. The 3 years overall survival was 74%. Median disease free survival was estimated 11 months (range 7–14 months). Significantly higher overall survival was noted in patient treated with mitotan ($P<0.0001$) comparing to untreated patients. Median overall survival was not statistically different between patient with low, medium and high Ki67 index in this study. In conclusion, we presented the survival in ACC patients in Serbia, our 7 years experience.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P871

### Leydig cell tumor of ovary: a rare case of virilization

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Introduction

Leydig cell tumors are a type of steroid cell tumor, account for 0.1% of all ovarian tumors. The clinical presentation is usually a hyperandrogenic state with signs of virilisation. The tumor has a benign behavior, with an excellent prognosis and reversion of symptoms after surgical treatment.

Case report

Woman, 81 years-old, nulliparus, with clinic hyperandrogenism and vaginal bleeding during the last year. She had history of dementia syndrome, dyslipidemia, psoriasis, primary infertility. Her family history was irrelevant. The physical examination revealed hirsutism, androgenic alopecia, masculine voice, and clitoromegaly without adnexial masses in gynecology examination. She had no features of Cushing syndrome. Total testosterone (7.4 ng/ml (0.06–0.82)) and delta 4-androstenedione (>10 ng/ml (0.30–2.99)) were elevated and other hormonal serum levels were normal namely DHEA-S, 17-hidroxiprogesterone, prolactin and TSH. Transvaginal ultrasound revealed intramural fibroid, without visualization of adnexial masses. Abdomino-pelvic computerized tomography (CT) revealed a nodule with 30 mm in the left ovary and normal adrenal glands, confirmed by abdomino-pelvic magnetic resonance. A complete hysterectomy and bilateral oophorectomy were performed. Histological examination revealed a Leydig cell tumor of the ovary. After surgery, the androgen serum levels normalized.

Discussion

The rapid onset of clinical hyperandrogenism with virilism and a serum testosterone level higher than 2.0 ng/ml raised suspicion of an androgen production tumor. In a hyperandrogenism presenting patient, a complete gynecology examination should be performed and an adrenal and ovary CT should be done to exclude an androgen production tumor.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P872

### Lutetium-177 DOTATATE for paraganglioma refractory to conventional chemotherapy: a single case report

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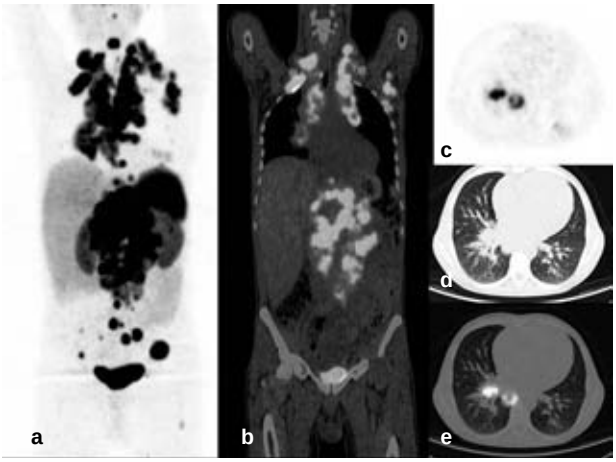
A 31-year-old man presented with a two month history of sweating, right-sided abdominal pain and supraclavicular swelling. CT scanning showed widespread lymphadenopathy with multiple retroperitoneal lymph nodes. Biopsy of a left supraclavicular node resulted in a diagnosis of metastatic paraganglioma. Raised urinary catecholamine as well as urinary metanephrine levels further confirmed the diagnosis.

A normocytic anaemia requiring repeated blood transfusions was believed to be due to bone marrow dysfunction. He was also found to have pituitary

insufficiency as evidenced by hypgonadotrophic hypogonadism, undetectable serum prolactin levels and a suboptimal cortisol increase in a glucagon suppression test (pituitary MRI had been unremarkable).
Further imaging demonstrated MIBG uptake in keeping with extensive lymphadenopathy above and below the diaphragm. In addition, Gallium-68 DOTATATE scanning showed uptake in lesions which were not MIBG avid.
He initially completed four cycles of conventional chemotherapy consisting of cisplatin and etoposide as well as palliative radiotherapy to para-aortic nodes. He continued, however, to suffer from extensive lymphoedema, abdominal distension and difficulty in breathing which had necessitated hospital admission and home oxygen therapy. Rising chromogranin A levels as well as repeated CT scans confirmed disease progression.
Therefore, conventional chemotherapy had been discontinued and he was commenced on Lutetium-DOTATATE treatment.
Up to now, he has received four cycles at intervals of 2–3 months. His clinical response was excellent with gradual improvement of both symptoms and quality of life. Prior to his third cycle, the lymphoedema had disappeared and there was significant reduction in the size of overall metastatic disease on repeat CT scanning. He is no longer transfusion-dependent and his only remaining symptom is of occasional night sweats.
This is a unique case which demonstrates successful therapy of a metastatic paraganglioma, refractory to conventional chemotherapy, with Lutetium-DOTATATE. Significant clinical improvement could be achieved with very low toxicity and treatment side effects.




68Ga-DOTATATE PET-CT Scan. Maximum intensity projection a) and fused coronal PET-CT image b) shows the extent of disease above and below the diaphragm. Multiple lymph nodes, both lungs and bones are involved. Lung metastases are demonstrated on axial PET c), CT d) and fused e) images.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P873

**Hypoglycemia by insulinoma: for the purposes of a case**

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Clinical case
Introduction
Insulinoma is a rare disorder with an estimated incidence of 1 case per 250 000 people per year.
Case report
37 years old female, with a history of irritable bowel syndrome, dyslipidemia and kidney stones. In December 2010, she developed a headache, dizziness and visual disturbances, predominantly in the evening and amnesia for some of the episodes. Initially interpreted as peripheral vertiginous syndrome, because of recurrence of symptoms, interfering in daily activity, she developed psycho-emotional instability and anxiolytic medication was prescribed. In April she had a similar episode accompanied by syncope and imbalance, she was assisted at the Health Center with 31 mg/dl. She was admitted for hypoglycemic investigation. She performed a fasting test that was stopped an hour later through symptomatic hypoglycemia (serum glucose levels of 25 mg/dl, insulin 36.3 μU/ml, C-peptide 5.06 ng/ml), with increase of the serum glucose levels $>25$ mg/dl after glucagon, suggestive of endogenous hyperinsulinism. CT showed nodular mass in the pancreatic head with 21 mm with the arterial phase uptake suggestive of insulinoma without mesenteric or retroperitoneal lymphadenopathy. She underwent tumor enucleation.. Pathological examination showed well differentiated insulinoma $1.8\times1.5\times1.2$ cm, low mitotic index, Ki 67 3%, with no necrosis and no vascular invasion or surgical margin, synaptophysin positive. After surgery she remains clinically well.
Discussion
This case illustrates the need for high clinical suspicion for insulinoma diagnosis. Neuroglycopenia and adrenergic symptoms are often confused with acute vertigo and anxiety disorders. Although it was the case with this patient, the delay in diagnosis was only five months. Some studies have shown average delays in diagnosis of 1.5 years. In this case, the patient has a tumor with good prognosis (pT1N0M0, stage IA), with an estimated survival at 5 years of 61%.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P874

**Atypical presentation of type 1 gastric neuroendocrine tumor should increase our awareness**

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Introduction
Among three types of gastric neuroendocrine tumors (GEPNETV) type 1 are most common. At the basis of the pathogenesis of type 1 is atrophic gastritis, which leads to achlorhydria and deficiency of intrinsic factor, resulting in G cell hyperplasia and hypergastrinaemia. Gastric endoscopy usually reveals the multiple and polypoid lesions ($<1$–2 cm) in the corpus. Five-year survival in these patients is not different from survival in the selected age group population. Type 1 GEPNET metastasizes very rare (2–5% of cases).
The aim of our study was to present atypical type 1 GEPNETV.
Case report
In the Department of Endocrinology we follow up 41 cases of GEPNETV, among them 30 of type 1. M.S., 33-aged male, in 2008 due to anemia and epigastric pain had gastroscopy. It reavealed three wide based polypoid lesions bigger than typical lesions (15–30 mm in diameter). In biopsy there was highly differentiated neuroendocrine tumor expressing chromogranin. Ki67 index $<1$%. In addition, atrophic gastritis was found. In abdomen CT and scintigraphy with somatostatin analogues (SS scintigraphy) no speciphic changes were found. Patient refused proposed surgery and for 1 year was lost for follow up. In the next gastroscopy in 2010 there were three polypoid lesions up to 30 mm in diameter, with similar biopsy to former examination. In 2011 total gastrectomy was performed. Histopatology revealed neuroendocrine cancer with metastases to the local lymph nodes. In postoperative SS scintigraphy no uptake of the tracer was found, patient is treated with somatostatin long acting analoque.
Conclusions
Atypical presentation of type 1 GEPNETV should increase our awareness and influence the treatment decision making.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P875

**A case of multiple endocrine neoplasia type 1 associated with multicentric VIPoma**

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Introduction
Multiple endocrine neoplasia type 1 syndrome (MEN1sy.) is a complex disease predisposing to a variety of multifocal neuroendocrine tumors (NET). Approximately 5% of pancreatic VIPomas and glucagonomas are associated with MEN1sy., most of them with metastases at diagnosis.
Case report
A 15-year-old boy was admitted with primary hyperparathyroidism, pituitary microadenoma (6 mm) and suspected MEN1sy. His father had primary hyperparathyroidism and pancreatic gastrinoma. While hospitalized, the patient had intense abdominal pain, facial flushing and massive diarrhea, severe volume depletion, hyperchloremic hypokalemic acidosis and aggravated hypercalcaemia. Endoscopic ultrasound (EUS) and MRI showed two tumors localised in the pancreatic head and tail. Octreoscan revealed one lesion in the pancreatic head. Gastrin and 5-HIAA values were in normal range. Chromogranin A (CgA) was increased (562 ng/l). Direct sequencing of MEN1 gene identified germline mutation at codon 395 of exon 8 (CAG→TAG). Somatostatin analogue therapy was started before surgery. Near total parathyroidectomy with profilactic thymectomy was performed. Parathyroid glands showed diffuse hyperplasia. Pancreatic resection with total tumor exscision was also performed. Histopathological analysis revealed four well-differentiated NETs, three of them showed VIP-ma imunoprofile, but one was pure glucagonoma (Ki67-1%, MI 0/50 HPF, CgA+++, VIP+++, glucagon++). Peritumorous pancreatic tissue showed microadenomatous proliferation. There were no regional lymphonodal metastases. Postoperatively the patient remained asymptomatic with persistant hypoparathyroidism and normal CgA values. After 18 months, MRI and EUS showed one lesion in the pancreatic tail, of the same dimensions (10 mm) as preoperatively, without other dissemination. Patient also had microprolactinoma. Prior and after pancreatic surgery (independently of VIP stimulation) serum PRL levels were up to 3000 mIU/l, without PRL response to TRH stimulation.
Conclusions
We present a case with multicentric pancreatic VIPomas and one glucagonoma in association with MEN1sy. The persistant solitary lesion in the pancreatic tail can be either clinically non-functional NET or microadenomatosis associated with MEN1sy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P876

**Disseminated insulinoma in 13-year-old patient**

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Neuroendocrine tumors are rare in young patients and usually have favorable course. We present a case of young, 13-year-old girl diagnosed with insulinoma. The diagnosis was stated on the basis of the presence of neuroglycopenic symptoms. An abdominal CT scan demonstrated a tumor localized in the head of pancreas. The patient received diazoxide as a preparation to surgery. Therapy was effective, but discontinued due to side effects. Patient underwent the Whipple operation. During this surgery pathological local lymph nodes and liver metastases were also stated. Histopathological examination confirmed well differentiated neuroendocrine tumor WHO grade1 with Ki67 index $<1\%$. Hypoglycemia persists after surgery. Four months after operation liver metastases grew up to 28 mm dimension and pathological lymph nodes were observed. PET–FDG was negative whereas PET-Ga68-DOTATATE was positive (about half of known disease foci showed tracer uptake). Despite the fact of only partially positive PET we decided to introduce somatostatin analogue into the treatment of hypoglycemia. The patient continues therapy with a very good control of glycemia. In the case of disease progression systemic therapy will be considered.
The presented case is an example of unfavorable course of rare neoplastic disease in young patient.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P877

**Management of ectopic Cushing's syndrome caused by bronchial carcinoid: four case reports**

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During the evaluation of hypercortisolism, distinction between Cushing's disease (CD) and ectopic ACTH syndrome (EAS) may be problematic. Detailed medical history, diagnostic procedures and pitfalls associated with EAS of four cases are presented. All the four patients had ectopic Cushing's syndrome due to typical bronchial carcinoids. Their mean age was 39 years, and the mean time from the presentation of Cushing's symptomes until diagnosis and definitive cure was 2.3 years (from 9 months to 5 years). The high-dose dxm suppression test was misleading in Cases 1 and 2, indicating CD. In Case 2, bilateral inferior petrosal sinus sampling (BIPSS) indicated a central source of ACTH overproduction, which led to hypophysectomy, despite of negative sella MRI findings. In Case 3, the BIPSS results were inconclusive and the sella MRI scan was negative, so renewed search for an ectopic origin was decided. In Case 4, the cortisol response to high-dose dxm was negative, but on the sella MRI pituitary microadenomas were visible. This led to a retrospectively unnecessary hypophysis surgery.
$^{111}$In-octreotide SPECT correctly localized lung carcinoid in every case. Hybrid imaging using SPECT–CT allowed an accurate anatomic description, detection of additional sites of disease, and higher specificity by exclusion of false-positive uptake at sites of physiologic tracer activity.
Conclusion
$^{111}$In-octreotide SPECT-CT was the most valuable and decisive method in the detection of ectopic ACTH-secreting tumors. A more favorable place in the diagnostic algorithm of ACTH-dependent hypercortisolism should be considered for such an image-fusion method applying simultaneous SPECT and CT acquisitions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P878

**Metastatic malignant insulinoma**

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Introduction
Insulinomas are the most common form of functioning pancreatic neuroendocrine tumors (NET) with an estimated incidence of 1–3/million per year. Less than 10% are malignant and rarely with distant metastases, carrying a poor prognosis.
Case report
We report a case of a 73-years-old woman attended at our ER for recurrent syncope, with irrelevant medical history. Several radiology exams were preformed, revealing numerous liver metastases and a probable metastatic lesion in L3 vertebral body, justifying a complete investigation for occult malignancy. The chest, pelvis and abdominal CT scan showed a lesion with tumor-like characteristics in the tail of the pancreas. The PET scan, bone scintigrafy and somatostatin receptor scintigrafy also suggested extensive metastization in the liver, cervical spine and both humerus of a primitive NET of the pancreas. Biopsy of a hepatic metastatic nodule was preformed, and the anatomopathological examination confirmed the diagnosis of a well differentiated NET (G2-WHO 2010(Ki-67 index of 6%)). The laboratory findings were:chromogranin A 91 nmol/l ($<6.0$), gastrin 392 pg/ml ($<90.0$), fasting insulin 34 μUI/l ($<30$), random insulin 54 μUI/l for a random glycemia of 33 mg/dl. Other biochemical tests were unremarkable and the screening for MEN1 was negative.
The patient was discharged under octreotide 100 μg, t.i.d. but kept frequent and severe hypoglycemias which motivated a re-admittance. In spite of an intensive treatment with octreotide 100 μg, t.i.d., diazoxide 100 mg, t.i.d., dexamethasone

0.5 mgq.d. and isotonic or hypertonic continuous glucose solutions, the patient kept severe hypoglycemia and chemotherapy was proposed because there was no surgical eligibility. After three cycles of chemotherapy(streptozocin 725 mg combined with epirubicin 87 mg), under monthly octreotide 30 mg, diazoxide 100 mg, t.i.d., prednisolone 20 mg, q.d. and a nutritional plan, the patient was discharged and remains with no further hypoglycemia or pain complaints (ECOG 0).
Conclusion
The rarity of this case and the absence of clear guidelines illustrate the difficulties in its treatment and the need for a multidisciplinary approach. The severity of the patients' symptoms imposed the use of multiple therapies and the option of a classic systemic chemotherapy, with good results. In spite of the poor prognosis carried by an advanced malignancy (T2NxM1 – ENETS and AJCC/UICC 2011), the patient is asymptomatic and has a good quality of life.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P879

### A rare case of hirsutism: mucinous cystoadenoma of the ovary with stromal luteinization and hilar cell hyperplasia

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Introduction
Mucinous ovarian cystoadenoma with stromal luteinization and hilar cell hyperplasia is a rare cause of hirsutism and virilization and only few cases are described in literature. We present a rare case of mucinous cystadenoma with hypertestosteronemia.
A 38-years old woman came to our observation for severe hirsutism and oligomenorrhea. Hormonal work-up showed severe hypertestosteronemia (total testosterone: 4.39 ng/ml) with high levels of DHEA-S (3574 ng/ml) and 17 OH progesterone (3.6 ng/ml). 17 OH progesterone after ACTH test stimulation was 18 ng/ml leading to the diagnosis of late onset congenital adrenal hyperplasia. However testosterone levels were, in our opinion, too high to be related only to a 21-hydroxilase deficiency. In the suspicion of an androgen secreting tumor, abdominal US and CT scan were performed, showing a right ovarian cystic mass of about 10 cm. Then the patient underwent right ovariectomy. Histology confirmed a mucinous cystoadenoma with hilar cell hyperplasia and stromal luteinization. After surgery, testosterone levels suddenly decreased, confirming our suspicion of an ovarian secretion of testosterone.
Conclusion
Mucinous cystoadenoma represents 15–20% of ovarian tumors. They often become very large and can extend up into the abdomen, so that the typical clinical presentation is usually related to the abdominal mass. Hypertestosteronemia is a rare clinical finding when hyperplasia of stromal cells and Leydig cells occur.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P880

### Neuroendocrine tumors of the gastrointestinal tract: a descriptive study

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Introduction and objective
Neuroendocrine tumors (NETs) are relatively rare tumors. The majority are located in the gastrointestinal (GI) tract and bronchopulmonary system. The aim of this report is to study the characteristics of gastrointestinal NETs at diagnosis.
Material and methods
Descriptive study about 18 patients diagnosed of gastrointestinal NETs during 1993–2011 in our hospital. Data regarding demographic, clinical, analytical, anatomopathological and diagnostic variables were collected. Statistical analysis was perfomed using SPSS 18.0, results are expressed as mean $\pm$ S.D.
Results
Eighteen patients (50% men) with a mean age at diagnosis of $52.7 \pm 14.4$ years were studied. Seven (38.9%) presented typical carcinoid clinical syndrome. The rest presented symptoms due to growth of the primary tumor such as appendicitis, intestinal obstruction or diffuse abdominal pain. The mean time since symptoms appeared until diagnosis was 10 months. The ileum was the most common location (61.1%) followed by stomach (16.7%), appendix (11.1%), rectum (5.6%) and colon (5.6%). Eleven (61.1%) had metastases at diagnosis, all of them hepatic. Six of the seven patients with carcinoid syndrome presented metastases at diagnosis. Tumors were diagnosed more frequently by CT scan (50%), ultrasound (16.6%) and MRI (5.5%). Inmunohistochemical markers resulted positive for: chromogranin A (88.9%), enolase (61.1) synaptophysin (44.4%) and cytokeratin (33.3%). Ki67 was determinated in 50% of cases. It was $<3\%$ in seven cases (38.9%) and $>20\%$ in two (11.1%). The mitotic rate was determinated only in four patients (22.2%), in three of them was $<2\%$ (16.7%) and between 2 and 20% in one case (5.6%).
Conclusions
The ileum is the most common location of NETs of gastrointestinal tract. The mitotic and proliferation rate must be a priority in the anatomopathological study as it may condition different therapeutical approaches. In our serie these tumors are diagnosed in an advanced metastasic stage probably due to a low suspicion of these tumors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P881

### Prediction of remission of gestational trophoblastic disease based on initial levels of prolactin

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Objective
A prospective study to research the relationship between initial levels of prolactin in the development and outcome of patients with gestational trophoblastic disease.
Material and method
A longitudinal study including 51 women attending the Reference Center of Gestational Trophoblastic Neoplasia of Rio de Janeiro- Brazil, with complete mole, and comproved histopatologic examination. All women were followed up until remission was achieved. The criteria of selection was the level of hCG above 100,000 mIU/ml, a risk factor included in the FIGO 2000 staging and risk factor scoring system for gestational trophoblastic neoplasia. Two groups were established: the first one with prolactin level $>30$ ng/ml and the second one with prolactin level $<30$ ng/ml. The serum levels of hCG and prolactin followed the same time interval established by the consensus protocol for the monitoring of gestational trophoblastic disease.
Results
Of 208 patients registered in the GTN Reference Center in Rio de Janeiro during the period analyzed, 51 presented with βhCG serum levels $\geq 100.000$ mUI/ml: 36 (70.6%) had prolactin levels above 30 ng/ml (group 1) and 15 (29.4%) had prolactin levels $<30$ ng/ml (group 2). In group 1, 25 presented with vaginal bleeding, 12 with uterus large for gestational age, four had hypertension and 18 hyperemesis. Six patients were diagnosed with bilateral ovarian cists. Spontaneous remission occured in 27 patients, nine requiring chemotherapy. In group 2, three patients presented with vaginal bleeding, none had uterus large for gestational age, none had hypertension, four patients had hyperemesis and two were diagnosed with bilateral ovarian cists; 14 went into spontaneous remission, one was submitted to chemotherapy.
Conclusion
In this study of 51 patients with complete mole there was not found any correlation between baseline prolactin levels, progression to spontaneous remission, indication for chemotherapy or time interval for remission.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P882

**Radioguided cortical-sparing approach for recurrent pheocromocytoma/paragangliomas**

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Background
A 27-years-old women affected by recurrent pheocromocytoma/paragangliomas. We describe the evolution of disease and the surgical approach. The patient underwent a two-steps bilateral adrenalectomy for pheocromocytoma. At 15 years she underwent a left adrenalectomy. At 19 a right adrenalectomy + removal of paracaval paraganglioma were performed. After 8 years of good health she experienced again headache, tachycardia and hypertension not responsive to therapy. A diagnosis of recurrent disease was made.
Methods
Nor-colesterol and MIBG scintigraphy were performed and stated respectively for a hyperfixation in the left adrenal bed and two spot of hyperfixation on paracaval and paraortic area (Default 1). The last two spots were concurdant with a MRI imaging. The patient underwent surgical exploration using an intraoperative radionuclear scanning probe. A target/background ratio (T/B ratio) was calculated. All lesion with a T/B ratio level more than 1.2 (239 mBq) were considered suspected.
Results
Three lesions with a value respectively of 270, 2020 and 2600 mBq were removed. Pathological report classified the latter two lesions as paragangliomas. At 5 years follow-up the patient has no biological evidence of disease and does not need any replacement therapy.
Conclusions
The easier finding of the lesions by radioguidance allowed the surgeon to perform a focused approach, obtaining a radical procedure, to achieve a successful treatment of a recurrent disease. Sparing the remnant of cortical adrneal tissue on the left side also had a great impact in the quality of life preserving the patient from life-long steroid replacement therapy (Figure 1).
Declaration of interest
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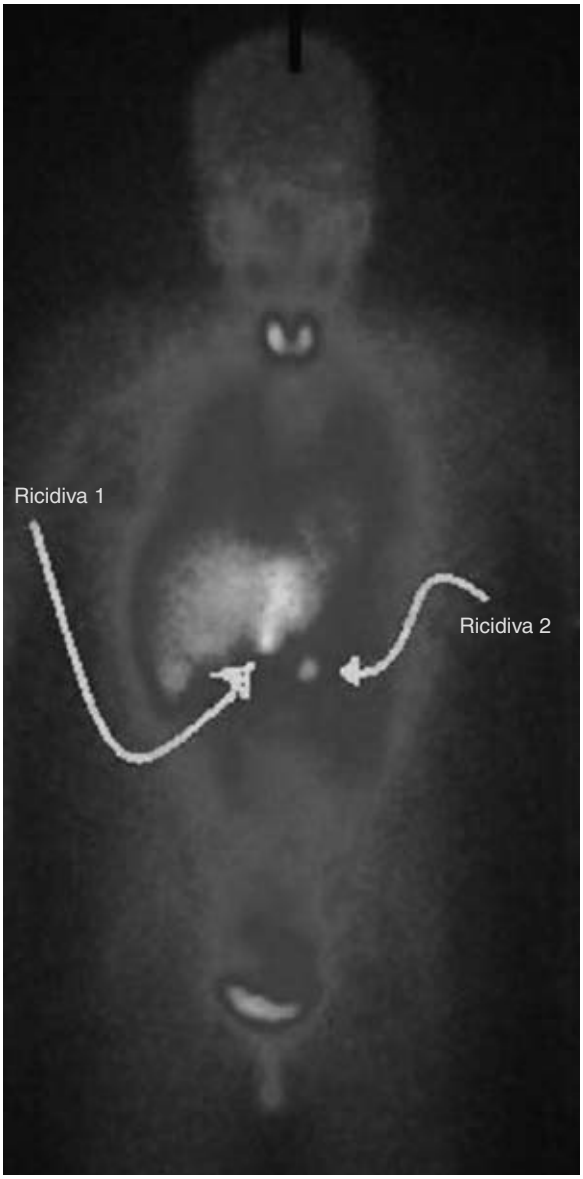


**Figure 1** MIBG Scan showing a recidive of paraganglioma

## P883

**Clinically palpable parathyroid adenoma**

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A palpable parathyroid mass, in a patient with primary hyperparathyroidism, is presumed to be parathyroid carcinoma until proven otherwise, with other less common causes including parathyroid cysts and adenomas.
In developing world primary hyperparathyroidism presented in different way not only in the disease age group as well as in clinical symptoms and signs. More cases of symptomatic PHPT are presented in younger age group and some time large adenomas can presented a palpable neck swellings.
We have 13 cases of clinically palpable parathyroid tumors out of 58 cases. In all palpable cases except one proved benign adenomas.
Large parathyroid adenomas can be presented clinically palpable swelling. Cytology and imaging of parathyroid adenomas may, on occasion, mimic follicular thyroid neoplasms.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P884

**Special diagnostic, therapeutic and evolutive aspects of insulinoma: a tunisian experience**

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Insulinoma is a very rare type of islet cell tumor. The aim of our study is to report our clinical experience with insulinoma in order to determine clinical, biological, radiological and therapeutic particularities of this tumor type.
It's a retrospective study, including five patients (three women and two men) referred to our department between 2005 and 2011 for hypoglycemia exploration. Mean age of our group is 39.8 ± 15.4 years (ranges: 20–60 years). Two women were type 2 diabetic patients.
All our patients showed neuroglycopenic symptoms associated with a blood glucose level < 0.5 g/l. Three patients had a weight gain of 16 ± 13.5 kg (ranges: 3–30 kg).
The fasting test was interrupted after 6.6 ± 4 h (ranges: 3–11 h), showing an inadequate high insulin (ranges: 4.2–240.5 UI/ml) and C-peptide (ranges: 2.91–14.4 ng/ml) secretion with hypoglycemia (ranges: 1.5–2 mmol/l). The screening for sulfonylurea was negative in all patients confirming the diagnosis of insulinoma. It was a sporadic form of insulinoma in all patients.
Endoscopic ultrasonography and CT-scan showed a tail pancreatic tumor measuring 17 mm in one case, and were negative in the other cases. In this group, the tumor was detected using per-operative ultrasonography. All our patients underwent enucleation without any per or post-operative complications.

Postoperative glucose metabolism was normalized in all patients even in diabetic patients.
The combination of hypoglycemia and endogenous hyperinsulinemia with a negative screening for sulfonylurea confirm the diagnosis of insulinoma. However, preoperative localization techniques can be negative especially in very small tumor. In this case, Intraoperative ultrasonography is helpful.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## Female Reproduction

### P885

**The ovarian failure associated Y235 residue of human BMP15 gene is target of evolutionary positive selection**

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BMP15 is a TGFβ-like oocyte-derived growth factor involved in ovarian folliculogenesis as a critical regulator of many granulosa cell processes. BMP15 is synthesized as a pro-protein which dimerizes and then is processed in the bioactive mature domain and a large prodomain. The proregion has an important role in the BMP15 processing by driving the dimerization and secretion of the active mature dimers. Since several mutations in the BMP15 gene have been found with different ovarian phenotipic effects, its role seems to differ between mono- and poly-ovulatory species. In fact, heterozygous natural mutations in mono-ovulating sheep cause increased ovulation rates, while poly-ovulating $bmp15^{-/-}$ null mice are subfertile. In humans, almost all the identified BMP15 mutations are located in the proregion, in heterozygosity and associated with Primary Ovarian Insufficiency (POI). To investigate the BMP15 role in controlling ovarian function in mammals, a phylogenetic analysis of sereval TGFβ/BMP family members was performed. BMP15 shows a very early divergence and a more rapid evolution than other members of the family. Using branch-site models from PAML packages, we detected signals of positive selection for the following residues: F146, L189, I229, Y235, R244. Among them, the Y235C mutation was previously identified in association with primary amenorrhea and ovarian dysgenesis. By luciferase reporter assay, the biological activity of the wild-type (wt) BMP15 was compared to those of mutant variants, obtained by replacing the positively selected aminoacid by alanine or cysteine. The L189A, Y235A and Y235C mutants showed a significant increase of BMP signalling compared to wt. In conclusion, present analysis evidences that Y235 residue is important for BMP15 biological activity, indirectly confirming its role in the onset of POI. Moreover, the BMP15 gene seems to have specialized in regulating proper ovarian function during evolution so that, if altered, poly-ovulation or POI could occur.
Declaration of interest
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### P886

**Coordinate regulation of heterogeneous nuclear ribonucleoprotein dynamics by steroid hormones in the human Fallopian tube and endometrium *in vivo* and *in vitro***

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Context
The regulation of heterogeneous nuclear ribonucleoproteins (hnRNPs) and their interactions with steroid hormone signaling in Fallopian tubes and endometrium are not clearly understood.
Objective
We determined whether hnRNP expression is regulated during the menstrual cycle and correlates with estrogen receptor (ER) and progesterone receptor (PR) levels in human Fallopian tubes *in vivo*. We also explored the mechanisms of hnRNP regulation in human endometrium *in vitro*.
Design and methodology
Fallopian tissue was obtained from patients in early, late, and postovulatory phases and mid-secretory phase, and endometrial tissue from premenopausal and postmenopausal women undergoing hysterectomy. We measured expression of hnRNPs and assessed their intracellular localization and interactions with ERs and PRs. We also determined the effects of human chorionic gonadotropin (hCG), 17β-estradiol ($E_2$), and progesterone ($P_4$) on hnRNP expression.
Results
In Fallopian tubes, mRNA and protein levels of hnRNP A1, AB, D, G, H, and U changed dynamically during ovulation and in the mid-secretory phase. In coimmunolocation and coimmunoprecipitation experiments, hnRNPs interacted with each other and with ERs and PRs in Fallopian tubes. After treatment with $E_2$ and/or $P_4$ to activate ERs and PRs, hnRNP A1, AB, D, G, and U proteins displayed overlapping but distinct patterns of regulation in the endometrium *in vitro*.
Conclusion
Our findings expand the physiological repertoire of hnRNPs in human Fallopian tubes and endometrium and suggest that steroid hormones regulate different hnRNPs directly by interacting with ERs and/or PRs or indirectly by binding other hnRNPs. Both actions may contribute to regulation of gene transcription.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### P887

**Role of the CRH receptors and brainstem noradrenergic nuclei in the gonadotropins secretion induced by acute stress at proestrus morning**

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Acute stressors can active the reproductive function depending on estrogen priming. Corticotropin-releasing hormone (CRH) has been implicated as mediator of stress-induced effects on hypothalamus–pituitary–gonadal axis (HPG), acting in the limbic system, paraventricular nucleus and noradrenergic (NA) neurons. Antalarmin and astressin2-B are selective CRH-R1 and CRH-R2 antagonists, respectively. We investigated the mediation of CRH-R1 and/or CRH-R2 receptors and of locus coeruleus (LC), A1 and A2 neuron groups in the effects of acute stress on LH and FSH secretion at proestrus morning. Wistar rats showing regular estrous cycles received a catheter in the jugular vein one day before acute stress. At proestrus morning, it was injected antalarmin (1 mg/kg i.v.), asstressin2-B (4.2 nmol i.c.v.) or vehicle and 30 min later was performed the restraint stress for 30 min. Blood samples were collected before, during and after restraint stress to measure FSH and LH by RIA. At the end of the experiment, the rats were perfused and brain were removed to carry out immunofluorescence for tiroxine hydroxylase (TH) and /FOS in LC, A1 and A2 cell groups. Restraint stress increased FSH and LH levels for 30 min. Antalarmin blocked stress-induced increase of FSH and LH whereas astressin2-B only blocked the increase of FSH secretion. The TH activity increased in the LC and A2 groups and a minor magnitude in A1 group. Only the antalarmin injection reduced this effect principally in the LC. These data indicate different roles of CRH receptors in the stress restraint-effects on gonadotropins secretion at proestrus. The CRH-R1 more than CRH-R2 receptor would be essential to the HPG axis stimulation by acute stress and this response would be mediated at least in part by NA neurons, principally in the LC.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### P888

**An invdup (15) associated with premature ovarian insufficiency**

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Premature ovarian insufficiency (POI) is a disorder in which amenorrhea, associated to elevated levels of gonadotropins, occurs before the age of 40 years.

The proband, a 36-year-old woman, was born at term after an uneventful pregnancy and delivery from healthy non-consanguineous parents. Growth and mental developmental milestones were normal. At the age of 12 years she presented menarche followed by regular menses, she had no miscarriage and had a successful pregnancy at the age of 27 years. At 35 years of age she experienced asymptomatic abrupt amenorrhea. Physical examination was unremarkable with no evidence of major malformations as well as facial dysmorphisms. Hormonal profile was normal except for LH and FSH values that were in the menopausal range (55.0 and 166.4 mU/ml respectively) and were confirmed over a period of one year.
Environmental, metabolic, iatrogenic and autoimmune causes of POI were excluded. FRAXA expansion was analyzed by standard methods and excluded.
Cytogenetic analyses showed the presence of a supernumerary marker chromosome (SMC) that was characterized by FISH and array-CGH (comparative genomic hybridization).
This marker chromosome derived from chromosome 15 and contained only heterochromatic material. The Prader Willi/Angelman region, that is associated with an abnormal phenotype, was not present. Patients with invdup(15) chromosomes with only the heterochromatin of the proximal 15q have a normal phenotype. Interestingly, an aspect that is sometimes overlooked in these cases is infertility and amenorrhea that is also described in cases with SMC derived by other acrocentric chromosomes and aneuploidies (trisomy 13, 18, 21 and 45,X0). This case constitutes a further example that etiology of POI is not always associated with a defective gene, but in some cases oocytes atresia can be the consequence of the abnormal meiotic pairing of chromosomes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P889

**Impact of thyroid status and thyroid autoimmunity on assisted reproduction technology outcome**

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Introduction
The current evidence on the association between thyroid function or thyroid autoimmunity (TAI) and reproductive outcomes after assisted reproduction techniques (ART) is scarce and inconclusive.
Objective
To record ART outcome according to function and TAI status of thyroid gland.
Methods
A group of 226 subfertile women that underwent any method of ART (IVF, ICSI, intra-uterine insemination (IUI)) from January 2006 to December 2010 were included in the study. Women were excluded if they had failed to complete the ART protocol or if they had a history of thyroid disease. In case more than one ART cycle was applied to a certain woman, only the first one was included in the study.
Results
Studied women were divided into subgroups according to serum TSH concentrations and presence of TAI. Nine had TSH $<0.05$ μIU/ml, 156 between 0.05 and 2.5 μIU/ml, 51 between 2.5 and 4.5 μIU/ml and nine $>4.5$ μIU/ml. Overall, 37% of women achieved biochemical pregnancy, 33% clinical pregnancies and 2% miscarried. The live birth rate was 29%. No difference was found among the four TSH or the two TAI subgroups regarding reproductive outcomes. Age was negatively correlated to numbers of retrieved ($r=-0.349$, $P<0.001$) and fertilized oocytes ($r=-0.344$, $P<0.001$).
Conclusion
This study was not able to demonstrate a relationship between thyroid function or TAI and reproductive outcome after ART. Nevertheless, further prospective trial should be made, studying thyroid function not only before but also during the ART cycle.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P890

**The association of thyroid hormones with menstrually related mood disorder, sexual abuse histories and mood symptoms**

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Introduction
Our previous research suggested that a history of sexual abuse (SA) may impact thyroid axis profiles differently in women with menstrually related mood disorder (MRMD) compared with non-MRMD women but was limited by a small sample of non-MRMD women with SA. The current study was designed to study a larger sample of MRMD and non-MRMD women with SA to evaluate whether MRMD status moderates the effects of SA on thyroid axis function.
Methods
A total of 109 women (aged $34\pm8$ years) were prospectively evaluated for current diagnosis of MRMD according to the DSM-IV criteria; for SA histories using a validated interview; for depressive symptoms using the beck depression inventory (BDI); and for serum concentrations of free $T_3$ ($FT_3$) and free $T_4$ ($FT_4$). The $FT_3/FT_4$ ratio, that provides an index of the peripheral conversion of $T_4$ to $T_3$, was also calculated.
Results
Fifty-seven women met criteria for MRMD (23 with SA histories and 34 without SA histories) and 52 women were enrolled as non-MRMD controls (18 with SA histories and 34 without SA histories). There was a significant MRMD$\times$SA interaction for $FT_3$ concentrations ($F(108,3)=4.07$; $P=0.04$), since non-MRMD women with SA histories had lower $FT_3$ concentration than all other groups (ps$<$ 0.05). Only for non-MRMD women with SA histories, lower $FT_3$ predicted higher BDI scores ($\rho=-0.51$, $P=0.046$).
MRMD women, when compared to non-MRMD women, had lower $FT_4$ concentrations ($F(108,1)=5.82$; $P=0.02$) and higher $FT_3/FT_4$ ratio ($F(108,1)=$ 14.99; $P<0.001$).
Conclusions
Our results suggest that MRMD diagnosis moderates the influence of SA on $FT_3$ concentrations. Lower $FT_3$ concentrations are associated with increased depressive symptoms in non-MRMD women who have SA histories. For MRMD women, regardless of SA history, there is increased peripheral conversion of $T_4$ to $T_3$, consistent with a defense reaction and more recently conceptualizations that PMDD represents a stress-related disorder.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P891

**Androgen profiling in adolescent females by liquid chromatography–tandem mass spectrometry**

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Polycystic ovary syndrome (PCOS) has an early onset. However, its prevalence in the youngest age groups has not been assessed, mostly due to the poor specificity of the criteria for defining PCOS in adolescents, including the testosterone references generated only on adult women. Moreover, the current references for testosterone were mostly built by immunoassays, known to have poor accuracy. This study was aimed at defining androgen and pro-androgen reference intervals in 149 healthy normal weight and drug-free young women, aged 16–20 y, by using liquid chromatography–tandem mass spectrometry (LC–MS/MS). All had 11–13 menses/year and no signs of hyperandrogenism. Median values (2.5th–97.5th centiles, ng/ml) for testosterone, androstenedione, DHEA, 17OHprogesterone and progesterone were 0.275 (0.129–0.519), 0.898 (0.407–1.665), 6.78 (2.58–18.34), 0.646 (0.210–2.443) and 0.132 (0.049–13.950) respectively. After excluding 36 subjects who did not report their last bleeding day and 9 subjects in ovulatory peak, subjects were sub-grouped according to menstrual phases: follicular phase (day 1–13, LH $<20$ μU/ml, estradiol $<100$ pg/ml, FSH $<10$ uU/ml, n=48); luteal phase (day 14 on, LH $<20$ uU/ml, estradiol $<100$ pg/ml, FSH $<10$ uU/ml, n=56). Follicular and luteal phase median values (2.5th–97.5th centiles, ng/ml) were: 0.247 (0.126–0.416) and 0.274 (0.131–0.553) for testosterone, 0.884 (0.384–1.514) and 0.839 (0.317–1.556) for androstenedione, 6.91 (2.84–21.09) and 7.01 (2.29–17.23) for DHEA, 0.375 (0.160–0.847) and 1.275 (0.261–2.516) for 17OHprogesterone, 0.067 (0.049–0.337) and 4.177 (0.060–14.575) for progesterone respectively. Confirming higher progesterone ($P<0.0001$) and 17OHprogesterone ($P<0.0001$), increased

testosterone was found in the luteal compared to the follicular phase ($P=0.037$). Within the luteal phase subgroup, 23 anovulatory (progesterone $<2$ ng/ml) and 20 normo-ovulatory subjects (progesterone $>7$ ng/ml) were found. Notably, testosterone was significantly higher in anovulatory than ovulatory subgroup ($P=0.021$). Using LC– MS/MS, we defined androgen and pro-androgen reference intervals in young females according to menstrual phase, highlighting the link between testosterone and ovulatory efficiency.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P892

### Expression of CRH/urocortin gene family in mouse gestational tissues during late pregnancy

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Introduction

There is increasing evidence for a role of the CRH/urocortin family in human pregnancy, since they have been implicated both in physiological processes and pathogenesis of diseases such as spontaneous preterm labour. However, little information is available on the presence or function of the CRH/urocortin genes in mouse pregnancy. The study aimed to investigate the expression of CRH, urocortins and their receptors in mouse utero-placental tissues during late pregnancy.

Design

All animal care and experimental protocols were approved by the appropriate animal ethics authorities. Placental, uterine and fetal membrane tissues were separated and collected from timed-pregnant mice on days 16–19 of pregnancy ($n=10$ for each group; parturition occurred on D20). RNA was extracted from the tissues and Taqman real time RT-PCR was performed using standard techniques.

Results

CRH and urocortin were expressed at low levels in the murine placenta and did not vary significantly during late pregnancy. Urocortin2 was the most abundant urocortin in mouse placenta and levels increased significantly from D16 to D19 ($P<0.001$). Urocortin2 levels were lower in fetal membranes, but increased significantly on D19 of pregnancy ($P<0.05$ D16, D17, D18 vs D19). In contrast, urocortin3 concentrations were higher in fetal membranes than in placenta and decreased significantly on D17–19 compared with D16 ($P<0.05$). CRH-R1 was predominantly expressed in placenta and concentrations did not vary significantly. However, CRH-R2 was more widely expressed and the level in the fetal membranes increased significantly on D18 compared with D16 ($P<0.05$).

Conclusions

Urocortin2 is a major urocortin in mouse gestational tissues, with the up-regulation in placenta and fetal membranes in late pregnancy suggesting a role for the gene in parturition. Moreover, the co-incident decrease in urocortin3 in fetal membranes suggests that the balance between the two urocortins, which both act via CRH-R2, may have a key role in pregnancy and parturition.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P893

### Hypoxia-induced oxidative stress regulates the expressions of calcium transport channels in the duodenum, kidney and placenta of rats

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Introduction

Preeclampsia is a pregnancy-specific disease characterized by *de novo* development of concurrent hypertension, proteinuria and oxidative stress in placenta. Hypoxia occurs during the development of placenta in the first trimester and is implicated in trophoblast differentiation. The oxidative stress, resulting from deficient remodeling of spiral arteries, is an important inducer of preeclampsia. The potassium-dependent sodium/calcium exchangers including NCKX3 and NCX1 play critical roles in the transport of intracellular calcium that is exchanged with extracellular sodium ions. Calcium related proteins, NCXs, calbindin, calcium pumping proteins (TRPV5-6, PMCA1b) transcripts are abundant in the smooth muscle, uterus, aorta and intestine.

Methods

The expressions of calcium related proteins in the kidney, duodenum and placenta after hypoxic stress in rats at gestation 19.5 day (GD 19.5) was examined by real-time PCR and western blot analysis.

Results

Hypoxic condition did not change fetal weight, however, it significantly increased the weight of placenta compared to normoxic condition. In GD 19.5, renal NCKX3 and TRPV6 expressions were increased, while the levels of NCX1 were decreased in hypoxic rats compared to normoxic pregnant rats. The expressions of CaBP-9k, TRPV5 and PMCA1b were not altered in normoxic- or hypoxic rat tissues. Duodenal expressions of CaBP-9k, TRPV5-6 and PMCA1 were decreased in hypoxic rats, while NCXs were not changed. The transcripts of NCKX3, TRPV5-6 and PMCA1b were highly expressed in the placenta of hypoxic rat.

Conclusions

The expressions of renal, duodenal and placental calcium related proteins appear to be modulated by hypoxia-induced oxidative stress, implying that calcium related proteins may be involved in preeclamptic oxidative stress.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P894

### Daily serum testosterone during the menstrual cycle by ID-LC–MS/MS

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Testosterone levels in normally cycling women are generally assumed to be elevated during ovulation. The clinical relevance of changing testosterone levels during the menstrual cycle, however, is unclear, due to poor performance of current direct immunoassays for testosterone at low concentrations.

An isotope dilution-liquid chromatography–tandem mass spectrometry (ID-LC–MS/MS) method with sufficient reliability and sensitivity[1] was used to measure testosterone in serum samples obtained daily during the menstrual cycles of 25 healthy women, characterized by biochemical and physical examination. Since ID-LC–MS/MS is not accessible to all clinical laboratories, we also evaluated the performance of the recently available direct immunoassay Architect 2nd Generation Testosterone.

Performance of the ID-LC–MS/MS method was comparable to the reference method[2] (weighted Deming regression: $y=1.007\times-0.056$ nmol/l; $r=0.9998$). Comparison of the 2nd generation immunoassay results to ID-LC–MS/MS yielded $y=1.095\times+0.104$ nmol/l ($r=0.90$). Testosterone levels were significantly higher mid-cycle, although a peak was not discernable in all individuals. Intra-individual variation exceeded the group average in the menstrual cycle; the ratio of extremes (max/min) found in individuals ranged from 1.6–3.7. Apart from a persistent positive bias, the immunoassay measured the same testosterone profiles. The reference interval was 0.30–1.69 nmol/l for ID-LC–MS/MS and 0.50–2.00 nmol/l for the immunoassay.

Our ID-LC–MS/MS method measured low testosterone levels accurately. The Architect 2nd generation testosterone immunoassay had acceptable performance across the entire range measured in women. On average, the elevation of mid-cycle testosterone levels is statistically significant, although not relevant for clinical practice since the day-to-day variation is higher and independent of menstrual cycle. In light of this, we recommend measuring testosterone on at least two independent occasions for diagnostic purposes.

1. Bui 2010 *AnnClinBiochem.*
2. Thienpont 2008 *ClinChem.*

Declaration of interest

I fully declare a conflict of interest. Details below.

Funding

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## P895

**NT-pro-BNP levels in patients with polycystic ovarian syndrome**

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Polycystic ovarian syndrome (PCOS) is a common endocrine disturbance in women of reproductive age, thought to be linked to increased cardiovascular risk in later life. Cardiovascular risk factors are usually present even at younger age and this suggests that the chronic disturbances in hormonal and metabolic status typical for the syndrome predispose the patients to development of early atherosclerosis and premature clinical presentation of cardiovascular disease. NT-pro-BNP was shown to have a high predictive value regarding cardiovascular events in patients with hypertension and left ventricular hypertrophy especially in those without overt cardiovascular disease.

The aim of the study is to investigate the importance of NT-pro-BNP as a predictive factor in relation to other classical cardiovascular risk factors.

Methods

Seventy Bulgarian women aged 18–45 years with PCOS (ESHRE-ASRM criteria) and/or obesity (BMI $\geq 30$ kg/m$^2$) participated in this cross-sectional study. NT-pro-BNP was determined with electrochemiluminescence sandwich immunoassay method.

Results

There was no statistical difference between NT-pro-BNP levels (mean $\pm$ S.D.) in obese ($46.9 \pm 57.0$), lean PCOS ($43.5 \pm 52.9$) and obese PCOS ($47.9 \pm 37.7$) patients, between patients with or without metabolic syndrome and between patients with different cardiovascular risk according to androgen excess and polycystic ovary syndrome (AE-PCOS) society consensus. The rate of patients with NT-pro-BNP levels above the cut-off of 103 pg/ml is not statistically different between the groups. NT-pro-BNP does not show significant correlation to age, weight, body mass index, waist-to-hip ratio, waist-to-stature ratio, systolic or diastolic blood pressure and results from OGTT except from immunoreactive insulin levels on 120 min. NT-pro-BNP correlates weakly with HDL-C, but not with other indices of lipid profile.

Conclusions

In this relatively young female population NT-pro-BNP levels did not show to be an important marker for increased cardiovascular risk.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P896

**Differential expression and regulation of $Mg^{2+}$ inorganic phosphate transport channels by hypoxia stress in human placental cells**

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Introduction

Preeclampsia is a pregnancy-specific disease characterized by *de novo* development of concurrent hypertension, proteinuria and oxidative stress in the placenta. Hypoxia occurs during the development of placenta in the first trimester and is implicated in trophoblast differentiation. Last trimester of gestation, the calcium and mineral transport from mother to fetus are dramatically increased in response to the accelerated demand for bone mineralization in the fetus. Two channels of this subfamily, TRPM6 and TRPM7, are permeable for various divalent cations, including $Ca^{2+}$, $Mg^{2+}$ and $Zn^{2+}$. Adequate phosphate homeostasis is of critical importance for a wide variety of functions including bone mineralization and energy metabolism. Signaling mechanisms mediating hormonal regulation of $Mg^{2+}$ and inorganic phosphate channels are not well understood in the placenta during pregnancy.

Methods

The expression of cell membrane $Mg^{2+}$ and inorganic phosphate channels was investigated when oxidative stress was induced in human placental cells BeWo, JEG3 and human placental primary cells (hPC). The mRNA and protein levels were examined by real-time PCR and western blot analysis.

Results

The transcriptional and translational levels of $Mg^{2+}$ and inorganic phosphate channels were altered in hypoxic condition. The expression of $Mg^{2+}$ channels was down-regulated by hypoxia. The transcripts of inorganic phosphate channels were increased in BeWo and JEG3 cells, while decreased in the hPC.

Conclusions

$Mg^{2+}$ and inorganic phosphate channels were distinctly expressed after oxidative stress in human placental cells, suggesting that altered expression of $Mg^{2+}$ and inorganic phosphate channels may be involved in placental hypoxic stress, a determinant factor causing preeclamptic abnormality in human placental cells.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P897

**Progesterone levels on the hCG day and outcomes IVF in women with polycystic ovary syndrome**

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Background

The purpose of this study was to determine the incidence of premature luteinization in patients with polycystic ovary syndrome and compared the main determinants of success in IVF in PCOS patients with and without premature luteinization.

Methods

Retrospective analysis of 180 PCOS women of Chinese Han origin with infertility who underwent controlled ovarian hyperstimulation (COH) with an exogenous gonadotropin/GnRH antagonist protocol. Hormone levels on the hCG day and IVF outcomes were assessed.

Results

The incidence of premature luteinization was 23.3%. Compared with PCOS patients without premature luteinization, PCOS patients with premature luteinization (PL) had a higher number of oocytes retrieved ($18.20 \pm 6.6$ vs $15.08 \pm 7.3$, $P = 0.037$) and a higher fertilization rate ($72.9 \pm 1.9$ vs $63.1 \pm 2.3$, $P = 0.033$), but clinical pregnancy rates were no statistical significance (53.3 vs 56.0, $P = 0.836$). Though the implantation rate was higher in no premature luteinization patients, but the difference was not statistically significant (37.7 vs 30.3, $P = 0.115$).

Conclusion

The PCOS patients with premature luteinization had a higher fertilization rate and high number of oocytes retrieved, and the similar implantation rate and clinical PRs as PCOS patients without premature luteinization.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P898

**State of care in women affected by early premature ovarian failure (ePOF) within the first two years of treatment in a center for reproductive medicine and endocrinology (RME)**

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Context

Early premature ovarian failure (ePOF) defined by cessation of ovarian function before age 18 is a rare condition. The link between puberty progression and POF hasn't been explored in details.

Aim of study

To describe pubertal development and clinical practices of women with ePOF in a referral center for RME within the first 2 years follow-up.

Description method/design

A mixed retrospective and prospective study was performed. A total of 358 consecutive POF patients were followed from 1997 to 2010. From this cohort 108 patients with ePOF (young girls, adolescents $<18$ years of age at diagnosis of POF) with karoytype excluding Turner syndrome, and no iatrogenic cause are the focus of our study.

Their clinical (age, pubertal evolution, circumstance diagnosis), hormonal (FSH, LH, $E_2$) and morphological features (pelvic ultrasonography, laparoscopy) were analyzed. We also documented their management's characteristics (type of/age of hormonal treatment).

Results
Primary amenorrhea (PA) was more often associated with Tanner breast's stage 1–2–3 and delayed or partial puberty (28.7%) than breast's stage 5 and normal puberty (12%). Secondary amenorrhea (SA) was associated to stage 5 and normal puberty (27.7%). SA's mean age was 16.22 (15–18) years with menarchy at 13.56 (10.5–17) years. The diagnoses are made on high FSH and mean level was 94.76 UI/l. LH was 32 UI/l and estradiol 17 ng/ml.
Mean age at start of hormonal treatment was 17.55 (15–28) years; progestogen's onset was one year later. Combined treatment started at 18 years old. During these two years 1/5 of patients were lost for follow-up.
Conclusion
The fact to complete puberty doesn't exclude PA. However in SA a normal puberty is most often found then in PA. Suboptimal care of these ePOF is documented therefore widespread guidelines to improve care of patients with early POF are needed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P899

**Nutritional status and reproductive morbidity among women (age 15–40 years) in Dhaka city**

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Introduction
Reproductive health is closely related with nutritional status of a country. Nutritional problems are different between developed and developing countries. In the context of Bangladesh there is no recent assessment. This study aims to assess the nutritional status and reproductive morbidity among women in Dhaka city.
Description of methods
A cross-sectional study was performed in 400 women, aged 15–40 years, in Dhaka city. Sample was selected using multistage cluster sampling method. For measuring nutritional status standard techniques of anthropometric indices were used. Social status and reproductive morbidity related data were collected using a pre-designed questionnaire. Results were expressed as frequency, bivariate correlation and $\chi^2$ test.
Results
The average age of the respondents is 25 years and monthly family income is 9216 TK. 31% of women were illiterate. Body mass index (BMI) is an important indicator for nutritional status of the women. This study found that, among the women the proportion of underweight is 19% and overweight is 17%. 32% respondents suffered pregnancy related complication during their pregnancy or delivery time. Anemia is a risk factor for pregnancy related complications. Based on their self-reported information, 74% women had anemia and 33% women had irregular menstruation during their reproductive age. 13% hypertension was found among the respondents in this study. Hypertension is a risk factor during pregnancy for pre-eclampsia and eclampsia. Among the women 3% had diabetes. About half of the respondents had poor appetite. That means they might be suffering from anorexia or bulimia nervosa. BMI is significantly ($P<0.05$) associated with respondents age ($r=0.25$), education level ($r=0.19$) and monthly family income ($r=0.21$). In this study anemia, hypertension, diabetes and respondents appetite are measuring variables of reproductive morbidity and BMI is a measuring variable of the nutritional status. BMI is significantly ($P<0.05$) associated with reproductive morbidity.
Conclusion
i) Among the respondents one third women are malnourished. ii) Approximately 60% women are suffering from reproductive morbidity in Dhaka city. iii) There is a strong association between socio demographic and nutritional status. iv) Reproductive morbidity is associated with nutritional status.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P900

**Anthropometric indices of visceral obesity and cardiovascular risk factors in patients with polycystic ovarian syndrome**

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Patients with polycystic ovarian syndrome (PCOS) have an increased risk for diabetes mellitus and often show an adverse cardiovascular risk profile. Among the anthropometric variables for evaluation of central obesity widely used are the waist circumference (WC), waist-hip ratio (WHR) and, more recently, waist-to-stature ratio (WSR).
The aim of the present study is to investigate the link between some anthropometric indices of visceral obesity and cardiovascular risk factors according to androgen excess and polycystic ovary syndrome (AE-PCOS) Society consensus.
Patients and methods
Sixty four PCOS subjects (45 lean; 19 obese) aged 18-40 years were included in this cross-sectional study. Obesity was defined as BMI $\geq 30$ kg/m$^2$ and visceral fat predisposition was considered according to WHR $>0.85$, WC $>80$ cm and WSR $>0.5$. OGTT with determination of immunoreactive insulin (IRI) and Homeostatic model assessment index (HOMA) were performed and testosterone, DHEAS and androstenedione were measured.
Results
Obese PCOS patients had significantly higher rate of visceral obesity than lean PCOS subjects although $\sim 1/3$ of lean patients had visceral predisposition despite lower BMI. Both WSR and WC but not WHR showed to be good markers of adverse metabolic profile in women with PCOS. The cut-off point for WSR of 0.50 is useful and the cut-off of 80 cm for WC is more appropriate than 88 cm in detecting cardiovascular risk in PCOS patients. Unlike fasting IRI on 0 min and HOMA, androgen levels did not correlate to increased cardiovascular risk (AE-PCOS) in PCOS patients. Of the studied androgens only DHEAS showed significant correlation to anthropometric indices – WSR, WC and WHR (($r=0.36$ ($P=0.012$); 0.32 ($P=0.027$) and 0.26 ($P=0.074$) respectively).
Conclusions
WSR and WC are stronger associated with composite cardiovascular risk factors as defined by AE PCOS consensus than WHR. WSR and WC have similar diagnostic value in detecting adverse metabolic profile in PCOS patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P901

**Health related behaviour and understanding of menopausal therapies among women with premature menopause**

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Premature menopause (PM) occurs spontaneously as premature ovarian failure (POF), or is medically induced (MIPM) by oophorectomy, chemotherapy or radiotherapy. PM is associated with increased health risks, including osteoporosis. This study explored understanding of menopausal therapies, medication use and health-related behaviour in women with and without PM.
Methods
Cross-sectional, questionnaire-based study involving 23 premenopausal, 25 POF and 29 MIPM women, aged 20–41 years.
Results
Mean age $\pm$ s.d. of each group was: control $=30\pm7.0$ years, POF $=35.8\pm4.3$ and MIPM $=37.4\pm3.2$ ($P<0.001$). Compared to premenopausal women (44%), more MIPM (79%), and POF women (56%) perceived hormone replacement therapy (HRT) was associated with increased breast cancer risk ($P=0.03$) and prevented fractures (56% POF, 40% MIPM, 13% controls, $P=0.008$). More controls (48%) reported not knowing risks/benefits of HRT compared to 16% POF and 11% MIPM groups ($P=0.006$). Most premenopausal women (86%) and MIPM women (75%) reported not knowing risks/benefits of bioidentical hormones compared to POF women (56%, $P=0.06$). All groups reported lack of knowledge regarding herbal therapies (67–74%, $P=0.9$).
More PM women (80% POF, 83% MIPM) reported currently taking prescription medication compared to 52% premenopausal women ($P=0.04$); most women overall reported taking non-prescription medication (61–72%, $P=0.7$). More PM women reported blood pressure checks (92% POF, 100% MIPM, 82% controls,

$P=0.03$), cholesterol tests (84% POF, 69% MIPM, 39% controls, $P=0.005$) and breast examinations (72% POF, 90% MIPM, 30% controls, $P<0.001$). Bone density tests were reported less frequently overall although more commonly in PM women (64% POF, 59% MIPM, 4% controls, $P<0.001$).
Conclusion
Differences in understanding of menopausal therapies and health-related behaviour exist between women with PM of differing aetiology and premenopausal women. While perceived understanding of HRT was greater than other therapies, targeted education is needed regarding specific risks/benefits of menopausal therapies. Health professional education, especially regarding the importance of bone density tests after PM, is also indicated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P902

**The effects of the therapy with ethinylestradiol 30 μg-drospirenone + metformin on endothelial dysfunction in the polycystic ovary syndrome**

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Cluj-Napoca, Romania.

Introduction
Recent data indicate that women affected by the polycystic ovary syndrome (PCOS) are at a greater risk for cardiovascular disease (CVD). The objective of this study was to evaluate the effect of the association ethinylestradiol 30 μg–drospirenone 3 mg (DRP/EE30 μg) plus metformin and weight loss on surrogate markers of CVD in PCOS.
Methods
Twenty-five young women with PCOS (mean age $22.76\pm0.83$ years, body mass index (BMI): $28.44\pm6.23$) completed this 6-month study. The oral contraceptive- DRP/EE30 μg (21 days/month) and metformin (1700 mg daily) were administered to the PCOS group. Additionally, the 15 overweight and obese patients (BMI $>25$ kg/m$^2$) were instructed in a diet of no more than 1500 cal daily. The main outcome measures were surrogate markers of cardiovascular disease and included endothelial function, i.e. flow-mediated dilatation (FMD) on the brachial artery and endothelin-1 levels, as well as body composition, insulin resistance and serum lipid profile. Variables were assessed at baseline, as well as after six cycles of treatment.
Results
The combination between DRP/EE30 μg plus metformin combined with weight loss triggered a significant improvement in the FMD values (FMD-PCOS basal $3.48\pm1.00$ vs FMD–PCOS 6 months$7.43\pm1.04$, $P=0.033$), as well as body composition and insulin insensitivity while negatively affecting lipid profile ($P<0.05$).
In conclusion, a 6-month course of metformin – DRP/EE30 μg (associated with weight loss) improves the endothelial dysfunction in PCOS. These results suggest that the medical therapy of PCOS should be reconsidered, especially for women with additional metabolic and cardiovascular risk factors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This work was supported, however funding details unavailable.

## P903

**Hormonal contraception and female sexual function**

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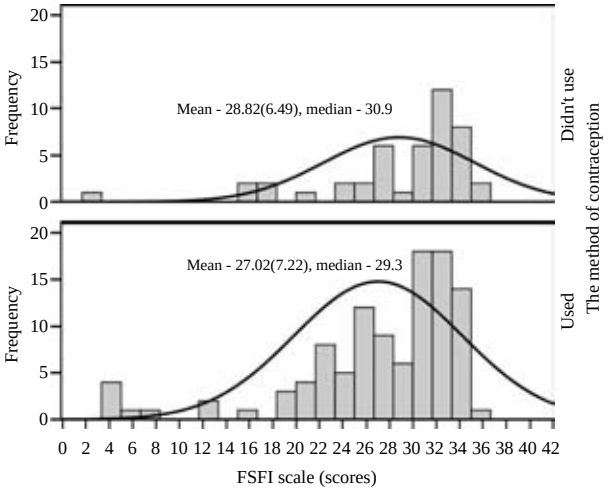
Summary
To establish the relations of women's sexual function with social factors and use of hormonal contraception.
Methods
There has been performed a pilot study. 20–43 years old participants with regular menstrual cycles (22–35 days) were randomly selected and have been interviewed. The test group consisted of 113 women who use any of hormonal contraception method. The control group consisted of 51 women not using hormonal contraception. Participants had to fill two questionnaires: socio-demographic and medical factors questionnaire prepared by the author and the female sexual function index (FSFI).
Results
The FSFI desire domain score of the women who used contraceptive agents comparing with women who did not use contraceptive agents was 3.95 (SN 1.08) vs 4.33 (SN 0.92; $P=0.006$), their FSFI arousal domain score was 4.19 (SN 1.48) vs 4.59 (SN 1.59; $P=0.005$), the FSFI lubrication domain score was 4.72 (SN 1.68) vs 5.32 (SN 1.2; $P=0.02$). The women's who had had permanent sexual partners comparing with women's who hadn't had permanent sexual partners mean of FSFI arousal domain score reached 4.4 (SN 1.45) vs 3.3 (SN 1.98; $P=0.01$), their mean of FSFI lubrication domain score reached 5.03 (SN 1.46) vs 3.55 (SN 2.17; $P=0.003$), the mean of FSFI pain domain score reached 4.88 (SN 1.61) vs 2.77 (SN 2.22; $P<0.001$). The means of FSFI arousal domain ($P=0.05$) and orgasm domain ($P=0.04$) scores were significantly different taking into consideration the BMI groups. The means of the women whose BMI $<18.5$ kg/m$^2$ were significantly higher than of those whose BMI 18.5–24.9 and $\geqq 30$ kg/m$^2$.
Conclusion
The presence of permanent sexual partners had a positive effect on the women's sexual function.

**Table 1** FSFI scores in participants according to investigative groups (dependent on having permanent sexual partner).

| Scale | Permanent sexual partner Had Mean (S.D.) | Permanent sexual partner Didn't have Mean (S.D.) | *P* value |
|---|---|---|---|
| Desire | 4.11 (1.04) | 3.6 (0.98) | 0.06 |
| Arousal | 4.4 (1.45) | 3.3 (1.59) | 0.01 |
| Lubrication | 5.03 (1.46) | 3.55 (3.17) | 0.003 |
| Orgasm | 4.4 (1.72) | 3.75 (2.27) | 0.3 |
| Satisfaction | 4.91 (1.29) | 4.36 (1.32) | 0.06 |
| Pain | 4.88 (1.61) | 2.77 (2.22) | <0.001 |
| FSFI total | 27.85 (6.92) | 23.77 (7.83) | 0.02 |




Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P904

**Metformin in treatment of polycystic ovaries syndrome in women of birthing age**

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Aim
To study the effectiveness of metformin in treatment of polycystic ovaries syndrome (PCOS) in women of birthing age.
Materials and methods
We followed 31 women with PCOS. Mean age of patients was 29.8 years old. All patients were evaluated for hormones levels (LH, FSH, TSH, estradiol, progesterone, testosterone, prolactin, coritsole, free thyroxine, dehydroepiahn-drostendione etc.), glucose tolerance test, lipids profile, uterus and ovaries ultrasonography, pituitary MRI etc.
All these evaluations were repeated in 6 months after metformin therapy. Medication was given 500 $mg^3$ times a day during meals for 6 months.
Results
Before treatment patients had significant changes both in hormonal and imaging evaluations. Hyperandrogenemia was revealed in 83.8%, hyperprolactinemia in 77.4%, elevated LH in (48.3%), hypoestrogenemia in 93.5% of patients. Of all patients, 23 females (74.1%) had impaired glucose tolerance test, whereas 16 women 51.6% had dyslipidemia. 22 patients (70.9%) had increased body mass and 13 women (41.9%) had arterial hypertension indicating that these patients with PCOS had metabolic syndrome.
In 6 months' time after therapy with metformin we observed reliable bilateral improvement of ovaries structure, including cysts regression, size normalization in all of patients (100%), while laboratory and hormonal measurements improved in 29 patients (93.5%), whereas body mass decreased in 20 women (90%) of 22.
Conclusion
(1) Metformin therapy significantly improves clinical and biochemical values in women with PCOS which confirmed by ultrasonography (2) it should be recommended to administer metformin therapy to women with PCOS in short breaks (3 months).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P905

**Letrozole induces metabolic and reproductive disorders in female rats: a dose response study**

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Continuous administration of 400 μg/day of letrozole (LET), a non-steroidal inhibitor of P450 aromatase, with start pre-pubertally to female rats, induces hyperandrogenemia and reproductive abnormalities similar to those observed in women with polycystic ovary syndrome (PCOS). However, these rats do not develop metabolic abnormalities, despite high circulating testosterone. A possible explanation may be that the dose was too high and thus completely inhibited the estrogen synthesis in insulin-sensitive tissues. Therefore, lower doses of LET may induce not only reproductive disturbances, but also metabolic abnormalities. Here, we present a dose-response study with continuous administration of three different doses of LET during 12 weeks and their effects on metabolic and reproductive variables. At 21 days of age, 61 female Wistar rats were divided into four groups: LET1: 83 μg/day; LET2: 100 μg/day; LET3: 200 μg/day or control: placebo. The body weight development and food intake (FI) were registered. Respiratory exchange ratio (RER) during 24-h was determined by indirect calorimetry and body composition by dual-emission X-ray absorptiometry. Blood samples were taken for analysis of sex steroids. Insulin sensitivity (IS) was evaluated by euglycemic-hyperinsulinemic clamp. All LET-groups were acyclic and gained more in body weight compared with control ($P<0.001$). Body fat and lean body mass in relation to body weight, FI and RER were comparable between groups. Testosterone concentration was higher in LET-groups after five ($P<0.001$) and 10 weeks ($P<0.001$) of LET-exposure, compared to control. The IS index was lower in all LET-groups compared to controls ($P<0.001$) and the mean adipose size in inguinal and mesenteric depot was higher in LET3 compared to controls ($P<0.01$). These results highlight the importance of the dose of LET for development of metabolic abnormalities. The LET doses applied in this study may be used to develop a rat PCOS model including both reproductive and metabolic abnormalities.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P906

**Increased prevalence of polycystic ovary syndrome in overweight and obese premenopausal women with nonalcoholic fatty liver disease**

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Background
Increased prevalence of abnormal aminotransferase levels and/or ultrasonographic evidence of hepatic steatosis have been found in women with polycystic ovary syndrome (PCOS), being more pronounced in overweight and obese patients. However, few data exist concerning the prevalence of PCOS in premenopausal women with nonalcoholic fatty liver disease (NAFLD).
Objective
To investigate the presence of PCOS in overweight and obese premenopausal women with NAFLD.
Patients/methods
Forty apparently healthy overweight and obese (BMI: 26.0–46.9 $kg/m^2$) premenopausal women (age: 18–45 years) with a history of none or minimal alcohol consumption, were evaluated prospectively for the presence of NAFLD with abdominal ultrasonography and biochemical testing, after excluding causes of secondary liver disease. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR). Hepatic steatosis was detected in 22/40 women, who were further studied for the presence of PCOS with hormonal evaluation and pelvic ultrasonography. Free androgen index (FAI) was calculated.
Results
PCOS was diagnosed in 10/22 patients with NAFLD (45.5%), according to the Androgen Excess Society criteria. Patients with NAFLD and PCOS compared to patients with NAFLD without PCOS were younger ($29.3\pm6.5$ vs $35.8\pm7.2$ years, $P=0.04$), more obese ($40.9\pm7.4$ vs $34.4\pm5.6$ $kg/m^2$, $P=0.03$), with an increased waist circumference ($108.7\pm10.4$ vs $95.3\pm15.9$ cm, $P=0.03$) and had higher levels of serum testosterone ($60.4\pm20.4$ vs $33.1\pm13.6$ ng/dl, $P<0.01$), lower SHBG levels ($17.6\pm6.0$ vs $30.5\pm6.3$ nmol/l, $P<0.001$) and higher FAI values ($12.7\pm5.2$ vs $3.8\pm1.4$, $P<0.001$).
Conclusions
These findings indicate an increased prevalence of PCOS in overweight and obese premenopausal women with NAFLD. Evaluation for PCOS should be considered in premenopausal NAFLD patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P907

**Association of the (TAAAA)*n* repeat polymorphism of *SHBG* gene with age at menopause in Greek postmenopausal women**

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Introduction
Sex hormone-binding globulin (SHBG) regulates the bioavailability of sex steroid hormones, which in turn regulate reproductive function. The potential association of SHBG gene polymorphisms with the age at menopause has not been examined.
Objective
The present study aimed to assess the possible relationship between the pentanucleotide (TAAAA)*n* repeat polymorphism in the promoter of the SHBG gene and the age at menopause in a Greek female population.

Methods
Two hundred and ten postmenopausal women aged 46–63 years were included in the study. The age (year and month) at the last menstrual period as well as anthropometric parameters were recorded in all participants. Genotyping of the (TAAAA)*n* repeat polymorphism was performed by PCR.
Results
Genotype analysis revealed (TAAAA)*n* pentanucleotide alleles of 6–11 repeats. Regarding the allele with seven TAAAA repeats, the age at menopause was higher in carriers of this allele (50.2 ± 3.1 years) than in non carriers (48.0 ± 4.8 years, $P = 0.026$). Furthermore, the age at menopause was lower in women carrying the allele with eight TAAAA repeats (47.5 ± 4.8 years) than in women not carrying this allele (48.8 ± 4.4 years, $P = 0.048$). The associations remained significant after statistical adjustment for body mass index (BMI) and smoking ($P = 0.019$ and $P = 0.043$ respectively). No association of the other alleles with the age at menopause was detected.
Conclusions
Our results indicate that the (TAAAA)*n* repeat polymorphism of SHBG gene is associated with the age at menopause in Greek postmenopausal women. Additional studies are required to explore the biological importance of this SHBG polymorphism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P908

**Prevalence of elevated glycated hemoglobin in women with polycystic ovary syndrome: according to the recently defined criteria**

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Background
Insulin resistance is a core pathophysiology of polycystic ovary syndrome (PCOS). The screening recommendations for type 2 diabetes in PCOS patients have varied according to organizations, but the AE-PCOS Society recommended that a OGTT be performed in obese patients or in lean patients with advanced age (>40 year), with a history of gestational diabetes, or with a family history of type 2 diabetes. Recently, the American Diabetes Association (ADA) newly includes hemoglobin A1c (A1C) as a component of diagnostic criteria of 'diabetes' (≥6.5%) or 'increased risk for diabetes' (5.7–6.4%). This study was performed to examine the prevalence and the risk factors for elevated A1C (≥5.7%) in women with PCOS compared to age matched control women.
Methods
A1C was evaluated in 154 patients and 469 controls.
Results
One-third (31.2%) of the PCOS patients had elevated A1C. The prevalence of elevated A1C was similar in obese PCOS and obese controls (23.5 and 20.0%, respectively, $P = 1.0$), but non-obese PCOS women (mean age 29.8 ± 5.4 years) had a higher prevalence of elevated A1C than non-obese controls (31.2 vs 6.6%, respectively, $P < 0.001$). The prevalence of elevated fasting plasma glucose was not different. The odds that a woman has an elevated A1C was 6.7 times higher if she has PCOS (adjusted OR 6.67, 95% CI 3.50–12.70).
Conclusions
Non-obese PCOS patients presented a significantly higher prevalence of elevated A1C than non-obese controls, whereas in obese subjects, the prevalence of elevated A1C was similarly elevated. Since substantial proportion of young and non-obese PCOS women are at an increased risk for diabetes, screening for type 2 diabetes may be necessary even in these young and non-obese PCOS patients. Future studies is mandatory to assess whether A1C is as effective as OGTT as a diagnostic tool in patients with PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P909

**Most of type 2 diabetes associated loci identified in genome-wide association studies are not associated with polycystic ovary syndrome**

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Objective
Insulin resistance is a core feature of polycystic ovary syndrome (PCOS), thus, it may be the common link between PCOS and type 2 diabetes (T2DM).
Although the etiology of PCOS has not been elucidated, a number of studies have suggested that genetic factors may play important roles in its etiology and pathogenesis. If PCOS and T2DM share a common genetic background, genetic variants determining the risk of T2DM may also be associated with PCOS. Recently, genome-wide association studies (GWAS) have reported a number of SNPs with reproducible associations and susceptibilities to T2DM, which were also replicated in Koreans. Thus, we compared the genotype distributions of the diabetogenic genes, identified in GWAS, between PCOS patients and age-matched controls in Korean women.
Measurements
Genotyping was performed on DNA samples from 377 PCOS patients and 386 age-matched controls.
Results
Genotype distributions for all investigated SNPs were in Hardy–Weinberg equilibrium in controls. None of the 12 SNPs in the six genes (KCNJ11, TCF7L2, SLC30A8, HHEX, FTO and CDKAL1), discovered in GWAS, were found to be associated with PCOS. For further analysis, PCOS patients were divided into two or three subgroups according to its genotype, and we also assessed the associations between the genotypes and insulin resistance or insulin secretary capacity. No SNPs were significantly associated with HOMA-IR, HOMA β-cell (%), or 75 g OGTT 2 h insulin levels in PCOS patients, neither other serum hormonal and metabolic markers such as androgen or glucose levels.
Conclusions
Our results suggest that the most of type 2 diabetes associated loci identified in GWAS are not associated with PCOS. Although our data do not demonstrate an association between diabetogenic SNPs discovered in GWAS and PCOS, the relationship between T2DM susceptibility gene and PCOS remains to be investigated because both diseases share a common link, insulin resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P910

**Optimization of a uterine leiomyoma xenograft model using different immunodeficient mice and grafting procedures**

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Introduction
Uterine leiomyoma (fibroid) is the most common solid tumor in reproductive-age women. Symptomatic leiomyomas affect millions of women through menorrhagia, abnormal uterine bleeding, and infertility. No effective treatment except surgery is available, partly because of the lack of effective animal models for screening new therapies. We established a new xenograft model by grafting primary cultured leiomyoma cells beneath the kidney capsule in super-immunodeficient mice; however, this model is not suitable for screening tests because of the expensive host and difficult grafting procedure. To resolve these issues, we investigated whether this model could be replicated using different types of immunodeficient mice and grafting procedures.
Methods
All specimens were obtained from patients who had undergone hysterectomy for symptomatic leiomyomas. We primarily cultured leiomyoma smooth muscle cells and mixed them with type 1 collagen gel to form cell pellets. We then grafted the pellets beneath the kidney capsule in non-obese diabetic /severe combined immunodeficient (NOD/SCID) mice, SCID mice, and BALB/c nude mice. Next we grafted the pellets beneath the kidney capsule and subcutaneous space in NOD/SCID and NOD/SCID IL2Rγ null (NOG) mice. All mice were ovariectomized at the time of grafting, and both 17β-estradiol and progesterone

were injected subcutaneously every week. Eight weeks later, we performed immunohistochemical evaluation of the xenografts with regard to their gross appearance, histology, and expression of estrogen and progesterone receptors.
Results
The xenografts beneath the kidney capsule were significantly enlarged in NOD/SCID mice compared with those in SCID or BALB/c nude mice. Furthermore, similar to subrenal xenografts, subcutaneous xenografts were enlarged in both NOD/SCID and NOG mice. Histologically, all enlarged xenografts within the mice mimicked human uterine leiomyomas and expressed both estrogen and progesterone receptors.
Conclusions
NOD/SCID mice are suitable hosts for the leiomyoma xenograft model. A subcutaneous xenograft is superior to a subrenal one. The easy grafting procedure can avoid the risk of intraoperative death of the host mice. The optimized xenograft model is suitable for screening new therapies that generally requires large number of subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P911

**The polymorphic variants K121Q of the plasma cell membrane glycoprotein and G972R of the insulin receptor substrate-1 modulate in opposite ways the clinical and biochemical profiles of women with polycystic ovary syndrome**

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Background
Insulin resistance (IR) is the common denominator of three multigenic disorders: type 2 diabetes mellitus, obesity, and PCOS. IR can result from genetic abnormalities involving proteins of the insulin signalling pathway, including PC-1 and IRS-1. The IRS-1 G972R variant had the highest world frequency and correlated with IR in PCOS Sicilian women (*Horm Metab Res*, 2010).
Objective and methods
To determine: i) the rates of the G972R IRS-1 and K121Q PC-1 polymorphisms in Sicilian PCOS women ($n=100$) and age-matched controls ($n=45$); ii) the association of the polymorphisms with the clinical/biochemical phenotypes of PCOS.
Results
The rate of the IRS-1 G972R polymorphism in PCOS and controls was 50 and 24.4% ($P=0.004$), while the rate of the PC-1 K121Q was 20 and 13.3% ($P=0.33$). Carriers were always heterozygous (Het), except four PCOS women (two IRS-1 homozygous (Hom), and another two PC-1 Hom). Distribution of genotypes (wild type (WT) or Het-Hom) in PCOS vs controls was: IRS-1/PC-1 WT/WT (44 vs 68.9%, $P=0.0055$), IRS-1/PC-1 Het-Hom/Het-Hom (14 vs 6.7%, $P=0.20$), IRS-1 Het-Hom/PC-1 WT (36 vs 17.8%, $P=0.027$) and IRS-1 WT /PC-1 Het-Hom (6 vs 6.7%, $P=0.88$). The IRS-1 Het-Hom/PC-1 WT PCOS women had: i) the worst metabolic profile (fasting insulin, HOMA-IR and triglycerides, significant vs IRS-1 WT/PC-1 Het-Hom and IRS-1/PC-1 WT/WT; lower Matsuda index, significant vs IRS-1/PC-1 WT/WT); ii) the worst hormone profile (higher total testosterone and lower estradiol, significant vs IRS-1/PC-1 WT/WT; lower FSH, significant vs IRS-1 WT/PC-1 Het-Hom and IRS-1/ PC-1 Het-Hom/Het-Hom); iii) the youngest age and the lowest hirsutism index (both significant vs IRS-1/PC-1 WT/WT).
Conclusion
Only the G972R IRS-1 polymorphism is significantly more frequent in PCOS women. When this variant is not associated with the K121Q PC-1 variant, the metabolic profile and testosteronemia are worse and age at presentation is anticipated. However, the modest hirsutism suggests relative androgen resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P912

**Exposure to soy isoflavones during postnatal life disrupts structural development of reproductive organs in female mice**

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Exposure to soy isoflavones (ISO), abundant in soy protein infant formula, during early postnatal life has benefits to bone health in female mice. Moreover, exposure during the first 10 or 21 days of life has been shown to interfere with structural development of female reproductive organs and results in heavier body weight. The study objective was to compare if shorter exposure to ISO, during the first 5 vs 10 days of life, results in lesser adverse effects on ovarian and uterine structure in adult mice. At birth, female pups (8–12 pups/l) were cross-fostered and litters were randomized to corn oil or ISO (7 mg/kg of body wt/day) treatment for the first 5 or 10 days of life. The 5-day protocol was selected to mimic previously published studies investigating the effects of environmental estrogens (i.e. diethylstilbestrol) on reproductive and skeletal development. Body and organ weights, and histology of ovaries and uterus were analyzed. There were no differences in the ovary or uterus weight, number of ovarian follicles, number of multiple oocyte follicle or percent of ovarian cysts with 5 or 10 day ISO intervention compared to respective controls. Ten day ISO group had higher body weight from 6 days to 4 months of age and higher percent of ovarian hyperplasia than their respective control. Lower number of ovarian corpus luteum and a higher incidence of abnormal changes were reported in the uteri of both ISO groups compared to their respective control. In conclusion, 5 and 10-day exposure to ISO had similar long-lasting adverse effects on the structure of ovaries and uterus in adult mice, identifying the first five days of life as a susceptible window for programming reproductive development. Only the 10-day ISO exposure resulted in greater body weight gain at adulthood.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P913

**The kisspeptin levels in girls**

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The kisspeptin, the peptide product of *KiSS-1* gene has recently emerged as essential gatekeeper of pubertal activation of gonadotropin-releasing hormone neurons and the reproductive axis. Mutations or targeted disruptions in the gene cause hypogonadism or premature sexual development and sterility.
Purpose
The study was undertaken to research the kisspeptin levels in healthy girls and girls with endocrine and gynaecological disorders.
Patients and methods
Serum kisspeptin levels were determined in and 69 girls (aged 7 months and 8 years) healthy, adolescent girls with menstrual disorders, girls with premature isolated thelarche, adolescent girls with dyshormonal mammary dysplasia and adolescent girls with polycystic ovary syndrome. The concentration of kisspeptin was measured using Kisspeptin-10 (metastin) and competitive enzyme immunoassay. The SPSS 16.0 Software package was used to perform statistical analyses. Results were analyzed using two-way ANOVA. Data are expressed as median, $P$ value of $<0.05$ were considered statistically significant.
Results
The investigation shows that healthy adolescent girls had kisspeptin median levels 0.03 ng/ml. Among the girls with secondary amenorrhea and opsomenorrhea kisspeptin levels was lower 0.01 ng/ml ($P=0.014$). Girls with premature isolated thelarche had kisspeptin levels 0.04 ng/ml ($P=0.04$); girls with mastopathy (dyshormonal dysplasia) had kisspeptin levels 0.05 ng/ml ($P=0.033$). Serum kisspeptin levels were significantly higher in adolescent girls with polycystic ovary syndrome than in control group (0.3 vs 0.03 ng/ml, $P<0.01$).
Conclusion
Kisspeptin levels is various for healthy adolescent girls, for girls with menstrual disorders, mastopathy, isolated thelarche, with polycystic ovary syndrome. In this study, we demonstrated that serum kisspeptin level was significantly lower in girls with amenorrhea and opsomenorrhea and higher in girls with premature thelarche, mastopathy and polycystic ovary syndrome. Serum kisspeptin may be used as a marker of sexual disorders, mammary diseases and polycystic ovary syndrome in girls.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P914

**Subcutaneous adipose tissue in women with polycystic ovary syndrome**

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The present study was performed to search whether SCAT increase in women with PCOS. Fifty-two women with PCOS patients and 53 matched healthy controls were participated in the study. Caliper device was used to measure skinfold thickness (SFT). Body fat distributions were determined by bioelectrical impedance analysis. SFT in all defined areas, total body and trunk fat free mass (FFM), and HOMA score were higher in women with PCOS, while adiponectin was significantly lower. SFT values correlated positively with HOMA score, and negatively with adiponectin. SFT in triceps and supscapular areas, trunk fat mass, trunk fat ratio, trunk fat mass and trunk fat free mass values were the most powerful predictors of HOMA score. In conclusion SCAT and FFM are increased in women with PCOS, with relation to insulin resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Clinical, endocrine and anthropometric measures of patients with PCOS and controls.

| Variable | PCOS ($n=52$) | Control ($n=53$) | P |
|---|---|---|---|
| Age (year) | 21.1±3.8 | 21.5±3.6 | 0.112 |
| BMI (kg/m$^2$) | 24.3±5.3 | 22.2±2.3 | 0.430 |
| WHR | 0.7±0.1 | 0.7±0.04 | 0.264 |
| Glucose (mg/dl) | 87.4±8.4 | 81.0±5.9 | 0.0001 |
| Total-C (mg/dl) | 159.2±37.0 | 166.0±35.9 | 0.175 |
| LDL-C (mg/dl) | 89.9±27.2 | 98.2±28.6 | 0.140 |
| HDL-C (mg/dl) | 49.4±10.8 | 55.2±11.0 | 0.02 |
| Triglyceride (mg/dl) | 99.7±59.1 | 62.7±19.8 | 0.0001 |
| LH/FSH | 1.5±1.0 | 0.81±0.5 | 0.0001 |
| Testosterone (ng/dl) | 66.1±27.7 | 41.5±13.2 | 0.0001 |
| HOMA-IR | 2.3±1.2 | 1.2±0.5 | 0.0001 |
| Adiponectin (μg/ml) | 9.1±3.8 | 12.9±6.7 | 0.008 |
| Total body fat free mass (kg) | 45.3±5.6 | 42.9±2.7 | 0.005 |
| Total body fat mass (kg) | 19.4±8.9 | 16.6±4.8 | 0.610 |
| Total body fat ratio (%) | 28.6±7.5 | 27.4±5.1 | 0.341 |
| Trunk fat ratio (%) | 24.6±8.7 | 24.1±6.1 | 0.711 |
| Trunk fat mass (kg) | 9.0±4.8 | 7.9±2.9 | 0.610 |
| Trunk fat free mass (kg) | 25.6±3.2 | 24.2±1.5 | 0.003 |
| MUAC (cm) | 27.9±4.5 | 25.7±2.6 | 0.015 |
| Biceps SFT (mm) | 10.5±4.8 | 7.7±2.5 | 0.0001 |
| Triceps SFT (mm) | 16.5±5.6 | 13.7±4.3 | 0.0001 |
| Supscapular SFT (mm) | 15.9±6.5 | 13.0±3.6 | 0.032 |
| Suprailiac SFT (mm) | 17.4±6.7 | 13.7±4.4 | 0.003 |

BMI, body mass index; WHR, waist-hip ratio; HDL, high density lipoprotein; LDL, low density lipoprotein; C, Cholesterol; LH, Luteinizing hormone; FSH, Follicle stimulating hormone; DHEA-S, Dehydroepiandrosterone sulphate; HOMA, homeostasis model assessment; MUAC, mid-upper arm circumference; SFT, skin-fold thickness.

## P915

**Plasma anti-Müllerian hormone concentration in women with polycystic ovary syndrome and type 1 diabetes mellitus**

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Introduction
A high prevalence of polycystic ovary syndrome (PCOS) and hyperandrogenism has been described in adult women with type 1 diabetes mellitus (T1DM). Anti-Müllerian hormone (AMH) is secreted by granulosa cells of antral ovarian follicles and its plasma concentration is correlated with the number of small antral follicles. AMH levels are increased in women with PCOS and higher plasma concentration has been observed in prepubertal girls with T1DM. The aim of this study was to estimate plasma AMH concentrations in women with PCOS with and without T1DM and its relation to hormonal profile.
Study participants and methods
We studied seven women with PCOS and T1DM (T1DM + PCOS), seven women with PCOS without T1DM (PCOS), 10 women with T1DM without PCOS (T1DM), and eight healthy women (control). The clinical examination, estimation of plasma AMH, sex hormones and ultrasonographic evaluation were performed in all study participants.
Results
Plasma AMH concentration was markedly higher in PCOS group vs controls ($P=0.041$) and was not different between T1DM + PCOS and control group, and both PCOS group (PCOS vs T1DM + PCOS). Plasma SHBG was higher in T1DM + PCOS in comparison to PCOS ($P=0.024$). Ovarian volume and follicles number were higher in PCOS and T1DM + PCOS vs controls ($P=0.013$, $P=0.045$ and $P=0.029$, $P=0.011$ respectively). T1DM + PCOS had increased plasma AMH concentration ($P=0.011$), higher ovarian volume and follicles number vs diabetic women without PCOS. In the entire studied group plasma AMH correlated significantly with FSH ($r=-0.385$, $P=0.03$), testosterone ($r=0.434$, $P=0.013$) and number of ovarian follicles ($r=0.705$, $P<0.0001$).
Conclusion
Obtained results suggest that AMH might be involved in pathogenesis of PCOS in type 1 diabetic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P916

**The role of subclinical hypothyroidism and vitamin D deficiency in the development of menstrual disorders in women with PCOS**

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Subclinical hypothyroidism (SH) may affect the gonadotropic axis at different levels, causing changes in the hypothalamic–pituitary unit, functions of the gonads, and changes on peripheral metabolism of sex hormones. Polycistic ovary sndrome (PCOS) is a complex endocrine syndrome characterized by the combination of reproductive aberrations, namely hyperandrogenism and chronic anovulation, with metabolic defects.
The aim
To determine the role of SH and vitamin D deficiency (VDD) in the development of menstrual disorders in women with PCOS.
Materials and methods
The study included 100 women with PCOS (diagnosed by respecting the Rotterdam criteria). Patients to be included in the study required not to receive any medication within the last 6 months. Fasting serum glucose, basal insulin, insulin during OGTT, HOMA-IR index, TSH, $FT_3$, $FT_4$, thyroid autoantibodies (anti-Tg, anti-TPO), FSH, LH, PRL, total testosterone, DHEA-S, androstendione and free testosterone were determined.
Results
The mean age of subjects was 30.91±9.21 years and mean BMI was 29.24±3.85 kg/m$^2$. The mean TSH was 5.89±2.01 mIU/l. Approximately 61% of PCOS had SH. 26% have elevated anti-Tg and 45% had elevated anti-TPO. Patients with SH had in the same time and VDD (<20 nmol/l). PCOS were divided in two groups: PCOS with SH/VDD and PCOS without SH/VDD. Most patients were with normal values of FSH, $E_2$, progesterone and PRL. The level of testosterone (3.08±0.95 vs 2.03±0.64 nmol/l), androstendione (3.94±1.03 vs 2.56±0.87 ng/ml), DHEA-S and LH was higher in PCOS group with SH/VDD. HOMA-IR index was increased in 26% PCOS with SH. Disruption of menstrual cycle, usually oligomenorrhea type, was present in 36% of subjects. SH was significantly correlated with disorders of the menstrual cycle in PCOS women ($r=0.76$). VDD and SH were significantly correlated with PCOS, estimated based on the LH/FSH ratio ($r=0.76$).
Conclusion
SH and VDD significantly correlated with disorders of the menstrual cycle in PCOS women, thereby confirming their role in the occurrence of menstrual disorders in women with PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P917

**Treatment of androgen excess in adolescent girls: ethinylestradiol–cyproteroneacetate vs low-dose pioglitazone–flutamide–metformin**

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Objective
To compare the effects of a classic therapy to those of a novel treatment for androgen excess in adolescent girls.
Study design
Randomized, open-labeled trial over 12 months.
Study participants
Adolescents ($n=34$; mean age 16 year, BMI 23 kg/m$^2$) with hyperinsulinemic androgen excess and without risk of pregnancy.
Interventions
Ethinyl estradiol-cyproterone acetate (EE-CA; Diane 35 Diario) vs a low-dose combination of pioglitazone 7.5 mg/day, flutamide 62.5 mg/day, and metformin 850 mg/day (PioFluMet) for 12 months.
Main outcome measures
Hirsutism and acne scores; androgen excess; circulating C-reactive protein and high-molecular-weight adiponectin; carotid intima-media thickness; body composition (by absorptiometry); abdominal fat partitioning (by MRI); gene expression in consecutive biopsies of subcutaneous adipose tissue at the abdominal level.
Results
EE-CA and PioFluMet reduced the clinical and biochemical androgen excess comparably but had divergent effects on C-reactive protein and high-molecular-weight adiponectin; on carotid intima-media thickness; on lean mass; on abdominal and visceral fat; and on the expression of genes, for example, those related to macrophage activation and lipid storage in adipose tissue. All these divergences pointed to a healthier condition on low-dose PioFluMet.
Conclusion
Over 12 months, PioFluMet compared favorably to EE-CA in adolescents with androgen excess and without pregnancy risk. Further study of low-dose PioFluMet seems warranted, not only to strengthen its safety side over the longer term and in larger cohorts, but also to verify further whether the efficacy of this low-dose combination holds enough potential to allow it to become a first-choice therapy for the majority of young adolescents with androgen excess, namely those who are not at pregnancy risk and who are nowadays nevertheless exposed to supraphysiological doses of estro-progestagens.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P918

**Hyperandrogenism is related to adipose tissue dysfunction as reflected by VAI in polycystic ovary syndrome patients**

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A large body of evidence suggests that in polycystic ovary syndrome (PCOS) there is a link between abdominal adiposity, visceral adiposity dysfunction and hyperandrogenism and this relationship could be bidirectional. However a well designed study of adipose tissue distribution reported no significant differences between PCOS and controls. Visceral adiposity index (VAI) is a recently reported marker of the cardiovascular risk, strongly correlated with visceral adipose tissue (VAT) dimension and it was suggested to be a measure of VAT dysfunction.
We hypothesised that there is an association between PCOS and VAT dysfunction as reflected by VAI and aimed to study the relationship between VAT dysfunction and hyperandrogenism in PCOS patients.
We studied 256 PCOS patients (mean age 24.7$\pm$5.34 years, mean body mass index (BMI) 28.76$\pm$7.68 kg/m$^2$) and 102 controls (mean age 28.27$\pm$7 years, mean BMI 30.1$\pm$8.53 kg/m$^2$) selected from our PCOS research database. VAI was calculated based on waist circumference (WC), BMI, triglycerides and HDL-cholesterol values.
We found that PCOS patients were younger ($P<0.001$) and had higher waist-hip ratio (WHR; $P<0.05$) compared to controls, but the two groups were similar in terms of BMI and WC. In PCOS group VAI value was significantly higher compared to controls ($P<0.01$) and the association was statistically significant ($P<0.01$) even after adjustment for age and WHR in logistic regression analysis. In PCOS group multivariate analysis showed that free androgen index (FAI) was independently associated with VAI after adjustment for BMI, WC and WHR.
Conclusions
PCOS is associated with higher VAI values, suggesting the presence of visceral adiposity dysfunction in these patients that seems to be related to hyperandrogenism independent of anthropometrical measures of adiposity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P919

**Effects of berberine on the clinical, metabolic and reproductive features of obese polycystic ovary syndrome women**

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Objective
Polycystic ovary syndrome (PCOS) is a multifaceted disease characterized by metabolic and reproductive disorders associated with hyperandrogenism and insulin resistance. Berberine (BBR) is an isoquinoline derivative alkaloid extracted from chinese medicinal herbs that has been used as an insulin sensitizer.BBR could represent a potential therapeutic tool for PCOS.
The aim of this study was to evaluate the effects of BBR on the clinical, metabolic and reproductive features of PCOS women compared to healthy control women.
Design and methods
Fifty PCOS obese women and fifty healthy obese women (control group) were enrolled in Salerno Hospital University outpatient of Endocrinology of Fertile Age and Monza 'San Gerardo' Hospital outpatient of Diabetic Clinic. PCOS women were undergone to BBR treatment (Berberol, 500 mg berberine hydrochloride and 105 mg Silymarin, Pharmaextracta, Italy) that was administered twice for 3 months. Obese healthy women were encouraged to lifestyle changes by: a restriction intake (hypocaloric diet of 1200 Kcal/day) and aerobic physical exercise (running or bicycle at least 5 h/week).
Clinical, (anthropometric and hormonal) metabolic and reproductive parameters were assessed before and after the period of treatment in both groups of obese PCOS and healthy women.
Results
Treatment with BBR showed decreased waist circumference (WC) and waist-to-hip ratio (WHR; $P<0.05$), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-c; $P<0.05$) as well as increased high-density lipoprotein cholesterol (HDL-c) and sex hormone binding globuline (SHBG; $P<0.05$) in comparison to healthy women. Similarly, BBR treatment showed a reduction in several metabolic parameters, like: fasting plasma glucose, fasting insulin, HOMA-IR and AUCINS ($P<0.01$) and also ovulation rate improved in PCOS group compared to healthy women ($P<0.01$).
Conclusions
BBR improved several clinical, metabolic and reproductive features in obese PCOS women and main effects could be related to the improvement of insulin sensitivity and reduction of hyperandrogenemia. BBR also seemed to have a greater effect on the changes in body composition and dyslipidemia. However, the underlying mechanisms of its actions remain to be clarified in wider and longer term clinical trials.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P920

**Large-for-gestational-age and macrosomic neonates born by women with gestational diabetes mellitus diagnosed by the new IADPSG criteria: a case–control study of 301 patients and 839 controls**

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Introduction
Gestational diabetes mellitus (GDM) has been associated with adverse maternal and fetal/neonatal outcomes. The aim of this case-control study was to compare women whose pregnancy was complicated with GDM, diagnosed by the new International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, with a control group of healthy pregnant women as far as large-for-gestational-age (LGA) neonates and macrosomia are concerned.
Methods
The study included 301 pregnant women with singleton pregnancies complicated by GDM and 839 controls. Women were followed-up every two (GDM group) or four weeks (control group). Fetal ultrasonography was performed every 4 weeks. The main metabolic parameters recorded in every visit were body mass index (BMI), fasting plasma glucose (FPG), home blood glucose measurements (HBGM) and glycozylated hemoglobin A1c (HbA1c). The main ultrasonographic parameters were estimated fetal weight (EFW), head (HC) and abdominal circumference (AC). Decisions on treatment modification in the GDM group were based on both metabolic and ultrasonographic parameters. Main study outcomes were cumulative incidence of LGA neonates and macrosomia.
Results
The cumulative incidence of LGA was 5.3% in GDM and 4.8% in control group ($\chi^2$, $P=0.755$). The cumulative incidence of macrosomia was 4.3% in GDM and 4.1% in control group ($\chi^2$, $P=0.866$). There were no significant differences in EFW, HC or AC between GDM and control groups in serial fetal ultrasounds from gestational week 16–37.
Conclusions
Women with GDM, diagnosed by the new IADPSG criteria and followed-up by both metabolic and ultrasonographic parameters, had cumulative incidence of LGA and macrosomia similar to that of women whose pregnancy was not complicated by GDM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P921

**Factors affecting time to pregnancy: lithuanian women cohort study**

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Numerous studies provided data on possible decline in human fertility over the last decades and geografic differences in it. So, recently research interest in these tendencies and possible factors affecting fertility is increased.
Objective
In this study, we aimed to explore women's fertility expressed as time to pregnancy (TTP) – the number of contraceptive-free cycles needed to conceive – in a large cohort of Lithuanian women and the effect of biological and social factors along with sexual behaviour on TTP.
Methods
Data were gathered from 1813 women who delivered babies during the 16 months-period at one hospital. The information was collected retrospectively by using self-administered questionnaires. Odds ratios were determined for many factors. Multivariate models were used to determine which factors had the most impact on TTP.
Results
The mean TTP was 3.88 months (S.D. = 9.0). Advanced age at conception was associated with an increase in TTP, especially in the age group between 30 and 35 ($P<0.001$). Women who had achieved higher levels of education had longer TTPs ($P=0.049$), as did women who had irregular menstrual cycles ($P<0.001$) and those who did not use contraception prior to conceiving ($P=0.01$). Financial and marital status did not affect fecundity, nor did contraceptive method.
Conclusions
Women's age and irregular menstrual cycle were the most important factors predicting TTP of 12 and more months. Surprisingly contraception use before conception negatively influenced TTP either.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P922

**Insulin and androgens but not androgen receptor CAGn polymorphysm are determinant factors for hirsutism severity in PCOS**

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Background
Hirsutism is a major feature of the polycystic ovary syndrome (PCOS) and the only accepted clinical sign of hyperandrogenism for its diagnosis, according to the androgen excess society (AES) recommendations. Previous studies demonstrated that hirsutism can be influenced by factors other than hyperandrogenism *per se*.
Aim
To detect factors influencing hirsutism severity in PCOS.
Patients and methods
Hundred and seventy-five PCOS patients (AES criteria), of Romanian origin. Hirsutism was defined as a modified Ferriman–Gallwey (mFG) score of $\geq 8$. Other clinical and laboratory parameters were assessed, including total testosterone (TT), free androgen index (FAI), DHEA-S, 17-hydroxyprogesterone (17OH-P), fasting glycemia, insulinemia and calculated homeostatic assessment for insulin resistance (HOMA-IR). Androgen receptor (AR) CAG repeat genotyping and X-chromosome inactivation analysis were performed in a subset of 94 PCOS.
Results
Univariate correlations revealed that mFG score was significantly associated with fasting insulin ($r^2=0.11$, $P<0.01$) and HOMA-IR ($P<0.01$), after adjustment for BMI. A multiple regression model using androgens (TT and DHEA-S) as independent variables and mFG score as dependant was significant ($r^2=0.11$, $P<0.01$), but mFG univariate associations with FAI, DHEA-S were non-significant. AR CAG repeat number biallelic means and X-weighted biallelic means did not differ significantly between hirsute and non-hirsute PCOS and were not correlated with mFG scores.
In a multivariate regression mFG model including fasting insulinemia, TT, DHEA-S, BMI and age as independent variables, only insulin was associated significantly with mFG score, while the whole model did not reach significance ($r^2=0.21$, $P=0.068$).
Conclusions
Our results show that both hyperinsulinism and hyperandrogenism are determinants of the degree of hirsutism in PCOS, but insulinemia appears as a better marker of hirsutism than serum androgens. Androgen-sensitivity, evaluated by the AR CAG repeat genotypes is not associated with mFG score.
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## P923

**Frequency of insulin resistance, assessed by the glucose clamp technique, in 132 PCOS women**

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Introduction
Insulin resistance is a common finding in women with polycystic ovary syndrome (PCOS) and is considered a major aspect in this condition, as it likely plays a role

in the pathogenesis of both hyperandrogenism and several metabolic abnormalities, frequently found in these women. Despite its significance, prevalence of insulin resistance in PCOS subjects is still unclear, as it was assessed in small samples and/or by using surrogate parameters of insulin action. Moreover, it remains also unclear whether this is a feature of lean PCOS subjects.
Methods
To address this issue, 132 consecutive PCOS women were submitted to a careful metabolic phenotyping, including a hyperinsulinemic euglycemic clamp, the gold standard for measurement of *in vivo* insulin sensitivity. Insulin infusion rate in the glucose clamp was 80 mU/m$^2$ per min, to completely suppress endogenous glucose production.
Results
Mean (S.D.) age of PCOS women was 24 (6) year, and BMI 29.6 (8.0) kg/m$^2$. Thirty-four percent of these subjects were lean, 24% overweight and 42% obese. Mean fasting plasma glucose was 87 (10) mg/dl and insulin 17 (12) mU/l. As compared to reference values of insulin-induced glucose utilization during the clamp, 84% of these subjects were insulin resistant. In particular, 61% of PCOS women with a normal weight, 86% of those overweight and 100% of the obese subjects showed an impaired insulin action. The metabolic syndrome, diagnosed according to the ATP-III criteria, was found in 5% of lean, 26% of overweight and 62% of obese patients. Among the specific metabolic abnormalities, the most common was reduction of HDL-cholesterol, found in 56% of subjects. Fifteen percent of these women had abnormal glucose levels, at fasting and/or after oral glucose.
Conclusions
Our findings show that insulin resistance is a very common finding in PCOS women, even in lean subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P924

**Propranolol prevents stress induced polycystic ovary syndrome**
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We have shown that repeated cold stress increases ovarian sympathetic tonus and induces typical polycystic ovary syndrome (PCOS) features. Lesion of the noradrenergic nucleus Locus Coeruleus decreases ovarian noradrenaline release and prevents stress-induced PCOS in rats exposed to chronic intermittent cold stress. The aim of this study was to assess whether the alterations on ovarian morphology, ovulation rate and hormonal secretion induced by cold stress could be prevented by the beta-adrenergic blocker propranolol. Female rats were exposed to repeated sessions of cold stress (4°C/3 h per day from monday to friday), during 4 weeks. The rats had free access to water (S groups) or water with propranolol (4 mg/kg; SP group). Then after, the animals were kept at room temperature for four additional weeks with free access to water. Unstressed animals were maintained at room temperature for 8 weeks with free access to water (US group) or water + propranolol in the first 4 weeks (US + P group). Rats were decapitated, trunk blood was collected for hormone measurements, the oviducts removed to assess the number of oocytes, and ovaries for histological analysis. In S group there was a decreased number of healthy preantral and antral follicles, an increased number of atretic preantral and antral follicles, pre-cystic and cystic follicles and follicles with hyperthecosis compared to US rats. The number of oocytes was reduced and estradiol and DHEA plasma levels were higher than in unstressed rats. Propranolol treatment *per se* did not alter the parameters evaluated in the US + P group but prevented the majority of alterations in the SP group. The number of all disarranged structures, oocytes and DHEA levels in propranolol treated rats were similar to the US rats. Therefore, blocking ovarian beta-adrenergic receptor was shown to be a good tool to prevent the onset of PCOS induced by sympathetic hyperactivation.
Declaration of interest
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## P925

**Variants of the *NR5A1* gene in a large cohort of patients with primary ovarian insufficiency**
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Premature ovarian insufficiency (POI) is a disorder which affects ~1% of women under 40 years of age. Genetic component has been suggested in the majority of cases of nonsyndromic forms, and recently mutations of *NR5A1* have been reported to be associated with POI. In order to evaluate the frequency of NR5A1 mutations in POI together with the functional characterisation of the existing variants, we conducted a genetic study on a large cohort of POI patients.
Patients and method
Hundred and eighty eight patients diagnosed with idiopathic POI and 82 control subjects from the general population were screened for NR5A1 mutations and *in vitro* functional analysis was performed for the identified variants. HEK293T cells were transiently transfected with either wild type (WT) or mutant NR5A1-containing plasmid. The DNA binding capacity of the variants was evaluated by electrophoretic mobility shift assay (EMSA), while the transcriptional activity was assessed by luciferase assay.
Results
Sequencing NR5A1 gene revealed the presence of four missense variants in three patients (including two missense variants previously described in a patient with POI), nine intronic changes in thirteen patients and six polymorphisms. The three patients with missense variants were 20–33 years old and presented secondary amenorrhea. There was no family history of reproductive disorders, adrenal failure or DSD. Ovarian ultrasonography showed the presence of follicles in two of the three patients. Functional analysis was carried out for three missense variants not present in the control group. EMSA showed no difference in the DNA binding between the studied variants and the WT-NR5A1. Furthermore, no significant differences in the transcriptional activity of the respective variants compared to the WT were found when the luciferase assays was performed.
Conclusions
The results of our study conducted in a large cohort of patients show that NR5A1is not a major cause of idiopathic POI. We suggest that genetic investigation of NR5A1 in POI might be restricted to familial cases.
Declaration of interest
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## P926

**Aberrant expression of TRAIL in placenta and maternal serum in early pregancy complications**
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Recurrent miscarriage (RM) is defined as $\geq 3$ consecutive pregnancy losses and occurs in 1–3% of fertile couples. No specific early biomarkers with high predictive value of threatening miscarriage have been identified. Using GeneChips (Affymetrix), we profiled whole-genome differential gene expression in RM placental tissue compared to uncomplicated pregnancies matched for gestational age. In cases of RM, significantly increased placental mRNA expression of TNF-related apoptosis-inducing ligand (TRAIL; $P<0.005$) and S100A8 ($P<0.001$) encoding for inflammatory marker calprotectin (S100A8/A9) was confirmed by Taqman RT-qPCR. Using ELISA, the concentrations of soluble TRAIL and calprotectin in maternal serum in normal first trimester ($n=35$) and failed pregnancies (early, $n=18$ and late miscarriage, $n=4$; tubal pregnancy, $n=11$) were determined. When compared to normal first trimester pregnancy, significantly higher maternal serum concentration of sTRAIL was detected at the RM event ($P<0.001$), in women with tubal pregnancy ($P<0.05$), and in pregnant women, who developed an unpredicted miscarriage 2–50 days after prospective serum sampling ($P<0.05$). Maternal serum levels of calprotectin were neither diagnostic nor prognostic to early pregnancy failures ($P>0.05$). sTRAIL may have a potential as a predictive biomarker in maternal serum for early pregnancy complications.

Declaration of interest
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## P927

**Blood cell mitochondrial DNA content and premature ovarian aging**

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Primary ovarian insufficiency (POI) is a critical fertility defect characterized by a progressive and silent impairment of the follicular reserve. POI aetiology is heterogeneous and largely unknown, but a maternal inheritance often characterizes idiopathic forms. Therefore, we hypothesized a possible involvement of a mitochondrial defect in the pathogenesis of this disease since mitochondrial biogenesis and bioenergetics play an essential role in ovarian folliculogenesis. Our aim was to verify whether the content of mitochondrial DNA (mtDNA) was significantly reduced in the peripheral blood cells of POI women. We recruited 101 women with an impaired ovarian reserve: 59 women with premature ovarian failure (POF) and 42 poor responders (PR) to ovarian hyperstimulation. A Taqman copy number assay revealed a significant mtDNA depletion ($P<0.001$) in both trial groups in comparison with two control groups: 43 women of similar age and with intact ovarian reserve (normal responders to ovarian hyperstimulation, NR) and 53 very old women with a previous physiological menopause (CPM). No variations in a mitochondrial DNA polymerase gene (POLG) were detected in POF women with mtDNA depletion. Due to the ethical impossibility to test the direct correlation of the mtDNA content between the oocyte obtained by assisted reproductive techniques and the blood cell from the same individual, we demonstrated that the mtDNA content in the peripheral blood cells is representative of the one in the granulosa cells. Our results indicate that blood cell mtDNA depletion is a frequent finding among women with premature ovarian aging, suggesting that blood cell mtDNA determination could become an useful tool for POI risk prediction.
Declaration of interest
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## P928

**Fetal growth parameters and outcome of pregnancies complicated with thyroid disease**

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Introduction
Thyroid dysfunction and thyroid autoimmunity (TAI) have been associated with adverse pregnancy outcomes for both the mother and the fetus. The aim of this study was to describe fetal growth parameters of pregnancies complicated with thyroid diseases/TAI and to construct predictive models for their outcome.
Methods
The study included 196 consecutive singleton pregnancies in women with thyroid diseases, during the period 2007–2010. A cohort of 809 singleton pregnancies in euthyroid women without TAI was used as fetal growth control. The main metabolic parameters evaluated were: thyroid hormones (TSH, $fT_4$), thyroid autoantibodies (TPOAb, TgAb, TSI) and doses of levothyroxine ($LT_4$) and antithyroid medications for women with hypothryroidism and hyperthyroidism respectively. The main fetal growth parameters evaluated were: estimated fetal weight (EFW), head circumference (HC) and abdominal circumference (AC). Predictive models were constructed for pregnancies complicated with hypothyroidism.
Results
In the study population, 16% of women were euthyroid, having either TAI or nodular thyroid disease, 19% hyperthyroid and 65% hypothyroid. Of the studied pregnancies, 88% resulted in live birth, 10% in spontaneous abortion, 1% in iatrogenic abortion and 1% in intrauterine death. Maternal complications included pregnancy-induced hypertension, pre-eclampsia, hyperemesis gravidarum, placental abruption and poly-hydramnion/oligoamnion. Neonatal complications included jaundice, tachypnoea, respiratory distress syndrome and hypothyroidism. Fetuses of mothers with hypothyroidism had increased AC in comparison to fetuses of normal pregnancies but did not differ with respect to EFW or HC. In pregnancies complicated by hypothyroidism, thyroid auto-antibodies or serum TSH did not predict neither pregnancy outcome nor occurrence of maternal or neonatal complications.
Conclusions
The increment in fetal AC in pregnancies complicated by hypothyroidism needs to be confirmed in further prospective studies. The outcome of pregnancies complicated by thyroid dysfunction does not seem to be predicted by hormonal or ultrasonographic parameters.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P929

**Endocrine effects of the FSHB −211 promoter polymorphism in females**

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Introduction
FSH is a key player in reproductive functions, the expression of its unique subunit FSHB is regulated by the FSHB promoter. Recently, a single nucleotide polymorphism (SNP) at a highly conserved position in the FSHB promoter (rs10835638; −211G>T) has been found to be associated with decreased serum FSH levels in men and with male infertility. Because to date no information is available on possible endocrine consequences of this SNP in women, we conducted this study to analyze well characterized female individuals.
Patients and methods
Three hundred and sixty five normally cycling women undergoing diagnostics prior to assisted reproductive techniques (ART) were included. Anthropometric and endocrine data were assessed. An independent cohort of 438 fertile females was additionally analyzed. Customized TaqMan technology was used to detect the FSHB SNP in DNA extracted from lymphocytes.
Results
Comparing genotyped patients of our study population with controls, allele and genotype frequencies were not different between groups (T-allele: 14.7 vs 16.6%; TT-homozygotes: 2.4 vs 3.4%). However, we observed strong associations of the T-allele with elevated serum FSH (0.99 U/l per T-allele, $P=0.006$) and LH (1.30 U/l per T-allele, $P<0.001$). Additionally, T-allele carrier status was significantly associated with a reduced serum progesterone (−1.96 ng/ml per T-allele, $P=0.047$) in the studied subjects.
Conclusion
This is the first report on genotype-phenotype correlations of rs10835638 in females. Unexpectedly, the association of this SNP with an elevated FSH in our subjects stands in contrast to previous findings in male. On the other hand, elevated LH-levels in T-allele carriers seem to be present also in males. Together with the observed associations of this SNP with progesterone these novel findings could be indicative of a gender specific regulatory feedback mechanism involving sexual steroids and progesterone responsive elements of the FSHB promoter.
Declaration of interest
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## P930

**The prevalence of endometrial hyperplasia and endometrial cancer in Danish women with PCOS or hyperandrogenism**

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Objective
PCOS may be associated with an increased risk of endometrial hyperplasia (EH) and endometrial cancer (EC), but substantial evidence remains to be established. We investigated the prevalence of EH and EC in a well-characterized group of women with PCOS and/or clinical/biochemical hyperandrogenism.

Design
Retrospective, trans-sectional study.
Setting
Out-patient clinic at the Departments of Endocrinology and Gynecology, Odense University Hospital, Denmark.
Population
Nine hundred and sixty three premenopausal women consecutively referred with the diagnoses PCOS and/or hirsutism during 1997–2008.
Methods
All women underwent a standardized evaluation program. In 2011, The Danish Data Bank of Pathology was used to identify patients with endometrial histology diagnoses (year range of diagnosis 1982–2011).
Main outcome measures
Histology diagnoses, demographic variables.
Results
EH was diagnosed in 10 (1.0%) women and EC in 1 (0.1%) woman. The median BMI in the EH/EC group was 30.6 kg/m$^2$ compared to 26.8 kg/m$^2$ in the total cohort. There were no differences between the cases and total cohort in terms of individual Rotterdam Criteria. In Denmark, 70 yearly cases of EC are diagnosed in women 40–55 years corresponding to a prevalence of 0.4% in the corresponding time period.
Conclusion
The results of the present study do not suggest a higher prevalence of EC in women with PCOS and/or clinical/biochemical hyperandrogenism compared to the general population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P931

### Conformational control of P450aromatase by lipid membrane interactions and protein associations

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Cytochrome P450 aromatase is a membrane bound enzyme that synthesises estrogens. This reaction involves electron delivery from NADPH via cytochrome P450 reductase (CPR). Little is known as to how the P450arom performs the aromatase reaction or how it associates with CPR in the endoplasmic reticulum (ER). In humans, there is only one P450arom, however pigs have three isozymes. The catalytic efficiency of one of these, the porcine gonadal P450arom, is much lower than the human and the porcine placental isozymes despite the high amino acid sequence homologies.
We have used *in vivo* and *in vitro* techniques to study the interaction of P450arom proteins and lipid membranes. Forster resonance energy transfer (FRET) studies explored the protein-protein interactions in the ER. A quartz crystal microbalance (QCM) was used to measure the mass of protein(s) binding to a lipid membrane and in association with the Western blot data the stoichiometry for P450arom and CPR was determined. QCM also provides information about the conformational and structural organisation of proteins in the lipid membrane. Molecular mechanics calculations were also used to further probe the human and porcine P450arom.
Our FRET studies showed that the human P450arom forms dimers *in vivo*. The QCM showed that all the recombinant P450arom enzymes examined bound tightly to the lipid membranes; both truncated and full length. The P450arom: CPR complexes bound to the membranes exhibited good catalytic turnover. However, the human P450arom 'associated' very differently with the lipid membrane than the porcine gonadal P450arom. The rate of porcine P450arom binding was most influenced by the amount of CPR present. Thus the structural organisation within the membrane is very different between porcine and human, despite the similarities in amino acid sequence. The minimum energy structures for the human and porcine gonadal P450arom also differ and thus may influence the mechanism of P450arom function.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P932

### Metabolic characteristics in women with polycystic ovary syndrome by phenotypes based on various diagnostic criteria

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The metabolic features of Polycystic ovary syndrome (PCOS) can vary based on the definition used. The aim of this study is to compare the metabolic characteristics of PCOS according to the phenotypes from various diagnostic criteria and help to establish a optimal diagnostic criteria.
Subjects with PCOS defined by the NIH criteria ($n=494$) and the Rotterdam criteria ($n=568$) and regular cycling non-hirsuite control women ($n=1000$) were recruited. All subjects underwent blood samples for fasting glucose, lipids, insulin and reproductive hormones and a transvaginal ultrasound.
Women with PCOS have significantly higher prevalence for abnormal glucose metabolism and metabolic syndrome (MetS) compared to control (4 vs 0% for diabetes 11 vs 3.4% for IFG/IGTand 19 vs 2.4% for MetS) and it is not different among PCOS women according to diagnostic criteria. Women with PCOS were divided into following four different phenotypes; i) oligomenorrhea(OM)/hyperandrogenism (HA)/PCO, ii) OM/ HA, iii) HA /PCO, and iv)OM/PCO. Women with PCOS by NIH criteria (OM/HA $\pm$ PCO) have higher frequency of obesity, diabetes, IGT/IFG and metabolic syndrome compared to subjects with OM/PCO or HA/PCO (Table 1).
Regarding metabolic derangement, PCOS without HA may be different phenotype. Further studies will be required to observe the development of various disease in women with PCOS diagnosed by different criteria to establish a fine definition which can represent morbidity of disease.

**Table 1** Metabolic characteristics according to various phenotypes of PCOS.

| | OM/HA/PCO ($n=346$) | OM/HA ($n=165$) | HA/PCO ($n=136$) | OM/PCO (413) | Controls ($n=962$) |
|---|---|---|---|---|---|
| Age (year) | 24±5$^{*,\S}$ | 23±4$^{*,\ddagger}$ | 25±4$^{\dagger}$ | 24±4$^{*}$ | 26±4 |
| BMI (kg/m$^2$) | 23.8±4.7$^{*,\dagger,\ddagger}$ | 23.4±4.5$^{*,\dagger,\ddagger}$ | 21.2±2.9 | 20.8±2.4 | 20.9±2.5 |
| FPG (mg/dl) | 87±13$^{\dagger}$ | 86±18 | 85±8 | 84±7 | 85±6 |
| PPG (mg/dl) | 111±36$^{*,\dagger,\ddagger}$ | 109±41$^{*,\dagger,\ddagger}$ | 98±17 | 96±20 | 95±18 |
| TC (mg/dl) | 183±31$^{*,\dagger}$ | 184±333$^{*,\dagger}$ | 178±28 | 174±28 | 174±28 |
| TG (mg/dl) | 100±64$^{*,\dagger,\ddagger}$ | 93±53$^{*,\dagger}$ | 78±36 | 72±36 | 73±34 |
| HDL-C (mg/dl) | 50±13 | 50±15 | 50±9 | 53±11 | 50±10 |
| LDL-C (mg/dl) | 113±28$^{\dagger}$ | 116±28 | 112±26 | 107±23 | 109±24 |
| FPI (IU/l) | 7.8±7.2$^{*,\dagger}$ | 5.9±5.7 | 4.4±3.6 | 4.4±3.6 | 3.8±4.7 |
| PPI (IU/l) | 51.6±46.1$^{*,\dagger}$ | 54.3±42.2$^{*,\dagger}$ | 33.9±23.9 | 33.9±23.9 | 27.4±27.3 |
| HOMA-IR | 1.7±1.9$^{*,\dagger}$ | 1.3±1.3 | 0.9±0.8 | 0.9±0.8 | 0.8±1.0 |
| IFG/IGT (%) | 11.0 | 11.5 | 4.4 | 3.1 | 4.3 |
| DM (%) | 5.2 | 1.2 | 0 | 0.2 | 0 |
| MetS (%) | 20.8 | 14.0 | 2.9 | 2.2 | 2.4 |

$^{*}P<0.05$ vs controls; $^{\dagger}P<0.05$ vs OM+PCO; $^{\ddagger}P<0.05$ vs HA+PCO; $^{\S}P<0.05$ vs OM+HA.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P933

### Prevalence of chronic autoimmune thyroiditis in patients with polycystic ovary syndrome

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Introduction
Polycystic ovary syndrome (PCOS) is a common endocrinopathy in women of reproductive age and is characterized by chronic anovulation and clinical and/or biochemical hyperandrogenism. Chronic autoimmune thyroiditis (AIT) is a common disease of organ-specific autoimmunity affecting predominantly women, that is attributed to an interaction between a genetic background and environmental factors. There are few data showing higher AIT prevalence in PCOS patients.

Objective
To investigate the prevalence of AIT in women with PCOS.
Patients/methods
We studied 272 women diagnosed with PCOS according to the NIH (1990) criteria and 164 age- and weight-matched control women. All patients underwent a hormonal and biochemical evaluation. AIT was diagnosed in women with high levels of anti-Tg and/or anti-TPO antibodies who were further evaluated with thyroid ultrasound. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR).
Results
No significant difference was found in the prevalence of AIT between the two groups (34/272 (12.5%) PCOS patients and 24/164 (14.6%) controls were diagnosed with AIT). PCOS patients with AIT compared to PCOS patients without AIT were significantly older ($P<0.01$), more obese ($P<0.001$) and had lower serum SHBG levels ($P<0.001$). Control women with AIT compared to control women without AIT were significantly older ($P<0.001$), had lower serum total testosterone and DHEAS levels ($P<0.05$ and $P<0.001$ respectively) and were more insulin resistant as assessed by HOMA-IR ($P<0.001$).
Conclusion
In our cohort, the prevalence of AIT was similar in PCOS women and normal ovulatory women. Further studies with a greater number of participants are needed in order to clarify a possible relationship between these two common entities.
Declaration of interest
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## P934

**Lean muscle mass in classic or ovulatory PCOS: association with central obesity and insulin resistance**

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Central obesity and insulin resistance are more prevalent in women with PCOS, affecting both normal weight and obese subjects. The metabolic effects of insulin are partially dependent on the amount of lean mass and insulin resistance seems to selectively affect the metabolic, but not the mitogenic, actions of insulin. Therefore, this age-matched case-control study assessed total and segmental lean muscle mass in classic or ovulatory polycystic ovary syndrome (PCOS) patients and investigated whether lean mass is associated with hormone and metabolic features. PCOS was diagnosed according to the Rotterdam Consensus criteria. Participants underwent anthropometric and clinical evaluation. Habitual physical activity was assessed with a digital pedometer, and body composition by dual-energy X-ray absorptiometry. Laboratory measurements included total cholesterol, cholesterol fractions, triglycerides, glucose, total serum testosterone, serum insulin, estradiol, luteinizing hormone, and SHBG. Energy intake was calculated using a food frequency questionnaire. Classic PCOS patients had higher body mass index (BMI), waist circumference, testosterone and lipid accumulation product values than ovulatory PCOS and controls ($P<0.05$). Energy consumption, homeostasis model assessment index, SHBG, free androgen index and triglycerides, total and trunk lean mass were higher only in classic PCOS women vs controls. Arm, leg, trunk, total or limb lean masses were not correlated with hormone levels in any of the groups. However, in PCOS women lipid accumulation product was positively correlated with total ($r=0.56$, $P=0.001$), trunk ($r=0.59$, $P=0.001$), arm ($r=0.54$, $P=0.001$), leg ($r=0.44$, $P=0.03$) and limb ($r=0.48$, $P=0.001$) lean masses. BMI was positively correlated with all lean mass segments and independently associated with total lean mass. Lipid accumulation product and BMI were independently associated with trunk lean mass variation. In conclusion, the increase in lean mass in classic PCOS appears to be associated with insulin resistance and central obesity rather than with energy intake, physical activity or androgens.
Declaration of interest
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## P935

**Candidate pathway genetic analysis of PCOS**

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Polycystic ovary syndrome (PCOS) is a characterized by hyperandrogenism and irregular menses and is associated with insulin resistance, type 2 diabetes and obesity. While there is a strong genetic component to PCOS, candidate gene analyses and genome wide association studies (GWAS) have identified only a handful of replicated PCOS susceptibility loci. Therefore, we employed a candidate pathway approach to identify additional PCOS susceptibility loci. This approach is more comprehensive than single candidate gene analyses but also more targeted than GWAS, there is a reduced penalty for multiple testing allowing the detection of genes with smaller effect sizes. We tested for association between PCOS and 4930 SNPs mapping to 299 genes belonging to four pathways (ovarian rigidity (117 genes), TGF β signaling (77 genes), insulin resistance, diabetes and obesity (28 genes), and inflammation (77)) in a European ancestry cohort of 931 women with PCOS according to NIH criteria and 957 control women. After adjusting for population substructure and body mass index, the strongest evidence for association was with genes previously implicated in obesity, diabetes or insulin resistance (39% of genes with $P$ values $<0.01$) and genes whose expression is altered in a murine model of ovarian rigidity (22% of genes with $P$ values $<0.01$). The five genes with the strongest evidence for association with were RAMP1 (receptor G protein-coupled activity modifying protein 1; rs13405506 allele T, odds ratio = 0.57, $P$ value = 0.00010), FTO (fat mass and obesity associated; rs8056199 allele A, odds ratio = 1.47, $P$ value = 0.00015), ADAMTS19 (ADAM metallopeptidase with thrombospondin type 1 motif, 19; rs246246 allele G, odds ratio = 0.56, $P$ value = 0.00035), TBC1D4 (TBC1 domain family, member 4; rs9318332 allele C, odds ratio = 1.43, $P$ value = 0.00054), and SERPINA12 (serpin peptidase inhibitor, clade A, member 12; rs12433377 allele G, odds ratio = 1.63, $P$ value = 0.00056). This study provides convincing evidence that the ovarian and metabolic environment contribute to the PCOS phenotype.
Declaration of interest
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## P936

**Sex hormones and sexual function in postmenopause**

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The aim of the study was to explore the relation of female sexual function to the concentration of estradiol ($E_2$), total testosterone (T), DHEA-S in the sample of Lithuanian women in postmenopause.
Methods and participants
Two hundred and forty six postmenopausal women who fulfilled inclusion criteria were asked to fill in female sexual functioning index (FSFI) and socio-demographic questionnaire. A blood sample from all participants was taken for $E_2$, testosterone, SHBG, DHEA-S analysis.
Results
The mean age of study participants was 55.5 (5.2) years (range 45–65 years). Week positive correlation of total testosterone (nmol/l) with desire ($r=0.13$, $P=0.04$) and arousal ($r=0.12$, $P=0.05$) was detected.
$E_2$ (pmol/l) positively correlated with desire ($r=0.17$, $P=0.01$), arousal ($r=0.17$, $P=0.008$), lubrication ($r=0.16$, $P=0.01$), orgasm ($r=0.13$, $P=0.04$), pain ($r=0.16$, $P=0.02$), total score of sexual function ($r=0.16$, $P=0.01$). $E_2$ did not influence sexual satisfaction in our sample.
DHEA-S (μmol/l) had positive effect on sexual desire ($r=0.14$, $P=0.02$), lubrication ($r=0.14$, $P=0.03$), pain ($r=0.15$, $P=0.02$) and total score of sexual function ($r=0.14$, $P=0.03$).
Risk to have sexual dysfunction (FSFI $\leq 26.55$) was lower for women with higher $E_2$ – odds ratio 0.99 (0.99–1.00; $P<0.02$), and higher DHEAs concentration – odds ratio 0.85 (0.76–0.95; $P< 0.004$). But these hormones were not significant predictors of sexual dysfunctions when results were adjusted for age.
Conclusion
Sex hormones concentration along with age factor are significant determinants of female sexual function in postmenopause.
We are grateful for Professor John Bancroft (The Kinsey Institute for Research in Sex, Gender and Reproduction) for his consultations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P937

**Hypoandrogenemia at women with hypogonadotropic hypogonadism**

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We estimated androgen status at 111 female patients (pts) with HH before and after hormonal therapy (HT) by estadiol 2 mg + dydrogesterone 10 mg in sequence manner not <12 months. Median age of pts was 28 y.o., mean duration of HH 6.2 year, isolated HH $n=56$ group 1, HH as a part of hypopituitarism $n=55$ group 2, 45 healthy women of the same age were included in control group. Total T, SHBG and DHEA-S levels were measured, free T levels were calculated using standard formula. To estimate the quality of life the General Health Questionnaire (GHQ-28) was used.
Initially in both 1 and 2 groups total and free T levels were significantly lower compared to the controls ($P<0.05$), decrease was more appreciable in group 2 (see Table 1). DHEA-S levels were significantly lower in group 2 but not in group 1 compared with controls. There are some data that estrogen therapy can provoke depression of androgen levels. During HT the total and free T levels did not changed considerably so position had not worsened. DHEA-S levels even increased and the difference was statistically significant in group 2.
Total T levels positively correlated with the quality of sleep ($r=0.47$; $P=0.02$), social and physical activities ($r=0.54$; $P=0.000003$) and negatively correlated with depression ($r=-0.67$; $P<0.001$) and nervousness ($r=-0.57$; $P=0.0001$). Free testosterone levels positively correlated with the positive emotional reactions ($r=0.33$; $P=0.04$), social and physical activities ($r=0.6$; $P<0.001$), quality of sleep ($r=0.46$; $P=0.003$) and negatively correlated with depression ($r=-0.7$; $P<0.001$) and nervousness ($r=-0.6$; $P<0.001$). DHEA-S levels positively correlated with the positive emotional reactions (0.46; $P=0.003$), social and physical activities ($r=0.5$; $P<0.001$), quality of sleep ($r=0.45$; $P=0.003$), daily activities ($r=0.56$, $P<0.001$) and negatively correlated with depression ($r=-0.55$; $P<0.001$) and nervousness ($r=-0.45$; $P=0.004$).
Thus, hypogonadotropic hypogonadism is the disease associated androgen deficit with which influences on quaity of life. Hypoandrogenemia is more severe in patients with hypopituitarism
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**Table 1** Estradiol + dydrogesterone therapy does not worsen androgen deficiency.

| | Group 1 ($n=56$) | | Group 2 ($n=55$) | | |
|---|---|---|---|---|---|
| Parameter | Before HT | After HT | Before HT | After HT | Control group |
| Total T (nmol/l) | 0.95 (0.7; 1.2) | 0.85 (0.6; 1.0) | 0.1 (0.1; 0.2) | 0.1 (0.08; 0.3) | 1.05 (0.8; 1.4) |
| Free T (pmol/l) | 7.0 (4.8; 9.0) | 6.9 (5.5; 8.3) | 1.4 (0.6; 2.0) | 0.75 (0.4; 1.6) | 10.0 (7.0; 16.0) |
| DHEA-S (nmol/l) | 3590 (2747; 6630) | 5001 (3430; 6510) | 128 (73; 850) | 270 (94; 1357) | 5590 (4030; 6630) |

## P938

**Nonalcoholic fatty liver disease in pregnant women.**

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Introduction
NAFLD has become the leading cause of liver disease in the Western world and has a strong association with obesity and diabetes. There are no studies of NAFLD in pregnant women.
Methods
Pregnant volunteers ($n=57$) were recruited in the first or third trimester and evaluated for the presence of NAFLD using abdominal ultrasound scans using accepted criteria. Liver biopsies were not performed since this invasive procedure would present undue risks in otherwise healthy pregnant subjects. Fasting liver enzymes (AST and ALT), triglycerides, adiponectin, leptin, glucose, and insulin, were drawn. Gestational diabetes was defined in third trimester subjects using criteria developed by Carpenter and Coustan. Subjects were subsequently subdivided into lean, obese, and gestational diabetics.
Results
Half of the subjects enrolled had ultrasound evidence of NAFLD. NAFLD was observed equally among those presenting in the first or third trimesters. Additionally, the prevalence of NAFLD was similar between those with normal BMI vs obese women. No liver enzymes elevations were encountered. Serum adiponectin levels were significantly higher in the lean subjects irrespective of gestational age while obese and gestational diabetic subjects had lower adiponectin concentrations. Serum leptin levels in the first trimester were significantly lower in lean subjects compared to the obese and GDM women. In the third trimester, levels were elevated (compared to first trimester levels) in the lean and obese, and gestational diabetic groups. No relationship between adipokine levels and the presence of NAFLD was detected.
Conclusions
This is the first study to report on NAFLD in pregnant women. Pregnant women with NAFLD have normal AST and ALT and hence, are not reliable diagnostic tests to distinguish NAFLD in pregnancy. Distribution of NAFLD is comparable in lean, obese and gestational diabetic subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P939

**AMH and INSL3 levels in ovulatory and anovulatory women with the polycystic ovary syndrome**

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Introduction
Anti-Mullerian hormone (AMH) and insulin-like factor 3 (INSL3) represent ovarian functional markers of granulosa and theca cells respectively. This study investigated AMH and INSL3 plasma levels in two groups of ovulatory or anovulatory women with polycystic ovary syndrome (PCOS), and their relationship with ovarian morphology and androgen levels.
Methods
AMH and INSL3 were measured in a cohort of 57 patients with PCOS classified in two groups according to progesterone (P) levels ($n$: 21 ovulatory; $n$: 36 anovulatory) and in 27 age and body-weight matched controls with normal menses. Progesterone levels were randomly measured in those with amenorrhea and on the day 21 of the cycle in those with oligomenorhea or normal cycles. Clinical and endocrine characteristics and ovarian morphology were compared between the two PCOS groups.
Results
Compared to controls, plasma AMH and INSL3 were higher in PCOS women ($P<0.001$ and $P=0.01$ respectively) and, among them, in those with chronic anovulation with respect to the ovulatory ones ($P=0.003$ and $P=0.01$ respectively). The anovulatory group had lower number of cycles per year ($P<0.001$) and presented with higher number of ovarian follicles ($P=0.004$) and serum levels of testosterone ($P=0.001$), LH ($P<0.001$) and FSH ($P<0.001$) compared with the ovulatory group. Intriguingly, levels of INSL3 correlated with those of AMH in all PCOS women ($r=0.45$; $P=0.001$). Moreover, both INSL3 and AMH significantly and positively correlated with LH values in all PCOS women ($r=0.31$; $P=0.02$ and $r=0.53$; $P=0.001$ respectively).
Conclusion
These data show for the first time that blood levels of INSL3 significantly correlate with AMH plasma concentrations, and that levels of both peptides are specifically altered in anovulatory women with PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P940

**Increased risk of glucose metabolism impairment after gestational diabetes in polycystic ovary syndrome**

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Objective
The aim of the present study was to test the hypothesis that the risk of persistent glucose impairment after gestational diabetes (GDM) is increased in patients with polycystic ovary syndrome (PCOS).
Research design and methods
The prospective case–control study included 42 pregnant patients with PCOS and GDM and 84 pregnant control patients with GDM but without clinical and biochemical hyperandrogenism, polycystic ovaries and oligo-anovulation. The cases and controls were matched one to two for age and body mass index. The glycemic profiles were studied in all subjects 6 weeks, 12 weeks and 18 months after delivery. The incidence and the relative risk (RR) were calculated for overall persistence of an abnormal glycemic pattern and for each specific alteration, i.e. impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and diabetes mellitus (DM).
Results
At 18 months after delivery, the incidences of IFG, IGT and IFG–IGT were significantly ($P<0.05$) higher in the cases than in the controls. At the 18-month follow-up, the RR for the composite outcome of glucose metabolism impairment in PCOS women was 3.45 (95% confidence interval (CI) 1.82–6.58).
Conclusions
Patients with PCOS are at increased risk for a persistent impaired glucose metabolism after GDM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P941

**Effect of antiandrogens on spontaneous apoptosis in cultured porcine granulosa cells**

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Androgens are one of the most important agents influencing folliculogenesis. They can modulate follicular function by interactions with various factors and promote granulosa cells differentiation. On the other hand, androgens can antagonize follicular development by inducing apoptosis in granulosa cells. Therefore, androgens can promote follicular atresia. The purpose of the present study was to test the hypothesis that androgen receptor antagonists (2--hydroxyflutamide (2-Hf) or vinclozolin (Vnz)) in the presence of T inhibit granulosa cell apoptosis. Granulosa cells, isolated from mature pig follicles (6–8 mm in diameter), were cultured for 24 h in McCoy's 5A medium supplemented with 10% fetal bovine serum to cause attachment cells to the plastic. After that, media were changed and T, 2-Hf, Vnz, or both T + 2-Hf and T + Vnz were added to the culture media. Finally, 24 h later cells were fixed for morphological assessment of apoptotic cells utilizing a Hoechst staining technique and measurement of caspase-3 activity in cultured granulosa cells were performed.
It was shown that the addition of 2-Hf or Vnz to the culture media caused an increase in the incidence of apoptotic bodies and caspase-3 activity. Furthermore, testosterone treatment also enhanced apoptosis in granulosa cells. On the other hand apoptotic bodies were very sparse among granulosa cells cultured with T + 2-Hf and T + Vnz. Moreover, caspase-3 activity was suppressed in cultures treated with combination of androgen receptor agonist and antagonists.
In conclusion, our results indicate that both 2-hydroxyflutamide and vinclozolin appear to be atretogenic, but when tested with high level of testosterone they trigger anti-apoptotic effects. The nature of this protective mechanism as yet is unknown and requires further research.
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## P942

**Prenatal exposure to anti-androgen flutamide influences connexin 43 expression in the porcine fetal gonads**

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All evidence so far indicates a role of different connexins during gonadal development, gamete formation and testicular and ovarian functions. Connexin 43 (Cx43) is one of the predominant gap junction proteins in the testis and ovary. It is well established that gonads development is highly sensitive to hormonal disruption induced by various endocrine active compounds during fetal period. Therefore, it was interesting to study whether communication via gap junctions is altered in gonads of pig fetuses exposed to anti-androgen flutamide prenatally. To perform this project, pregnant sows were injected with flutamide (50 mg/kg body weight, seven times, every day) starting at 83 or 101 day of gestation. On days 90 and 108 the fetuses were excised during a surgical procedure. Fetal gonads were fixed in Bouin's fixative for immunohistochemistry or frozen in liquid nitrogen for real-time PCR analysis. Immunohistochemistry was performed on tissue sections using a polyclonal rabbit antibody against Cx43 (dilution 1:2000; Sigma–Aldrich) followed by a goat anti-rabbit IgG (dilution 1:300; Vector Lab.) and the standard ABC method. Binding of antibody to the antigen was visualized by diaminobenzidine. To assess Cx43 mRNA expression real-time PCR was performed using the TaqMan Gene Expression Assay (Applied Biosystems).
In the fetal ovaries, Cx43 protein was localized between interstitial cells surrounding egg nests and between granulosa cells of primary follicles. In the fetal testes, Cx43 was detected between Leydig cells. In the fetal gonads Cx43 expression was up-regulated after flutamide treatment.
Detection of changes in Cx43 gene expression in developing porcine fetal gonads after exposure to flutamide may indicate the role of androgens in the regulation of cell-to-cell communication within the testes and ovaries.
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Declaration of interest
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## P943

**Long term health status of POI Patients**

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Premature ovarian insufficiency (POI) is a disorder which affects approximately 1% of women under 40 years of age. Besides infertility and estrogen deficiency altering the quality of life, the impact of this disease on the long-term health status of these women, especially bone status, has received little attention. We therefore designed a cross-sectional study of patients with POI, 5–10 years after a first evaluation in our department. We evaluated their anthropometric, hormonal replacement treatment (HRT) and bone health status. One hundred and ninety four patients were proposed to participate to this study, and 130 agreed to participate (77%). The mean follow-up period was $8 \pm 2.5$ years (4.6–19.3). Eighty five patients had bone mineral density (BMD) determination. Forty six patients (54%) presented osteopenia, 8 osteoporosis (10%) and 31 (36%) had normal BMD. No fractures were reported. Thirty women (59%) with vitamin D deficiency exhibited bone demineralization. Forty five patients had BMD evaluation at the time of POI diagnosis and during the present evaluation. Thirteen (28.8%) of them had stopped their HRT for over a year. Twenty three (51%) had progressed to osteopenia or osteoporosis. In univariate analysis, discontinuation of HRT and POI duration were associated with a significant loss of BMD, while BMI was associated with a gain in BMD. In multivariate analysis, time since HRT discontinuation was significantly associated with a loss of BMD; BMI and occurrence of a pregnancy were associated with a gain in BMD, but POI duration had no significant impact.
In conclusion, our study was the first to analyse long-term consequences on these women's health of POI. We showed that impairment of BMD is common in POI women, especially in patients who stopped their HRT. These patients must be carefully monitored to improve their final outcome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P944

**Neonates of women with polycystic ovary syndrome display increased oxidative stress markers and metabolic aberrations comparable to gestational diabetes offspring**

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Context
Polycystic ovary syndrome (PCOS) and gestational diabetes (GDM) are two common metabolic disorders. The serum oxidative stress markers advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs) have been found to be elevated in women with PCOS and GDM compared to controls. However the impact of these harmful molecules to their offspring is under investigation.
Objective
To evaluate AGEs and AOPPs serum levels along with the hormonal/metabolic profile, their possible relationship as well as their potential impact in a cohort of PCOS(N) and GDM (N) neonates and their mothers PCOS(M) and GDM(M). As control was studied a group of normal mothers C(M) and their neonates C(N).
Design and setting
Prospective controlled study conducted in an Academic Medical Center.
Patients
The study population comprised 151 mothers/neonates pairs.
Main outcome measures
Anthropometric, metabolic, hormonal parameters and oxidative stress markers.
Results
AGEs and AOPPs were higher in PCOS(M) and GDM(M) mothers compared to controls(CM; $P<0.05$; AGEs: PCOSM: $7.19\pm1.8$ vsGDM: $7.28\pm2.4$ vs CM: $5.68\pm1.3$ U/ml, AOPPs: $10.18\pm1.76$ vs $10.06\pm2.02$ vs $5.3\pm1.8$ μmol/l) respectively. The same significant difference ($P<0.05$) was observed in the corresponding neonates groups (N; AGEs: PCOSN: $5.67\pm1.42$ vs GDMN: $5.74\pm1.52$ vs CN:$5.05\pm1.28$ U/ml, AOPPs: $9.56\pm1.49$ vs $11.24\pm5.49$ vs $6.2\pm2.51$ μmol/l respectively). A strong relationship between mothers' and neonates' AGEs ($r=0.605$, $P<0.001$) and AOPPs ($r=0.735$, $P<0.001$) was disclosed. Analogous findings were observed regarding androgens and insulin resistance in the subgroups of mothers and neonates.
Conclusion
The present study demonstrated that in PCOS neonates, the oxidative stress status and metabolic/hormonal profile was similar to that of GDM neonates and strongly associated with their mother's oxidative status. These findings may have clinical implications, since PCOS diagnosis can be achieved before pregnancy, thus enhancing pre-pregnancy health precautions for a better health outcome in their offspring.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P945

**Impact of dietary advanced glycation end products modifications on metabolic and hormonal profile in women with polycystic ovary syndrome**

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Objective
To investigate the impact of dietary intervention of advanced glycation end products (AGEs) intake on hormonal and metabolic profile in women with polycystic ovary syndrome (PCOS).
Methods
After baseline evaluation, 23 women with PCOS (mean$\pm$S.D., age: $23.4\pm5.7$ years; body mass index (BMI): $26\pm5.7$ kg/m$^2$) underwent the following consecutive 2-month dietary regimens: a hypocaloric diet with *ad-libitum* AGEs content (Hypo), an isocaloric diet with high AGEs (HA) and an isocaloric diet with low AGEs (LA). Metabolic, hormonal and oxidative stress status was assessed as well as AGEs levels were determined in all subjects, after the completion of any dietary intervention.
Results
Serum levels of AGEs, testosterone, oxidative stress, insulin and HOMA-IR index were significantly increased on HA compared to Hypo diet and subsequently decreased on LA diet (compared to HA; $P<0.05$ for all parameters). BMI remained unaltered throughout the HA and LA periods compared to Hypo period. Dietary AGEs were associated with serum AGEs ($r=0.45$, $P<0.001$), insulin ($r=0.3$, $P=0.005$), HOMA-IR index ($r=0.31$, $P=0.006$) and oxidative stress ($r=0.32$, $P=0.002$). Testosterone was correlated with insulin ($r=0.36$, $P=0.001$) and HOMA-IR index ($r=0.36$, $P=0.002$).
Conclusions
Modifications on dietary AGEs intake are associated with parallel changes in serum AGEs, metabolic, hormonal and oxidative stress biomarkers in women with PCOS. These findings support the advice of a low AGEs dietary content along with lifestyle changes in women with PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P946

**Inhibin-α gene promoter polymorphisms in patients with idiopathic premature ovarian failure**

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Background
Premature ovarian failure (POF) is a complex disorder defined as the cessation of ovarian function before or at the age of 40. Although the etiology of POF remains unknown in a large proportion of cases, it has been suggested that variations in the Inhibin α gene (INHA) may affect the ovarian function of women. This study was performed to investigate whether the genetic polymorphisms of the INHA gene are associated with idiopathic POF in a Korean population.
Methods
The subjects consisted of 159 idiopathic POF patients and 233 postmenopausal controls. Genotyping for $-16C>T$ polymorphism was performed by MGB primer/probe Taqman assay, and $-124A>G$ polymorphism was identified using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. Haplotypes were deduced by using the Haploview version 4.1.
Results
There were no significant differences in the genotype distributions or allele frequencies of the *INHA* gene $-16C>T$ and $-124A>G$ polymorphisms between the POF and the control group. Haplotype analysis also showed no significant difference between groups.
Conclusion
The distribution of the *INHA* gene promoter polymorphisms in a Korean POF population was not significantly different from controls, implying that the INHA gene polymorphisms may not be associated with the risk of idiopathic POF.
Keywords: inhibin-α, polymorphism, premature ovarian failure.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P947

**Skeletal frame size in females with polycystic ovary syndrome**

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Background
Body composition of females with polycystic ovary syndrome (PCOS) varies considerably. The literature lacks of skeleton size description in women with PCOS. Objective of the present study was to investigate skeleton robustness in PCOS women.
Material and methods
Hundred and one PCOS women (by androgen excess society criteria, mean age 26.85 ± 3.91 years) and 81 healthy control women (mean age 27.95 ± 3.65 years) were investigated in Vilnius city in 2009–2011. Height, body mass, seven longitudinal and ten transversal skeletal parameters were measured using standard anthropometric methods. 11 derivative indices including body mass index (BMI), frame index (FI), metric index (MI) and chest-to-pelvis ratio (ChPR) were calculated. Gonadotropins and androgens tests were carried out.
Results
Height did not differ between PCOS and controls. BMI of PCOS women was significantly higher compared to controls. PCOS women had longer trunk by 2.74 cm due to higher pelvis, 2.05 cm shorter arms and 1.90 cm shorter legs, wider shoulders (by 2.16 cm), chest (by 3.3 cm) and pelvis (by 1.81 cm), higher FI, MI and ChPR ($P < 0.01$). After the adjustment for BMI women with PCOS presented 0.67 cm wider chest, but 0.98 cm narrower pelvis, higher FI, MI and ChPR than healthy women ($P < 0.05$). 72.3% of women with PCOS had large frame size. 44.6% of PCOS women had picnomorphic somatotype, whereas 85.2% of the controls had leptomorphic somatotype. The study showed an inverse correlation between transversal skeletal measurements and luteinizing hormone, positive correlation between transversal skeletal measurements, FI, MI and free androgen index in women with PCOS. Area under ROC curve discriminating PCOS for FI was 0.830.
Conclusions
Women with PCOS have larger frame size, longer trunk, higher and narrower pelvis, shorter extremities in comparison to healthy women. Skeletal parameters may aid the identification of PCOS in women; frame index demonstrates the best PCOS predictive ability.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P948

**Quality of life and the frequency of anxiety and depression in women with PCOS**

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Background
Polycystic ovary syndrome is the most common endocrine disorder among women in their reproductive years. Apart from reproductive and metabolic manifestations, recently more attention has been paid to psychological features of this syndrome. The aim of the study was to evaluate the effect of PCOS on quality of life and to estimate the frequency of anxiety and depression in Polish women with PCOS.
Methods
One hundred seventy five women with PCOS aged 16–44, diagnosed accordingly to the Rotterdam criteria, recruited from the Department of Endocrinology, Diabetology and Isotope Treatment, Wroclaw Medical University and Outpatient Endocrinological Department of Provincial Health Care Specialist Clinics in Wroclaw, Poland, completed two questionnaires: Polish versions of hospital anxiety and depression scale (HADS) and the World Health Organization's Quality of Life-BREF (WHOQOL-BREF). The control group consisted of 30 healthy women.
Results
PCOS patients reported the lowest QoL in the psychological health domain. Environment and social relationships domains were also lower. The overall quality of life was 3.64 ± 0.76 in the five-point Likert's scale. 36.3% showed clinically relevant HADS anxiety scores and 9.6% had clinically relevant HADS depression scores, 8.3% had comorbid anxiety and depression. Lack of anxiety was significantly related with the better QoL.
Conclusions
Polycystic ovary syndrome decreases quality of life. PCOS may also increase risk for anxiety or comorbid anxiety and depression.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P949

**Examination of the generation of vaginal epithelium of the new vagina formed using oxidized regenerated cellulose**

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Purpose
For vaginoplasty of Rokitansky syndrome, we use oxidized regenerated cellulose instead of bio-craft that has been used in the past because it requires a less invasive and more physiological surgery. However, the mechanism of epithelialization of this procedure is still not clear. There is a report that differentiation and proliferation of the vaginal epithelium of newborn mice is promoted by administration of basic fibroblast growth factor (bFGF).
To characterize the epithelium of new vagina and to determine how it is formed, we examined the new vaginal epithelium for the presence of keratin, estrogen receptor α (ERα) and bFGF.
Methods
Four patients with Rokitnsky syndrome who underwent this operation were enrolled in this study. Patients' consent was obtained with the approval of the ethics committee.
Samples of vaginal epithelium were removed at the time of the operation, in the reproducing phase, and after completion of epithelialization. Samples of the vaginal epithelium and bdominal skin of patients who underwent total hysterectomy were also obtained as controls with their consent.
Immunostaining of ERα, keratin 13 and keratin 14 were performed using human monoclonal antibodies. Real-time PCR of four subtypes of fibroblast growth factor receptor (FGFR; FGFR-1, FGFR-2, FGFR-3, and FGFR-4) was also performed.
Results
Immunohistochemical staining revealed that keratin 13 and ERα were expressed in normal vagina and the new vagina, while keratin 14 was expressed only in the abdominal skin.
It appeared that the expression of FGFR-1, was markedly increased in the reproducing phase and decreased after the completion of vaginal epithelialization, while there were no significant changes in the other FGFR subtypes.
Conclusion
The characterization of the new vaginal epithelium with this surgery was demonstrated to be similar to that of normal vagina. Moreover, it was suggested that FGF may be an important growth factor which promotes new vaginal epithelialization.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P950

**Efficacy of sex hormone replacement therapy on induction of puberty in patients with Turner syndrome**

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**Abstract**
Introduction
Since primary amenorrhea because of ovarian dysfunction is a common complication in women with Turner syndrome (TS), sex hormone replacement therapy (HRT) is mandatory for induction of puberty, such as acquisition of

secondary sexual characteristics and increased bone density. However, appropriate treatment protocols have not yet been established. To evaluate the efficacy of HRT on puberty induction in our department, a retrospective analysis was conducted.
Methods
The clinical profiles of 62 TS patients with primary amenorrhea and five patients with spontaneous cycles were examined with their consent. Uterine length (UL) was measured by an ultrasonic examination and bone mineral density (BMD) of the lumbar vertebrae (L2–4) was measured by DEXA. Student's *t*-test was used for statistical analyses.
Results
There were 48 patients who had received HRT and 14 patients who had not. The mean ($\pm$S.D.) age at first visit was 24.4$\pm$4.7 years and at initiation of HRT was 19.7$\pm$3.0 years. The mean height and BMI were 145.7$\pm$4.6 cm and 21.9$\pm$2.8 kg/m$^2$ respectively. At the time of the first visit, the UL of patients receiving HRT (45.2$\pm$10.1 mm: $n=36$) was significantly longer than that of patients without HRT (37.0$\pm$6.0 mm: $n=8$). The BMD of patients receiving HRT (0.810$\pm$0.087 g/cm$^2$: $n=47$) was significantly higher than that of patients without HRT (0.7129$\pm$0.090 g/cm$^2$: $n=14$). However, both were significantly lower than in the patients with spontaneous cycles (1.007$\pm$0.09 g/cm$^2$). After receiving HRT in our department, the UL ($n=44$) of the patients increased significantly from 43.7$\pm$9.7 to 55.2$\pm$4.6 mm and the BMD of the patients without HRT increased from 0.7172$\pm$0.117 to 0.7924$\pm$0.083 g/cm$^2$, while there were no significant changes in the BMD of the patients with HRT.
Conclusions
HRT was effective for acquisition of secondary sex characteristics and increased bone density. It is suggested that early initiation of HRT may be more effective.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P951

### Different effects of electrical and manual acupuncture stimulation on estrous cyclicity and neuroendocrine function in rats with DHT-induced polycystic ovary syndrome

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Both low-frequency electro-acupuncture (EA) and manual acupuncture improve menstrual frequency and decrease circulating androgens in women with polycystic ovary syndrome (PCOS). We sought to determine whether low-frequency EA is more effective than manual stimulation in regulating disturbed estrous cyclicity in rats with PCOS induced by 5$\alpha$-dihydrotestosterone (DHT). To identify the central mechanisms of the effects of stimulation, we assessed hypothalamic mRNA expression of molecules that regulate reproductive and neuroendocrine function. From age 70 days, rats received 2-Hz EA or manual stimulation of the needles five times/week for 4–5 weeks; untreated rats served as controls. Specific hypothalamic nuclei were obtained by laser microdissection, and mRNA expression was measured with TaqMan low-density arrays. Untreated rats were acyclic. During the last 2 weeks of treatment, seven of eight (88%) rats in the EA group had epithelial keratinocytes, demonstrating estrous cycle change ($P=0.034$ vs controls). In the manual group, five of nine (56%) rats had estrous cycle changes (NS vs controls). mRNA expression of the opioid receptors Oprk1 and Oprm1 in the hypothalamic arcuate nucleus was lower in the EA group than in untreated controls. mRNA expression of the steroid hormone receptors Esr2, Pgr, and Kiss1r was lower in the manual group than in the controls. In rats with DHT-induced PCOS, low-frequency EA restored disturbed estrous cyclicity but did not differ from manual stimulation group. Thus EA cannot be considered superior to manual stimulation. The effects of low-frequency EA may be mediated by central opioid receptors, while manual stimulation may involve regulation of steroid hormone/peptide receptors.
Declaration of interest
I fully declare a conflict of interest. Details below.
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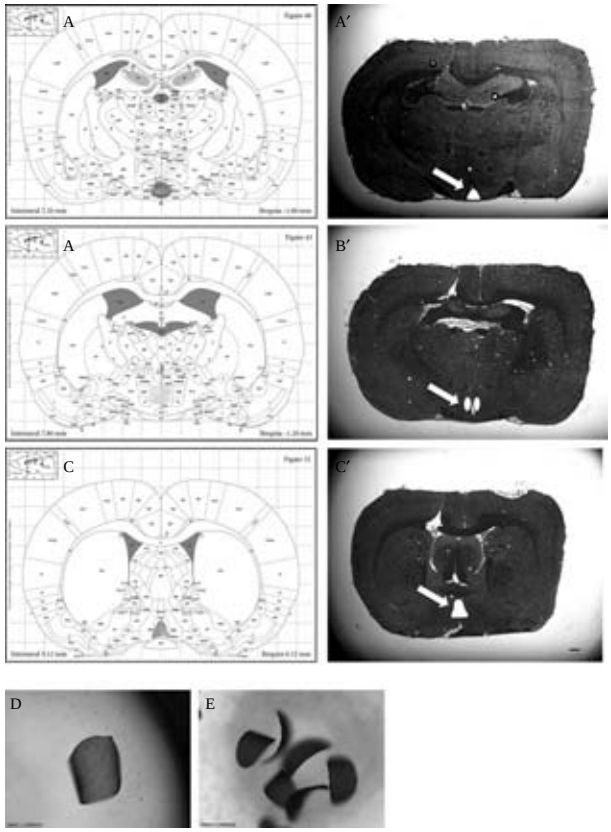


**Figure 1.** Laser microdissection of rat hypothalamic nuclei according to The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson 2009) of rat hypothalamus. (A) Arcuate nucleus (Arc). (B) Medial preoptic area (MPOA). (C) Anteroventral periventricular (Avpv). Scale bar, 500 μm. (D and E). Dissected tissue catapulted into the AdhesiveCaps. Scale bar, 300 μm.

## P952

### The influence of endocannabinoid receptor 1 gene variations on anthropometric and metabolic parameters in women with polycystic ovary syndrome

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Introduction
Polycystic ovary syndrome (PCOS) is one of the most commonly diagnosed endocrinopathies in women of reproductive age and is associated with an increasing number of metabolic comorbidities. About 50% of PCOS patients are obese, while insulin resistance affect up to 70% of these women. The endocannabinoid system contributes to human energy homeostasis. CNR1 is a biological candidate for human obesity and related metabolic disorders.
Aims
The aim of the study was to determine the relationships between CNR1 polymorphisms (A4895G (rs806368); A1422G (rs1049353); rs806381; rs10485170; rs6454674; rs2023239) and anthropometric and metabolic parameters in PCOS women.
Material and methods
In total, 130 women diagnosed with PCOS according to the Rotterdam criteria are recruited from the Endocrinology Department of the Medical University in Wroclaw, Poland. Medical history, physical examination, assessment of anthropometric parameters (body mass, height, waist and hip circumference,

BMI, WHR), metabolic parameters (glucose and insulin, insulin resistance index – HOMA, lipidogram) were carried out in all subjects. Genetic studies to detect six CNR1 gene polymorphisms were assessed. In order to amplify the genetic material the PCR technique was used. To polymorphisms identification minisequencing was used. Reaction products were separated on Genetic Analyser ABI 3100. For statistical analysis ANOVA test was used.
Results
The total cholesterol and LDL cholesterol levels in PCOS women carrying T/T genotype of rs2023239 CNR1 polymorphism were higher than in those with C/T and C/C ($P=0.02807$). There were no statistic differences in other metabolic parameters (glucose, insulin and HOMA levels) and also in value of BMI and WHR between the variants of rs2023239 CNR1 polymorphism. The other of studied polymorphisms of CNR1 gene were not associated with anthropometric and metabolic parameters in PCOS women.
Summary
On the basis of our study, it seems to CNR1 polymorphisms were not associated with obesity and metabolic disorders, including insulin resistance, in PCOS women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P953

**Androgens profile in Cushing's disease with hirsutism**

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Introduction
Cushing's disease (CD) is a condition due to an ACTH-secreting pituitary adenoma and leading to hypercorticism. Hirsutism is common in CD but its exact frequency has been poorly studied. It results from the stimulating effect of ACTH on adrenal production of androgens.
The aim of this study is to determine the androgenic profile in hirsute patients with CD.
Patients and methods
It is a retrospective study of nine female patients having a CD with hirsutism. Positive diagnosis of CD was made in the association of an ACTH-dependant hypercorticism, with a positive response to the high-dose-dexamethasone-suppressing test, and a pituitary adenoma on MRI.
Testosterone and DHEAS assays were carried out in all patients and δ-4-androstenedione assay was performed in five of them.
Results
Mean age at diagnosis was 32 (ext: 24–45). Hirsutism was severe in at least three patients. Menstrual irregularities were present in six of the nine patients and minor signs of hyperandrogenia (seborrhoea, acne) in two of them. Pituitary adenoma was a macroadenoma in 5/9 cases. ACTH was elevated in all patients with a mean ACTH value at 113.4 pg/ml (ext: 65–184). Mean values of testosterone, DHEAS and δ-4-androstenedione were respectively: 2.56 nmol/l (ext:1.4–3.3), 461 ng/ml (ext: 125–821) and 348 ng/ml (ext: 219–487). Six patients had a concomitant elevation of at least two androgens. Both testosterone and DHEAS were elevated in these six patients and δ-4-androstenedione was elevated in three of them. Only one patient had normal rates of testosterone and DHEAS.
Conclusion
Androgens are almost consistently mildly elevated in patients with CD and hirsutism. All hirsute patients with CD have an elevated ACTH, the majority of them has a pituitary macroadenoma. Further studies comparing these parameters in a larger cohort of patients having a CD, with and without hirsutism and hyperandrogenia, would be more conclusive.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P954

**Action of elevated leptin levels on steroidogenic enzymes expression in porcine small and medium size follicles collected from prepubertal and cycling pig**

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Cystic ovarian disease is a common reproductive disorder in women and females of domestic animals, leading to temporal or permanent infertility. Over one in three women with polycystic ovarian syndrome (PCOS) are overweight and have higher serum leptin concentration. Leptin may thereby mediate some of the adverse effect of obesity on ovarian function.
In the presented data, we decided to compare the action of two high doses of leptin (20 ng and 40 ng/ml) on progesterone ($P_4$), androstenedione ($A_4$), testosterone (T) and estradiol ($E_2$) secretion by small- and medium-sized follicles collected from pubertal and cycling pig. Taking into consideration previously published data reported that alterations in the content of steroid hormones in the cystic ovaries of swine was accompanied by changes in the expression and cellular distribution pattern of steroidogenic enzymes we evaluated action of leptin on CYP11A1, CYP17, 17β-HSD and CYP19 protein expression by western blot. During prepubertal time, leptin in both doses had no effect on steroids secretion by medium-size follicles, why increased $P_4$ and testosterone secretion with concomitant decrease in $A_4$ secretion and no effect on $E_2$ secretion by small size follicles. It was accompanied by increasing the expression of CYP11A1 in both size of follicles and 17 β-HSD in small follicles. In cycling animals, increase in $P_4$ and testosterone secretion with concomitant decrease in $A_4$ secretion and no effect on $E_2$ secretion by small and medium size follicles was noted. It was accompanied by increasing the expression of CYP11A1 and 17 β-HSD in both size of follicles.
These results suggested that leptin by action on above-mentioned enzymes, and influencing the ovarian steroid synthesis, may play an essential role in the creation and/or course of cystic ovarian disease both in prepubertal and regular cycling animals.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P955

**The role and mechanisms of action of leptin in control of ovarian functions**

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The results concerning the role of leptin in mediating effect of food restriction on ovarian functions, in controlling these functions, as well as the endocrine and intracellular mechanisms of leptin action are reviewed.
In our experiments, food restriction resulted in a reduction in rabbit and chicken plasma leptin, GH, IGF1 and gonadal steroid hormone levels and ovulation rate. Malnutrition (serum deprivation) inhibited hormone release and the expression of proliferation- and apoptosis-related substances (PCNA, cyclin B1, bax, bcl-2, ASK-1, MAP kinase, protein kinase A, CDC2 kinase, transcription factors p53, CREB1, STAT-1 and NFkB) and promoted the accumulation of heat shock proteins mRNA in cultured rabbit, human, porcine and chicken ovarian cells. Administration of leptin or IGF1 both *in vivo* and *in vitro* altered hormone release and the expression of these proliferation- and apoptosis-related substances, oocyte maturation, response of ovarian cells to gonadotropin and other hormones, and prevented effect of malnutrition. Immunoneutralisation of IGF-I reversed the effects of leptin on the secretory activity of human granulosa cells. Inhibitors of protein kinases PKA, MAPK and CDC2, as well as the transfection of ovarian cells with cDNA constructs encoding ASK-1, p53, STAT-1 and CREB-1 were able to affect ovarian functions listed above, as well as to promote or prevent effects of leptin and IGF1.
The present data suggest that leptin/IGF1 axis plays an important role in control of ovarian functions in mammals and birds. It is proposed, that food restriction can control reproductive functions via changes in leptin output, which in turn, through IGF1, protein kinases, transcription factors and heat shock proteins, can affect ovarian cell proliferation, apoptosis, secretory activity, response to hormonal stimulators and fecundity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P956

**What is the earliest predictor of atherosclerosis in normal weight polycystic ovary syndrome; carotid intima media thickness or visceral fat ratio?**

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Purpose
The aim of this study in new diagnosed adult women with polycystic ovary syndrome (PCOS) was to evaluate carotid intima media thickness (CIMT) and visceral fat thickness (VFT) measurement by ultrasonography and which method can be used for atherosclerosis risk follow-up.
Methods
A case-control study was conducted on 22 PCOS women (18–30 years of age) and 22 controls. BMI was matched volunteer controls. Two different criteria were used to assess atherosclerosis risk. CIMT and VFT were measured by ultrasonography. In addition, visceral fat ratio (VFR) was calculated. The variables were compared using the $\chi^2$ test and Mann–Whitney $U$ test.
Results
In PCOS women, impaired lipid profiles were not determined. Also, the mean CIMT and VFT were no significantly higher in PCOS subjects. But, VFR was significantly higher in PCOS women ($1.21 \pm 0.33\%$) compared to controls ($0.85 \pm 0.3\%$, $P = 0.001$).
Conclusions
These findings indicate that CIMT which used early diagnosing of atherosclerosis have not increase in newly diagnosed PCOS women. In the other hand, VFR is increasing in newly diagnosed PCOS women. So, VFR can be used for diagnosis and follow-up of suspicions atherosclerosis as an early diagnostic method.
Keywords: Polycystic ovary syndrome (PCOS), atherosclerosis, carotid intima media thickness (CIMT), visceral fat thickness (VFT), visceral fat ratio (VFR).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P957

**Prevalence of hyperandrogenism, polycystic ovary syndrome and metabolic syndrome in female-to-male transsexuals**

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Introduction
Gender identity disorder (GID) is a disagreement between biological sexual differentiation and self-declared gender identity. The aetiology of GID remains unclear, although endocrinological, neuroanatomical and psychosocial factors are all thought to be causally involved. Polycystic ovary syndrome (PCOS) is characterized by chronic anovulation, polycystic ovarian morphology, and biochemical and/or biological signs of hyperandrogenism. Most women with PCOS also exhibit insulin resistance and hyperinsulinaemia, which is independent of obesity. Several studies have found a high prevalence of PCOS in FTM patients. In addition, it has been suggested that hyperandrogenism could be related to the development of GID in male transsexuals
Objectives
To determine the prevalence of hyperandrogenism, polycystic ovary syndrome (PCOS) and metabolic syndrome (MS) in female-to-male transsexuals (FMT).
Design and methods
In total, 77 FMT were assessed clinically and biochemically to hyperandrogenism, before the beginning of the treatment with testosterone. We also assessed cardiovascular risk factors and other parameters of MS.
Results
About 26.0% of the sample had overweight, and 19.5% were obese patients. The prevalence of hyperandrogenism was 49.35% and those of PCOS was 36.4%, and 51.9% of patients had MS.
By adjusting the parameters of MS and PCOS, for the body mass index (BMI), we observed that the higher BMI, regardless of the concentrations of free testosterone (FT), increases insulin resistance (HOMA-IR 2.43 vs 2.93 vs 3.85, $P < 0.001$). Of all patients, 27.3% had HDL-cholesterol below 50 mg/dl.
Conclusions
The general hyperandrogenism, and PCOS in particular, are highly prevalent in FMT. The high prevalence of PCOS appears to be related to body weight.
The hyperandrogenism is associated with the development of MS, and other factors such as insulin resistance and decreased HDL-C, which globally increase the cardiovascular risk. These data suggest that gender dysphoria, at least in FMT, could be related to hyperandrogenism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P958

**Examination of the role of gynecologists in the management of patients with disorders of sex development**

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Purpose
Disorders of sex development (DSD) are defined as having, 'A chromosome, germinal gland, or anatomical structure that is atypical in the native state'. There are two types of patients with DSD who consult a gynecologist: patients diagnosed in childhood (group A), and patients diagnosed based on unusual secondary sex characteristics in adolescence (group B). In group A, gynecologists are required as a member of a multidisciplinary medical team for DSD. On the other hand, in B group they should take the initiative in the diagnosis, treatment, and follow-up for unusual presentations of secondary sex characteristics. To examine the status of the management of DSD patients, a retrospective analysis was conducted.
Methods
In total, 106 patients with DSD who consulted in our department were enrolled in this study with their consent. Clinical data were extracted from their medical records.
Results
There were 53 patients (50%) in group A, most of whom (45 patients, 84.9%) had Turner syndrome and who were referred by pediatricians for hormone replacement therapy (HRT). There were 53 patients (50%) in group B, of whom 15 patients had an abnormality of the uterus and vagina, ten had Rokitansky syndrome, and eight had androgen insensitivity syndrome. All patients in group B were referred by gynecologists in other hospitals for diagnosis, medical treatment, and follow-up of primary amenorrhea. HRT was administered to most patients in group A while surgical treatments, such as removal of vaginal septum, vaginoplasty or prophylactic gonadectomy were performed.
Conclusion
In our department, the percentage of DSD patients diagnosed in childhood and in adolescence were the same (50%). It was demonstrated that management based on the individual anatomical or functional abnormality was important. DSD patients also require reproductive care, such as management of ovarian function and fecundity by gynecologists throughout their life.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P959

**Polycystic ovary syndrome, hyperprolactinemia, hyperinsulinemia and congenital adrenal hyperplasia**

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Objective
To research the frequencies of HPRL, HI and CAH in PCOS, as well deducing their significance.
Material and methods
For five years, 96 patients and their clinical histories were examined by the author, as each patient was visited.
HI was proven by 3 h OGGT, testing glucose and insulin simultaneously, ovarian ultrasonography, testosterone, androstenedione, DHEA, 17-hydoxyprogesterone, PRL and LH/FSH were also investigated.
The percentages and the confidence intervals (IC) were calculated with $P < 0.05$.
Results
Percentages: HPRL in PCOS: 52.08% (50/96); CI = (0.393–0.602). All cases with HI: 78.12% (75/96); CI = (0.6732–0.8502). All cases with CAH: 34.3575% (33/96); CI = (0.26–0.4596). Only HI: 29% (28/96); CI = (0.2042–0.3994). Only HPRL: 5.2% (5/96); CI = (0.0168–0.1178). Only CAH: 7.29% (7/96); CI = (0.0302–0.145). HPRL + HI + CAH: 16.66% (16/96); CI = (0.0984–0.2574). HPRL + HI: 26.04% (25/96); CI = (0.1766–0.361). HPRL + CAH: 4.166% (4/96) ; CI = (0.0114–0.1034). HI + CAH: 6.25% (6/96); CI = (0.0232–0.1316). None of these three: 5.2% (5/96); CI = (0.0168–0.1178).
Thus HPRL is very common in PCOS
These findings are basically coincidental with other authors. We can deduce PCOS may be the offspring of a complex aetiology: HI, CAH and HPRL, separately or jointly, all cause hyperandrogenemia by different pathways, although there is another group with none of these three causes. Recently, another cause: stress, was proven in rats; this was neutralized by cutting the ovarian nerves. Is this similar in human females?
Conclusion
PCOS is most frequently caused by HI, but other aetiologies are possible, among them CAH and HPRL. All three can be found together or separately; furthermore, stress may be another cause in some patients. Hyperprolactinemia is very common, appearing in around 52.08%, IC = 39.3–60.2%.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P960

**The future of HRT**

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Objective
This work sets forth the current status and perspectives of HRT.
Material and methods
In total, 547 medical works dealing with this subject are evaluated.
Results
The beneficial effect of estrogens on the lipids, as well as the antagonistic influence of progestogens, have long been known.
After MWS, HERS and WHI, the beneficial effects on several organs – the arterial system included – have been confirmed. Currently, it is clear that the sooner HRT is established, the better.
The ASRM have recently published a matched 10 000/10 000 study reporting Alzheimer diminishment by 34% and only an eight case (41/33) increase in breast cancer diagnoses. This small increase is related by several works to continuous, but not cyclic, therapy and does not exist in Japanese women; this is thus attributed to a western life style. Tobacco tar was once blamed for this increase, because it yields alquilestrogens in the first passage of estrogens through the liver; percutaneous administration of estradiol prevents this increase in breast cancer frequency and also thromboenbolism peril.
In addition, when HRT is given in cycles, using bio-identical hormones, the carcinogenic effect is non-existent; continuous dosage stimulates breast and PRL secretion, the contribution of which latter to breast cancer growth is now well known.
Presently, lung cancer is attributed to HRT; this is surprising because the lung is not a hormonal target organ, but estrogen receptors were reported by these authors to be found in the lung.
Male HRT is commended by most authors, however, PSA, prostate size, and red blood cells must be controlled.
Conclusion
The cyclic therapy with bio-identical hormones via the skin might be the future of HRT, because it is the safest method and permits longer use of the HRT, solving the problems of breast cancer increase and thromboembolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P961

**Plasma adiponectin levels in women with polycystic ovary syndrome: impact of metformin treatment in a case–control study**

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Objective
An interaction between adiponectin, steroid synthesis or action and measures of insulin resistance (IR) have been reported in the pathogenesis of polycystic ovary syndrome (PCOS). The present study was done to determine plasma adiponectin concentration (PAC) in women with and without PCOS and to assess its correlation to the hormonal and metabolic parameters including measures of IR. The effect of metformin for 6 months in PCOS was also evaluated.
Patients
In total, 72 selected women were classified as follows: 17 obese (body mass index (BMI)) $>25$ kg/m$^2$ with PCOS; 19 normal weight (BMI 18–22.9 kg/m$^2$ with PCOS; 17 obese BMI $>25$ kg/m$^2$ without PCOS and 19 normal weight (BMI) 18–22.9 kg/m$^2$ without PCOS.
Interventions
Blood samples were collected from all women with PCOS between 0800 and 1100 h, after an overnight fast.
Main outcome measures
Serum level of LH, FSH, TSH, total $T_4$, testerosterone, 17-$\alpha$-hydroxyprogesterone (17OHP), DHEAS, insulin, adiponectin and glucose. Measures of IR included fasting serum insulin (FSI), glucose-to-insulin ratio, and homeostasis model assessment (HOMA).
Result
Waist-hip ratio (WHR), insulin, and HOMA index were significantly higher in the lower adiponectin group than in the higher adiponectin group. By using stepwise multiple regression analysis, in model 1 (including BMI, FSI, fasting plasma glucose (FPG) with other variables such serum as testerosterone and DHEAS), the weight and contributions from other variables, namely FSI and FPG were significant independent determinants of fasting PAC (adjusted $r^2 = 0.66$); and in model 2 (including BMI, HOMA, FPG only as an index of IR with other variables such as serum testerosterone, DHEAS), BMI, HOMA, were significant independent determinants of fasting PAC (adjusted $r^2 = 0.59$). FPG, HOMA Index and FSI were significantly lower after metformin treatment in both obese and non-obese PCOS while adiponectin levels increased significantly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P962

**Impaired beta cell function in Chinese women with polycystic ovary syndrome**

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Objective
The role of pancreatic insulin secretion in Chinese women with polycystic ovary syndrome (PCOS) is not clear. To evaluate insulin sensitivity (IS) and β-cell function in lean and obese women with PCOS, either separately or by using an disposition index (DI).

Methods
Cross-sectional study involving 137 Chinese women with PCOS and 123 normal women were examined by anthropometry, lipid profile, sex hormone, high-sensitivity C-reactive protein, oral glucose tolerance tests (OGTTs), and insulin tolerance tests. Statistical analysis used one-way ANOVA, Kruskal–Wallis, and Mann–Whitney *U* tests, as appropriate.
Results
After controlling for BMI status, the Matsuda index was significantly lower in women with PCOS in comparison to those of normal women ($P<0.01$). With regard to the insulin resistance in the homeostasis model it was found that the assessment index was significantly higher in women with PCOS in comparison to those of normal woman ($P<0.001$). On the other hand, early phase insulin secretion (insulinogenic index) remained significantly lower in lean women with PCOS than those of both lean and obese women of the control group ($P=0.007$, and $P=0.10$ respectively). The mean Log HOMA-F values were significantly lower ($P=0.045$) in obese women with PCOS than those with BMI-marked women. Further, all disposition indexes (DIs) derived from non-fasting state indexes in women with PCOS were significantly lower than those of BMI- and WHR-matched control women ($P<0.001$ for all). Lastly, DIs derived from fasting states indexes in obese women with PCOS were significantly lower than those of lean women with PCOS.
Conclusion
Impaired β-cell function was detected in both the lean and obese women with PCOS. However, more serious primary defect in insulin action was observed in lean women with PCOS compared to obese women with PCOS. These findings imply that early screening and intervention for PCOS could be therapeutic for Chinese women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P963

### Epicardial adipose tissue thickness in patients with polycystic ovary syndrome

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EAT has been linked to the presence and severity of cardiovascular disease. EAT has been linked to the presence and severity of cardiovascular disease. We searched if the patients with PCOS have increased EATT, or not.
Total of 41 subjects with PCOS and 46 age and body mass index (BMI) matched healthy controls were enrolled. EAT was measured by echocardiography.
EATT and HOMA-IR score were significantly higher ($P=0.0001$ for both) while plasma adiponectin concentration was significantly lower ($P=0.048$) in PCOS patients. EATT correlated positively with total cholesterol, triglyceride, insulin, LH, LH/ FSH, HOMA-IR score and negatively with SHBG ($P<0.05$ for all), whereas it displayed no correlation to plasma adiponectin level ($P=0.924$). Triglyceride level and LH/FSH ratio were the significant determinants of EATT in logistic regression analysis ($P=0.0001$, $P=0.024$ respectively).

**Table 1** Anthropometrics and basic laboratory results of patients with PCOS and healthy controls.

| Parameter | PCOS subjects ($n=41$) | Controls ($n=46$) | *P* value |
|---|---|---|---|
| Age (year) | 20.9±3.2 | 21.3±3.0 | 0.159 |
| BMI (kg/m$^2$) | 23.6±4.3 | 22.4±2.6 | 0.138 |
| Waist-to-hip ratio | 0.7±0.0.4 | 0.7±0.04 | 0.923 |
| Fasting plasma glucose (mg/dl) | 87.3±8.1 | 82.2±6.4 | 0.002 |
| Total cholesterol (mg/dl) | 162.1±38.7 | 159.7±32.6 | 0.947 |
| HDL-cholesterol (mg/dl) | 50.0±11.7 | 53.8±9.7 | 0.102 |
| LDL- cholesterol (mg/dl) | 90.7±28.5 | 93.0±27.7 | 0.710 |
| Triglyceride (mg/dl) | 105.7±71.7 | 64.1±21.2 | 0.002 |
| LH (mIU/ml) | 7.4±4.8 | 5.3±3.1 | 0.016 |
| FSH (mIU/ml) | 4.9±1.9 | 5.5±1.6 | 0.151 |
| Total testosterone (ng/dl) | 63.1±28.8 | 43.4±15.6 | 0.0001 |
| SHBG (nmol/l) | 32.9±16.8 | 59.8±26.3 | 0.001 |
| DHEA-S (μg/dl) | 345.0±135.0 | 283.5±95.1 | 0.018 |
| LH/FSH | 1.6±1.0 | 1.0±0.6 | 0.002 |
| HOMA-IR value | 2.2±1.2 | 1.3±0.5 | 0.0001 |
| Insulin (mIU/ml) | 10.3±5.1 | 6.5±2.2 | 0.0001 |
| Adiponectin (μg/ml) | 10.0±3.7 | 12.5±6.1 | 0.048 |
| EAT thickness (mm) | 4.4±1.5 | 3.2±1.1 | 0.0001 |

BMI, body mass index; HDL, high density lipoprotein; LDL, low high density lipoprotein; SHBG, sex hormone binding globulin; HOMA-IR, homeostasis model assessment of insulin resistance; EAT, epicardial adipose tissue.

EATT is increased in patients with PCOS and may be an effective tool to determine those PCOS subjects at the risk of cardiovascular events.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P964

### High plasma level of long pentraxin 3 is associated with insulin resistance in women with polycystic ovary syndrome

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PCOS is characterized by insulin resistance. Inflammation has been anticipated to play role in the pathogenesis of both insulin resistance and atherosclerosis. Pentraxin 3 (PTX3) is an inflammatory mediator synthesized in a variety of cells and tissues. In the present study, serum PTX3 level and its relationship with insulin resistance were investigated in patients with PCOS. In total, 40 patients with PCOS and 40 age and body mass index (BMI) matched healthy controls were enrolled in the study. Plasma levels of PTX3, hs-CRP and HOMA-IR scores were all significantly higher ($P=0.021$, $P=0.002$, and $P=0.0001$ respectively) in women with PCOS compared to healthy controls. Blood PTX3 level correlated positively with hs-CRP, BMI, waist-to-hip ratio, HOMA-IR, and negatively with high-density lipoprotein cholesterol level ($P<0.05$, for all). After adjustment for age and BMI, PTX3, total testosterone levels and BMI remained as independent predictors of HOMA-IR scores ($P<0.05$, for all). PTX3 level is increased in patients with PCOS in concordance with insulin resistance.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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**Table 1** Clinical and endocrine features of PCOS patients and their controls.

| Variable | PCOS ($n=40$) | Control ($n=40$) | *P* |
|---|---|---|---|
| Age (year) | 21.3±4.7 | 21.9±3.5 | 0.062 |
| BMI | 24.7±5.3 | 22.5±2.4 | 0.232 |
| WHR | 0.73±0.5 | 0.73±0.1 | 0.705 |
| Fasting plasma glucose (mg/dl) | 88.3±8.1 | 82.3±6.8 | 0.001 |
| Total cholesterol (mg/dl) | 160.4±33.9 | 165.7±34.4 | 0.513 |
| LDL-cholesterol (mg/dl) | 89.1±25.1 | 98.3±29.5 | 0.162 |
| HDL-cholesterol (mg/dl) | 48.8±11.1 | 53.7±9.3 | 0.705 |
| Triglyceride (mg/dl) | 109.8±74.1 | 68.3±23.7 | 0.004 |
| LH/FSH ratio | 1.5±1.0 | 0.9±0.6 | 0.002 |
| Estradiol (pg/ml) | 66.8±78.3 | 64.1±87.0 | 0.679 |
| Testosterone (ng/dl) | 68.1±29.9 | 41.9±13.4 | 0.0001 |
| DHEA-S (μg/dl) | 337.3±147.1 | 268.9±82.2 | 0.018 |
| Pentraxin 3 (ng/ml) | 1.0±3.6 | 0.8±0.3 | 0.021 |
| hs-CRP (μg/ml) | 1.9±1.6 | 1.0±1.0 | 0.002 |
| Insulin (mIU/ml) | 11.3±4.7 | 6±1.8 | 0.0001 |
| HOMA-IR | 2.5±1.1 | 1.3±0.5 | 0.0001 |

BMI, body mass index; WHR, waist to hip ratio; HDL, high density lipoprotein; LDL, low density lipoprotein; C, cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance.

## P965

### Sertoli cells ovarian tumor- an ovarian incidentaloma?

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Introduction
Infertility investigations include uterine–ovarian ultrasound. The incidental finding of a tumor in a young female might associate a potential severe prognosis in otherwise completely asymptomatic patient.

Aim
We present a case of a young female with an accidentally discovered ovarian tumor.
Case report
S.M. 35-year female patient is admitted for infertility. Her family and personal medical history is irrelevant. The biochemistry and endocrine profile is normal, including plasma testosterone, LH, FSH, TSH and 21-day plasma progesterone of 11.7 ng/ml. The pelvic ultrasound pointed a uterus of 39 by 39 mm, normal right ovary and a hypoecogen solid tumor of 31 by 30 mm with penetrating vessels at the level of the left ovary. The pelvic CT showed a left ovarian, homogenous, well shaped, iodophile tumor of 29 by 27 by 32 mm, and no lymph nodes. Left ovarectomy was performed and the pathological report showed a Sertoli cells (annular tubes variant). The immunohistochemistry revealed a ki67 of 1–3%, positive ck7 and vimentin into the tumor cells and negative CROMO, CEA, ck20, inhibin, 34βE12 and positive actin into the stroma but not into the tumor. The benign profile of the tumor did not make necessary any additional therapy, except for follow up. For the moment, no fertility procedures are indicated.
Conclusion
Practitioners should be aware of the possibility that an ovarian incidentaloma might be associated with fertility issues.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P966

**Clinical, hormonal and metabolic profile in overweight and obese women with polycystic ovary syndrome**

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Polycystic ovary syndrome (PCOS) is a heterogeneous condition affecting 5–10% of reproductive -age female population.
The aim of this study was to determine the clinical features, metabolic and hormonal profile in obese and overweight women with PCOS.
Patients and method
A total of 102 women (age 31.28 ± 6.05 and BMI 33.02 ± 5.93) with PCOS and 110 (age 32.35 ± 4.96 and BMI 33.05 ± 5.87) matched controls were investigated. In all patients the basal glucose, oral glucose tolerance test, cholesterol (total, HDL, LDL), triglycerides, testosterone, FSH and LH were determined in the follicular phase of the menstrual cycle. Also, the blood pressure, presence of acanthosis nigricans and smoking were recorded in both groups.
Results
PCOS patients compared with controls had increased levels of: total cholesterol (214 ± 40.08 mg/dl PCOS vs 206.61 ± 38.72 mg/dl non-PCOS $P < 0.05$), 2 h OGTT glucose (122.21 ± 36 mg/dl PCOS vs 112.06 ± 24.30 mg/dl non-PCOS $P < 0.05$), LH (8.37 ± 4.56 mUI/ml PCOS vs 4.14 ± 2.84 mUI/ml non-PCOS $P < 0.001$), testosterone (1.65 ± 1.14 ng/dl PCOS vs 0.34 ± 0.32 ng/ml non-PCOS $P < 0.001$). A significant difference existed also for high blood pressure (20% PCOS vs 13% non-PCOS $P < 0.05$) and acanthosis nigricans (16% PCOS vs 5% non-PCOS $P < 0.001$). No difference was mentioned regarding smoking between the PCOS and non-PCOS groups.
Conclusions
Comparing the two groups there is a significant difference regarding the incidence of hypertension and achantosis nigricans. Even the values of total cholesterol and 2 h OGTT glucose are in the normal range, the differences between are statistical significant pointing out the necessity of screening for glucose intolerance and cardiovascular risk factors in all PCOS patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P967

**Serum 17β-estradiol concentration and depression symptoms presence in climacteric women**

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We studied 74 women admitted to the Department of Gynecological Endocrinology University of Medical Sciences of Poznan because of climacteric symptoms. All the studied women were older than 40 years of age, all were still menstruating or had their last menstruation not later than 1 year ago.
The aim of the study was evaluation of possible relationship between serum 17β-estradiol concentration and depression symptoms presence in climacteric women.
We evaluated climacteric syndrome severity with the use of Kupperman index, the severity of depression symptoms with the use of Hamilton scale, serum FSH, LH and 17β-estradiol concentration with the use of immunoenzymatic assay.
The mean value of Kupperman index in the study group was 27 points (S.D. ± 14 points), the mean value of Hamilton scale was 12 points (S.D ± 6 points). The mean serum FSH concentration was 53.2 IU/l (S.D ± 41.8 IU/l), the mean serum LH concentration was 33 IU/l (S.D ± 22.4 IU/l) and the mean 17β-estradiol serum concentration was 87.9 pg/ml (S.D ± 123.3 pg/ml). Among the depression symptoms included in the Hamilton scale we found statistically relevant differences in 17β-estradiol concentration in relation to sleep disturbances, lack of interest and psychic symptoms of fear and restlessness (all Mann–Whitney $U$ test $P < 0.05$).
We concluded that 17β-estradiol plays an important role in the etiology of depression symptoms in climacteric women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P968

**Assessment of insulin resistance and carotid intima media thickness, cardiovascular risk factors in PCOS with normal OGTT**

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The patients with polycystic ovary syndrome (PCOS) are at increased risk for insulin resistance, central obesity, dyslipidemia and hypertension. In our study, we aimed to detect whether women with PCOS who had normal OGTT results have had insulin resistance. Moreover we investigated the association of probable insulin resistance with cardiovascular risk indicators such as hs-CRP and carotid intima media thickness (CIMT).
In total, 34 untreated PCOS patients (mean age 24.06 ± 5.20 years) who had normal oral glucose tolerance test were included in our study. The control group was comprised of 20 healthy subjects with concordant age and BMI values with patient group. Exclusion criteria were the following: use of any drug (such as oral contraceptive, glucocorticoids, antiandrogens, anti-hypertensive drugs), history of diabetes mellitus, hypertension, anaemia, renal disease, liver disease, and systemic illness. Complete blood count, fasting blood glucose, hs-CRP, fasting insulin and hormone profile analyses were performed in patient and control groups. The HOMA-IR index was calculated in both groups. In this study, insulin resistance was determined in 22 (58%) patients with PCOS and normal OGTT results. Elevated hs-CRP levels and increased CIMT were determined in the patients with PCOS who had insulin resistance but the difference was not statistically significant. The hs-CRP levels were significantly higher in the patient group than control group ($P < 0.05$). A strong positive correlation between BMI and hs-CRP levels, CIMT was found in the patients ($P = 0.009$ and $P = 0.006$ respectively).
In conclusion, we suggested that there is a close relationship between PCOS and insulin resistance and cardiovascular morbidity even if OGTT results are normal.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P969

**Relation of hyperandrogenism and metabolic derangements in women with polycystic ovary syndrome**

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Introduction
Polycystic ovary syndrome (PCOS) is associated with a higher risk for development of metabolic syndrome. In this study, we evaluated the degree of metabolic disorders in women with different PCOS phenotypes.
Methods
We analyzed 162 women with PCOS (BMI: 24.9±6.1 kg/m$^2$, age:25.6±5.6 years) diagnosed on the basis of ESHRE/ASRM criteria, and 39 women without PCOS who comprised the control group (CG; BMI: 21.4±4.1 kg/m$^2$, age: 28.4 ±5.4 years). Women with PCOS were divided into three subgroups according to the presence of hyperandrogenism (HA): A – with biochemical HA ($n=97$), B – with clinical HA ($n=28$), and C – without clinical or biochemical HA ($n=37$). In all subjects basal blood samples were collected in follicular phase of menstrual cycle for determination of glucose, insulin, total cholesterol (TC), HDL, LDL, triglycerides, apolipoproteins (Apo) A1, A2, B and E, lipoprotein (a), C-reactive protein (CRP), testosterone and SHBG. We used the standard formula to calculate HOMA, FAI, and lipid ratios TC/HDL, LDL/HDL, triglycerides/HDL, ApoB/ApoA1. PCOS and CG differed ($P<0.01$) in age and body mass index (BMI), and the calculations were done with the correction for these parameters.
Results
In PCOS subgroups, significant difference was found in concentrations of Apo A1 ($P=0.017$) due to the difference between subgroups A and C (1.49±0.24 vs 1.72±0.32 g/l). PCOS subgroup A had significantly higher ($P<0.001$) FAI index compared to subgroup B and C (A: 14.4±11.0%, B: 4.3±1.0%, C: 4.3±1.4%). FAI and ApoA1 had significant positive correlation ($\rho=-0.32$, $P<0.01$).
Conclusion
Women with hyperandrogenic PCOS phenotype have a significantly lower value of favorable ApoA1 in comparison to the PCOS phenotype without hyperandrogenism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P970

**Micronized progesterone: a beneficial new modality of treatment for Egyptian patients with hyperemesis gravidarum**

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Aim
Progesterone is thought to be associated with hyperemesis gravidarum (HG). Pregnancies in which progesterone is administered for luteal phase support do not exhibit an increased incidence of HG, suggesting that high progesterone levels alone do not cause HG. The aim of the present study was to evaluate the therapeutic role of progesterone therapy as a new modality in the management of hyperemesis.
Subjects
In total, 40 Egyptian women below 40 years of age, pregnant 20 weeks with no preconception history of any other medical illnesses. They were randomly subdivided into: Group I: 20 patients managed by i.v. fluids and micronized progesterone 300–400 mg vaginally/day for 2 weeks. Group II: 20 pregnant women who received the traditional lines of treatment of HG. A control group composed of 15 pregnant women.
Methods
Assessment of fasting serum progesterone and serum estradiol level was done. Transabdominal ultrasound examination was carried out to exclude vesicular mole and multiple pregnancies.
Results
Before treatment: the mean value of serum estradiol in patients treated with micronized progesterone was found to be not significantly different from that of patients treated conventionally. The mean serum progesterone values of patients treated with micronized progesterone was found to be not significantly different from that of patients treated conventionally or that of control subjects. Patients treated conventionally had a significantly higher $E_2$/progesterone ratio than normal controls. After treatment: The $E_2$/progesterone ratio was significantly lower in patients treated with micronized progesterone when compared to those patients treated conventionally, but it was still significantly higher than that of control subjects.
Conclusions
Serum levels of estradiol are significantly higher in hyperemetic patients compared to normal pregnant women. When balance is restored by increasing natural progesterone levels, these symptoms typically disappear. Therefore, micronized progesterone has a significant beneficial effect to treat Egyptian pregnant women suffering from HG.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P971

**Significance of obesity in the genesis of metabolic syndrome in women with polycystic ovary syndrome**

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Introduction
Metabolic disturbances that are common in polycystic ovary syndrome (PCOS), represent a risk factor for cardiovascular disease. The aim of this study was to define contribution of obesity for the development of dyslipidemia and insulin resistance in PCOS.
Methods
PCOS was diagnosed using ESHRE/ASRM criteria. We evaluated 158 women with PCOS who were divided into two subgroups according to body mass index (BMI) cut off value 25 kg/m$^2$: non-obese PCOS ($n=93$; 20.65±2.0 kg/m$^2$; 24.9±4.6 years) and overweight/obese PCOS ($n=65$; 31.0±4.6 kg/m$^2$; 26.7 ±6.7 years). Controls group consisted of 30 healthy, non-obese (19.9 ±2.1 kg/m$^2$, 27.5±5.3 years). In all subjects fasting blood samples were collected in follicular phase of menstrual cycle for determination of glucose, insulin, total cholesterol (TC), LDL, HDL, triglycerides, apolipoproteins (Apo) A1, A2, B and E, Lp(a), C-reactive protein (CRP), testosterone and SHBG. Blood pressure (BP), waist circumference (WC), free androgen index (FAI), homeostatic model (HOMA index), and ratios TC/HDL, LDL/HDL, triglycerides/HDL, ApoB/ApoA1 were also determined.
Results
In comparison to non-obese PCOS, obese PCOS had significantly higher levels of TC, LDL, triglycerides, ApoB, ApoE, CRP, HOMA, FAI, TC/HDL, LDL/HDL, triglycerides/HDL, ApoB/ApoA1, and significantly lower levels of HDL and ApoA1 ($P<0.001$). After adjustment for age, non-obese PCOS had borderline higher ApoB/ApoA1 than Controls ($P=0.051$). In PCOS group BMI significantly correlated with TC, HDL, triglycerides, HOMA, ApoB, and CRP ($P<0.001$). There were no significant correlations between FAI and metabolic indices. HOMA significantly correlated only with BMI ($P<0.001$).
Conclusion
Proaterogenic lipid profile and insulin resistance are metabolic consequences of PCOS. Obesity significantly worsens metabolic profile in women with PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P972

### Lipid accumulation product as a marker of metabolic syndrome in women with polycystic ovary syndrome

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Introduction
Women with polycystic ovary syndrome (PCOS) have higher prevalence of metabolic syndrome (MS) than healthy, age and BMI matched women. The aim of this study was to determine if novel abdominal adiposity index – lipid accumulation product (LAP), could predict MS in women with PCOS.
Methods
PCOS was diagnosed using ESHRE/ASRM criteria. We evaluated 159 PCOS women (PCOS group: $24.9\pm6.2$ kg/m$^2$; $25.8\pm5.4$ years) and 56 healthy women (control group: $22.9\pm5.8$ kg/m$^2$; $27.6\pm5.3$ years). MS was diagnosed according to JIS criteria (waist circumference cut off 80 cm) in 39/159 (25%) PCOS women, while no woman in controls group had MS. PCOS group was divided in subgroups: PCOS with MS ($31.9\pm5.4$ kg/m$^2$; $27.9\pm7.1$ years) and PCOS without MS ($22.8\pm4.6$ kg/m$^2$; $25.0\pm4.6$ years). In all subjects blood pressure (BP) and waist circumference (WC) were determined. Blood samples were collected in follicular phase of menstrual cycle for determination of basal glucose, insulin, HDL-cholesterol, triglycerides, testosterone, SHBG and homeostatic model (HOMA) index. LAP was calculated using formula ((WC-58)$\times$ triglycerides). All statistical analyses were adjusted for age and body mass index.
Results
PCOS had significantly higher LAP than controls ($37.3\pm43.5$ vs $18.5\pm17.1$, $P<0.001$). PCOS without MS had significantly lower LAP than PCOS with MS ($20.6\pm17.5$ vs $90.6\pm57.3$, $P<0.001$) and significantly higher LAP than controls ($P=0.016$). JIS criteria for MS, testosterone, SHBG and LAP entered binary logistic regression analysis which showed that independent predictors for MS in PCOS group were: LAP ($P=0.002$), systolic BP ($P=0.003$) and HDL-cholesterol ($P=0.004$). In PCOS, LAP significantly correlated with HOMA ($P=0.42$, $P<0.001$).
Conclusion
LAP is useful surrogate marker for the assessment of metabolic syndrome in women with PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P973

### Serum 25-hydroxyvitamin D and hsCRP levels in young obese women with polycystic ovary syndrome

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Insulin resistance is one of the common features of the polycystic ovary syndrome (PCOS), and recent studies indicate the possible role of vitamin D in the pathogenesis of insulin resistance and inflammatory response. We aimed to investigate the association between 25-hydroxyvitamin D (25(OH)D) and insulin resistance and inflammatory marker in obese women with PCOS and controls.
We recruited 50 obese women with PCOS (age $25\pm5$ years, BMI $27.5\pm2.3$ kg/m$^2$) and 50 age, BMI-matched controls (age $26\pm5$ years, BMI $27.3\pm2.1$ kg/m$^2$). Anthropometric measurements were obtained and metabolic and hormonal parameters were determined. Serum 25(OH)D levels were measured by IRMA (COBRA Quantum 5002) and hsCRP by TIA (ADVIA 2400). Glucose tolerance status was assessed by 75 g OGTT, and insulin resistance index was calculated from OGTT data.
The prevalence of insufficient 25(OH)D levels ($<30$ ng/ml) were 90% in women with PCOS and 88% in controls. Serum 25(OH)D and hsCRP concentrations are not different between women with PCOS and controls. 25(OH)D and hsCRP did not correlate with metabolic and hormonal parameters, and no significant association was found between 25(OH)D and insulin resistance index and hsCRP in both women with PCOS and controls.
25(OH)D insufficiency was prevalent in young obese women, but 25(OH)D and hsCRP levels were not associated with insulin resistance or PCOS itself. Future studies will be required to ascertain the relationship between 25(OH)D and insulin resistance in large number of subjects with various age and BMI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P974

### Association of metabolic disturbances and carotid intima-media thickness in women with polycystic ovary syndrome

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Introduction
Polycystic ovary syndrome (PCOS) is associated with higher risk of cardiovascular disease. Measurement of carotid intima-media thickness (CIMT) is a simple non-invasive procedure for assessment and measurement of atherosclerotic plaque. It is a valuable tool for evaluation of cardiovascular disease risk, as increase in CIMT is associated with cardiovascular events, mainly stroke. In this study we evaluated association and influence of metabolic disturbances on CIMT in women with PCOS.
Methods
PCOS was diagnosed using ESHRE/ASRM criteria. Ultrasound CIMT measurement was obtained on right and left common carotid artery in 98 women with PCOS (group PCOS: $24.6\pm5.5$ kg/m$^2$; $25.9\pm5.1$ years) and 37 healthy women (group controls: $22.9\pm5.1$ kg/m$^2$; $27.2\pm4.1$ years). Groups did not differ in age ($P=0.65$) and body mass index (BMI, $P=0.71$). In all subjects fasting blood samples were collected in follicular phase of menstrual cycle for determination of glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, C-reactive protein (CRP), testosterone and SHBG. Homeostatic model (HOMA index), free androgen index (FAI), mean CIMT and blood pressure (BP) were determined.
Results
In comparison to controls, PCOS had significantly higher mean CIMT ($0.56\pm0.68$ vs $0.47\pm0.59$, $P=0.022$), systolic BP ($P=0.042$), triglycerides level ($P=0.01$), and lower HDL-cholesterol level ($P=0.024$). All determined parameters entered multiple linear regression analysis which revealed significant model ($R^2=0.12$) where triglycerides level was the only independent predictor of CIMT in PCOS group ($P=0.002$).
Conclusion
CIMT is a surrogate marker for atherosclerosis. In this study we showed that women with PCOS have significantly higher CIMT in comparison to healthy, age and BMI matched women. Higher CIMT in PCOS women is associated with higher triglycerides level.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P975

### Prevalence of dyslipidemia in Algerian PCOS women

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting 6–10% of reproductive aged women. In young women with PCOS, multiple risk

factors for cardio vascular disease (CVD) including dyslipidemia may be found and prevention is needed.
Objective
This study aims to investigate the prevalence of dyslipidemia in Algerian PCOS women.
Materials
A prospective study was performed (since 2004–2008) on 181 PCOS and 90 age and BMI matched controls.
The data of anthropometric measurements, oral glucose tolerance test, serum lipids levels, total cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein cholesterol were collected from PCOS and controls women.
Results
PCOS women have higher prevalence of hypertriglyceridemia (59.6 vs 29% $P=0.001$) and low HDL cholesterol (12.3 vs 6.8% $P=0.002$) than their counterparts. At the same time the mean level of triglyceridemia was higher ($1.04\pm0.08$ mmol/l vs $0.91\pm0.04$ $P=0.02$) and the mean level of HDL.C was lower ($1.28\pm0.04$ vs $1.52\pm0.004$, $P=0.01$) than the controls.
The prevalence of hypertriglyceridemia increased with BMI respectively 5.9, 14.6, and 16.9% for BMI $<25$; BMI 25–30 BMI more than 30 $P=0.002$. The prevalence of low level of HDL-C also increased with BMI respectively 45.5, 56.09 and 70.4% for BMI $<25$; BMI 25–30 BMI more than 30 $P=0.01$.
Conclusion
In our study the prevalence of dyslipidemia was found to be high in PCOS women. PCOS should be screened for dyslipidemia. Diet, physical exercise and if necessary lipid lowering medication should be used to normalize dyslipidemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P976

**Serial serum levels of vitamin D in lean and obese pregnant women**

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Objective
Obesity is a major problem in pregnancy. Obesity has been associated with decreased vitamin D levels, diabetes and reproductive risks. Women were followed through pregnancy with frequent blood samples to determine any differences between lean and obese subjects. We present data on vitamin D levels through gestation in these subjects.
Methods
Normal pregnant women at 6–9 weeks GA provided blood samples every 2 weeks and body weights were recorded. We report serial serum levels of vitamin D in lean ($n=8$) and obese ($n=6$) pregnant women, and in one subject with gestational diabetes. Samples from singleton ($n=35$) and twin ($n=31$) pregnancies between 15 and 20 weeks of gestation were also analyzed in order to determine whether increased placental mass (twins) would increase vitamin D levels over singleton pregnancies. Vitamin D levels were measured by chemiluminescent immunoassay (immunodiagnostic systems).
Results
For each subject, the mean of all serial results was calculated and then the mean $\pm$S.E.M. of individual means was calculated for the lean and obese groups. Mean ($\pm$S.E.M.) vitamin D levels for the lean subjects was $28.39\pm1.98$ ng/ml and for the obese subjects was $19.80\pm3.05$ ng/ml. Of six lean subjects, five had a least two values or more above the sufficiency level for vitamin D ($>30$ ng/ml) and only one subject in the obese or gestational diabetic subjects had a value $>30.0$ ng/ml. Most subjects, either lean or obese, had a slight increase in serum vitamin D levels over the course of gestation. In the singleton and twin comparison at 15–20 weeks, there were no differences vitamin D levels plotted against BMI.
Discussion
Vitamin D levels are greater in lean than in obese pregnant women. Vitamin D levels are generally insufficient in the obese group and slightly increased in the lean group.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P977

**Erythrocytosis and thrombocytosis secondary to hypertestosteronemia caused by ovarian leydig cell tumor**

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Testosterone has a dose-dependent stimulatory effect on erythropoiesis but the mechanism is poorly understood. Although both erythroblasts and megakaryocytes express androgen receptors, testosterone's effect on thrombopoiesis in humans has never been studied.
We report a case of 53-years-old woman presented with androgenic alopecia, hirsutism, facial redness and hoarse voice. She was a non-smoker and had a history of well-controlled diabetes mellitus type 2. Endocrinological evaluation disclosed total serum testosterone level of 34.8 nmol/l (normal range 0.1–1.4), free serum testosterone of 693 pmol/l (normal range 1–33) and suppressed FSH and LH levels. Blood routine samples taken on two separate occasions disclosed erythrocytosis and trombocytosis. White blood cell count, erythrocyte sedimentation rate and C-reactive protein were all within normal ranges. Abdominal and pelvic computed tomography, transvaginal ultrasonography, and pelvic magnetic resonance imaging failed to localize tumor. Normal dehydroepiandrosterone-sulphate levels indicated ovarian origin of hypertestosteronemia. Therefore, laparoscopic bilateral ovariectomy was performed. Focal tumor mass was not found on macroscopic examination. Microscopic examination disclosed a part of tumor tissue in the hilus of the left ovary. Tumor mass was torn apart and cauterized during surgery and therefore it is true size could not be measured. Tumor tissue stained positively for inhibin and calretinin and the diagnosis of hilus Leydig cell tumor was established. Testosterone level decreased to 1.7 nmol/l, RBC decreased from 5.7 to $4.7\times1012$/l, hematocrit level from 0.520 to 0.420, haemoglobin from 170 to 141 g/l and PC decreased from 480 to $378\times109$/l level normalized 2 weeks after the surgery and remained normal 3 months postoperatively.
Ovarian Leydig cell tumors are rare. Their association with erythrocytosis was reported in one case and concomitant erythrocytosis and thrombocytosis have never been reported. Substantial, immediate and persistent decrease in both RBC and PC after tumor removal suggests testosterone's synergistic effect on erythropoiesis and thrombopoiesis. Further studies are worthy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P978

**Quality of life in patients affected by polycystic ovary syndrome**

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Background and aims
Polycystic ovary syndrome (PCOS) is characterized by phenotypic heterogeneity and a wide variety of consequences. About half of PCOS women are overweight or obese and their obesity may be a contributing factor to PCOS pathogenesis. The aim of this study was to evaluate if PCOS alone affects the patients' quality of life and to what extent obesity contributes to worsen this disease.
Methods and results
In total, 200 women with PCOS, 100 with BMI $>25$ and 100 with BMI $<25$ were recruited. They were evaluated with the 36-items short-form health survey (SF-36) and the polycystic ovary syndrome questionnaire (PCOQ20) and were compared with a healthy control group of 80 women, adequately matched.
The univariable analysis evidenced that PCOS patients, compared with controls, had decreased scores, indicating lower HRQoL, in all domains, excluding Bodily pain and Social role functioning; the most significant differences ($P<0.001$) between PCOS and control patients with BMI $\geq25$ were evidenced for emotional role functioning and summary mental health (SF-36), weight and acne (PCOQ20). Multivariable regression analysis evidences that in subjects with BMI $\geq25$, high values of BMI itself predict the possibility of being affect by PCOS; low scores in physical functioning, Physical role functioning, vitality, emotional role functioning and Mental health were identified as multivariate predictors of PCOS. Therefore, PCOS determines a relevant impairment of psycho-social functions in women; moreover, higher is the BMI, worse is the score of mental and emotional domains.
Conclusions
PCOS itself is a great cause of psychological morbidity and contributes to an overall diminished HRQoL; overweight-obesity, when present, considerably

concurs to worsen it. PCOQ20 is a very sensitive test to evaluate HRQoL in PCOS patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P979

### Effects of dopamine agonist administration during seasonal cyclicity in the mare

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Prolactin is associated with seasonal transitions into and out of anestrus in the mare. The earliest anestrous transitional changes are declining progesterone secretion and a concomitant prolactin decline. Ovarian changes include slowed follicular development and prolonged luteal activity. Dopamine antagonist administration during the autumnal transition prevented a seasonal progesterone decline, suggesting a cause-and-effect between prolactin and progesterone production. The present study sought to mimic decreased autumn prolactin secretion during summer cyclicity to investigate the prolactin–progesterone secretion relationship, possible prolactin influence over ovarian events and sexual behavior.
In total, 20 mares were divided into treatment (T) or control (C) groups (May 15–Aug 10) and assessed regularly for sexual behavior (teasing), endocrine function (plasma collection and RIA) and ovarian function (ultrasonography). Treatment mares received cabergoline, a long-acting dopamine agonist (5 mg orally every third day). Comparisons between groups (repeated measures ANOVA) included plasma prolactin and luteal progesterone concentrations, length of estrous cycle and its luteal and follicular phases, average daily follicular growth rates and numbers of small, medium and large-sized follicles. Sexual behaviors were scored as positive and negative and aggregate daily scores were compared between groups.
Plasma prolactin declined ($P<0.05$) within 24 h post-cabergoline treatment and remained lower than controls during the study. Luteal progesterone was not statistically different between groups, but there were some indications of progesterone decline in response to cabergoline. Although estrous cycle intervals were not different between T and C groups, late summer luteal phases tended to be shortened in T mares. Late diestrus (day 15) follicular growth wave suppression was demonstrated in T mares. Sexual behavior was different ($P<0.05$) between groups insofar as T mares demonstrated greater sexual ambivalence to the stallion, often displaying both positive and negative behaviors simultaneously.
Results suggest that prolactin/dopamine does influence some aspects of reproduction in the mare.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P980

### Differences in gonadal axis reaction to ketoconasole between PCOS and healthy individuals

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Background
Ketoconazole is a synthetic antifungal drug, and powerful inhibitor of steroidogenesis. Oral ketoconazole blocks both ovarian and adrenal androgen biosynthesis, and glucocorticoid synthesis, leading to a reduction in circulating testosterone and cortisol levels. In subjects with an intact HPA axis, ketoconazole by decreasing cortisol levels leads to compensatory rise an ACTH level.
Objectives
To assess influence ketoconasole induced changes in steroid synthesis on the hypothalamo-pituitary–gonadal axis and adrenal activity in PCOS.
Methods
The study group consisted of 44 women (i. PCOS group, $n=21$; ii. control group, $n=23$). During the follicular phase of menstrual cycle, $2\times400$ mg of oral ketoconazole was given. We measured FSH, LH, testosterone, DHEA-s, progesterone and 17-OH progesterone before ketoconazole and the morning after ketoconazole. Changes in the hormone concentrations were analyzed using Wilcoxon test, and presented as median (range) of the hormone change after ketoconazole. Positive values indicate increase and negative decrease after ketoconazole.
Results
FSH: $0.61\pm1.19$ vs $0.84\pm1.86$ IU/l; $P=0.47$; LH: $2.34\pm3.81$ vs $-0.11\pm2.4$ IU/l; $P=0.02$; testosterone $-1.19\pm0.83$ vs $-0.35\pm0.48$ nmol/l; $P<0.01$; DHEA-s $-1.28\pm1.19$ vs $-0.99\pm2.11$ nmol/l; $P=0.18$; progesterone $15.86\pm15.10$ vs $18.71\pm22.60$; $P=0.89$; 17OH progesterone: $1.71\pm1.79$ vs $0.95\pm1.07$; $P=0.15$; testosterone was significantly more suppressed in PCOS compared to control group. There was significant increase in LH in PCOS compared to control group.
Conclusions
Androgen synthesis in PCOS is more sensitive to ketoconazole inhibition than in non-PCOS subjects. This implies that it has a potential role in the PCOS treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P981

### Metabolic and hormonal parameters in lean PCOS women

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Background
A minority of women with polycystic ovary syndrome (PCOS) is lean, and several individuals have no signs of hyperandrogenaemia. Earlier we showed that lean PCOS patients have no increased visceral adiposity. The aim of the present study was study is to characterize the metabolic and hormonal status a group of lean PCOS cases.
Methods
In this cross-sectional study ten Caucasian women with PCOS and ten controls carefully matched for age and BMI were studied. Mean age was $31\pm2$ years, and all had BMI between 19 and 25 kg/m$^2$ and similar total fat contents, according to bioelectrical impedance analysis (BIA) and magnetic resonance imaging (MRI). Glucose, insulin, testosterone, SHBG, gonadotrophins, anti-Müllerian hormone (AMH), inhibin B and adipokines (adiponectin, leptin, TNF-α, IL6 and RBP-4) were assessed by standard techniques.
Results
BMI's were $21.6\pm1.1$ vs $21.8\pm2.1$ kg/m$^2$, waist-hip ratios $0.83\pm0.07$ vs $0.85\pm0.03$, fat mass 13.2 (4.9–19.7) vs 14.4 (4.0–22.5) kg and lean body mass 52.4 (45.2–59.4) vs 53.4 (44.4–59.7) kg for PCOS and control women, respectively (all $P=$NS). Fasting blood glucose and insulin levels were similar, and HOMA-IR was $0.55\pm0.19$ and $0.63\pm0.18$. Also the levels of adipokines were similar between both groups. PCOS cases had significant higher free androgen index (FAI), lower estradiol and lower FSH, but similar inhibin B. AMH levels were $11.1\pm3.0$ ng/ml in PCOS vs $3.3\pm1.8$ ng/ml in controls ($P<0.01$). Pooled ovarian volumes correlated positively with AMH levels, calculated for the largest ovary of each individual ($r=0.75$, $P<0.0001$) vs the smallest ovary ($r=0.70$, $P<0.001$), while positive correlations were only demonstrated between Inhibin B levels and the largest ovaries ($r=0.58$, $P<0.01$).
Conclusions
We observed no differences in metabolic parameters and insulin resistance between lean PCOS cases vs controls. Also in lean PCOS patients, AMH levels are increased. Similar ovarian-AMH relationships but different volume-inhibin B relationships confirm that both hormones originate from different ovarian cells (early antral vs granulosa cells). The differences in FSH need to be explored further.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P982

**Adiponectin, resistin, leptin and selected cytokines in association with insulin resistance in lean women with polycystic ovary syndrome**

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The polycystic ovary syndrome (PCOS) is associated with features of the insulin resistance syndrome and altered glucose homeostasis. Factors that play an important role in these processes are still emerging. Adipokines and pro-inflammatory cytokines may be involved in development of insulin resistance in PCOS. The purpose of this study was to determine if a relationship exists between adiponectin, resistin, leptin, interleukin (IL) 4, IL6, IL10, tumor necrosis factor α (TNF α) and insulin resistance indices in lean women with PCOS.

Methods

Fasting insulin, glucose, C-peptide, lipid profile, FSH, LH, prolactin, testosterone, sex hormone binding globulin, 17-hydroxyprogesterone, adiponectin, resistin, leptin, IL4, IL6, IL10, TNF α serum concentrations were analyzed at basal conditions in 12 lean women with PCOS (BMI $<25$ kg/m$^2$) and 17 age- and weight-matched healthy controls. Oral glucose tolerance test was performed. Homeostasis model assessment insulin resistance (HOMA-IR), Matsuda and Cederholm index were calculated. Statistics: Mann–Whitney *U* test and Spearman correlation.

Results

Matsuda and Cederholm index were significantly lower in lean women with PCOS than in control group ($P<0.05$ for both). Fasting glucose, insulin, C-peptide and HOMA-IR did not differ between groups. Adiponectin tended to be lower in PCOS ($P<0.054$). Resistin was significantly lower in PCOS than in control group ($P<0.05$). Leptin, IL4, IL6, IL10, TNF α levels did not differ between groups. In women with PCOS i) IL4 correlated significantly negatively with fasting glucose ($P<0.01$), ii) leptin correlated significantly positively with LDL cholesterol ($P<0.05$) and significantly negatively with Matsuda index ($P<0.05$).

Conclusions

In lean women with PCOS, the insulin sensitivity was decreased, however, serum levels of resistin were lower than in control group, leptin levels were associated with markers of insulin resistance and IL4 was connected with fasting blood glucose. Supported with the grants NS/9839-4 and CZ.2.17/1.1.00/32386.

Declaration of interest

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## P983

**Solution of an old endocrinological puzzle the rise and fall of an endocrinological doctrine Dr. László Makay, DVM H-2000 Szentendre, Sztaravodai u. 42, Hungary Tel./Fax: +36 26 316 791 E-mail: makay27@freemail.hu**

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Already a long time ago, the subtle adaptability of the reproductive endocrine system made it possible to elaborate a hormonal castration method for female pigs, which was based on the administration of a high-dose oestrogen (diethylstilboestrol) injection. The phenomenon was believed to be the result of a negative feedback reaction. However, this theory could not explain why more than 20% of the animals treated did not respond properly to the treatment. Therefore, the goal of these investigations was to get proper insights into the functioning of the relevant reproductive endocrine system. Although the treatment is no longer in use and the studies took place long ago, the results of the study presented here demonstrate a specific, so far unknown reproductive endocrine feedback phenomenon. Accordingly, the pig's reproductive endocrine system can respond to the administration of high-dose oestrogen injection by developing either of two different, opposite kinds of reactions, i.e. two distinct mechanisms of action. Whenever the reproductive endocrine system is able to do so, it always reacts by achieving a positive feedback reaction (the successful variant of reactions) rather than a negative one as had been believed previously. In the absence of this ability the negative feedback reaction (the unsuccessful variant of reactions) develops. However, this latter can be prevented by stimulating the activity of the reproductive endocrine system.

These findings disprove an old endocrinological doctrine and establish a new one.

Left: Ovaries of untreated gilts.
Middle: Follicular depression evoked by treatment.
Right: Luteal phase elicited by treatment.

Images of the ovaries
Left: ovaries of untreated gilts.
Middle: follicular depression evoked by the treatment (negative feedback reaction).
Right: luteal phase brought about by the treatment (positive feedback reaction).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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# Growth hormone IGF axis: basic

## P984

**Evidence of direct mitogenic activity of insulin and the insulin receptor in prostate cancer cell lines**

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Background

In addition to its normal spectrum of metabolic effects, insulin has been identified as a growth factor capable of promoting mitogenic activities. Thus, hyper-insulinemia, a consequence of insulin resistance, is regarded as a potential risk factor for the development of cancer in patients with diabetes. However, the mechanism of action of insulin in prostate cancer has not yet been completely elucidated. The aim of this study was to investigate whether insulin can directly induce mitogenic activity in prostate cancer-derived cell lines and to evaluate the role of the insulin receptor (IR) in mediating this activity.

Methods

A number of prostate cancer cell lines (LNCaP, P69, C4-2 and PC3), representing early and advanced stages of the disease, were employed. Insulin doses ranged between 0 and 500 ng/ml. Insulin-stimulated proliferation rates were measured by hemocytometer cell counting and MTT assay. Cell-cycle dynamics were evaluated by propidium iodide staining and FACS analysis. Activation of the IR was assessed by immunoprecipitation assays. Expression levels of the receptor were measured by western immunoblotting.

Results

Insulin induced cell proliferation in a dose-dependent fashion in the LNCaP and C4-2 cell lines, but not in P69 or PC3 lines. Cell cycle analyses showed that insulin can positively influence LNCaP and C4-2 lines to progress towards the G2/M phase. Immunoprecipitation assays show that in all of the cell lines expressing the IR, insulin activates IR but not IGF1R.

Conclusion

In the model studied, insulin exhibited direct mitogenic activities mediated exclusively through the IR. Further research is needed to fully dissect the molecular mechanism underlying the biological actions of insulin in prostate cancer.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P985

**Insulin receptor compensates for IGF1R inhibition and induces mitogenic activity in prostate cancer cell lines**

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Background
IGF1R targeting emerged in recent years as a promising therapeutic approach in prostate cancer (PCa). The insulin receptor (IR) shares high structure homology with the IGF1R and activates similar signaling cascades. In recent studies we have shown that insulin's mitogenic activities in PCa cells are mediated via the IR. Given the central role of IR in regulation of metabolism, most anti-IGF1R therapeutic strategies were designed to specifically inhibit IGF1R, but not the IR. Therefore, the aim of this study was to investigate IR ability to compensate and mediate IGF1 mitogenic signals after IGF1R inhibition.
Methods
We employed P69 and C4-2 prostate cancer-derived cell lines, which express both IGF1R and IR. To specifically inhibit IGF1R we used monoclonal antibody IMAC-A12 (ImClone) or Thyrphostin (AG1024). Activation of receptors was assessed by immunoprecipitation assays. Expression levels of receptors and activation of signaling cascades were measured by western immunoblotting. Cells viability was measured by MTT assays.
Results
IGFI mainly activates its own receptor but also led to significant cross-activation of the IR. Results of IP assays showed that specific inhibition of IGF1R in C4-2 cells did not affect cross-activation of IR by IGF1. In P69 cells, on the other hand, there was a minor inhibition of cross-activation. In neither cell line, IGF1R inhibition affected the activity of signaling cascade proteins Akt and ERK. Finally, MTT assays revealed that, despite a significant reduction in IGF1R abundance on cell membranes, IMAC-A12 had no effect on cells viability.
Conclusion
In our model, specific inhibition of IGF1R had no effect on the activation of IR by IGFI, providing evidence for a compensation mechanism by IR following IGF1R inhibition. The evidence for a compensatory mechanism between the two receptors may be of future use for the local targeting of both IGF1R and IR in PCa.
Declaration of interest
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## P986

**Designing long acting recombinant proteins: from bench to clinics**

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One major issue regarding the clinical use of many peptides is their short half-life span in the body, due to the rapid clearance from the circulation. Thus, at the clinical level, there is a need for a regime of frequent injections of the peptides into the patients to overcome this low stability factor. To overcome this problem, we succeeded to add the signal sequence of *O*-linked oligosaccharides to the coding sequence of the hormones. The cassette gene that has been used contains the sequence of the carboxyl-terminal peptide (CTP) of human chorionic gonadotropin-β (hCG-β) subunit. The CTP contains 28 amino acids and four *O*-linked oligosaccharide recognition sites. It was postulated that the *O*-linked oligosaccharides add flexibility, hydrophilicity and stability to the protein. On the other hand it was suggested that the four *O*-linked oligosaccharides play an important role in preventing plasma clearance and thus increasing the half-life of the protein in circulation. Using this strategy we succeeded to ligate the CTP to the coding sequence of follitropin (FSH), TSH, erythropoietin (EPO) GH, interferon β and thus to increase the longevity and bioactivity of these proteins *in vivo*. Interestingly, the new analog of FSH was found not immunogenic in humans and it is already passed successfully clinical trials phase III and approved by The European Commission (EC). In addition, our results indicated that long acting GH is not toxic in monkeys and it passed successfully clinical trials phases I and II and the protocol for phase III was approved. The preliminary results regarding interferon β seem to be promising. Thus, using this technology seems to be promising in designing long acting peptides. Development of long acting peptides will diminish the cost of these drugs and perhaps reduce the number of injections in clinical protocols.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P987

**GH harmonisation: it sounds simple…,?**

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Objective
The concentration of GH (hGH) is important in the attribution of hGH therapy, for which in The Netherlands a national committee is responsible. Without this authorization re-imbursement is impossible. Therapy is permitted if all hGH concentrations after two stimulation tests are below 20 mU/l and in some clinical conditions independent of hGH. However, different assays produce different results and nationwide hGH results varied with CV ~30%. Consequently, authorization for hGH therapy depends on the laboratory that analyzes the samples, leading to social inequality. The national committee asked the Section of Endocrinology of the Dutch Foundation for Quality Assessment in Medical Laboratories to diminish nationwide variation.
Method
Since, standardization of assays is impossible, it was decided to harmonize hGH assays. A commutable sample was developed, being defined as behaving in all assays exactly as patients samples would do. The sample was obtained by mixing sera from healthy volunteers after 20 min of intensive exercise. Such samples contain all relevant isoforms due to *de-novo* synthesis of hGH. This sample was diluted with serum with low hGH concentration to obtain concentrations around the clinical decision limit of 20 mU/l. Next, it was freeze-dried and distributed to all participating laboratories. This harmonization sample was used to recalculate all hGH concentrations with a harmonization factor being the ratio of the measured and attributed concentration. In external quality control assessment schemes the reduction of variation was verified.
Results
After the introduction of the harmonization sample the nationwide variation of hGH assays dropped to <10%. Consequently, it was decided that laboratories must use the harmonizing method to assure that patients eligible for hGH therapy receive authorization with respect to social equality.
Conclusion
Harmonization of hGH assays is a valid method to reduce variation and to promote equality between patients when prescribing hGH therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P988

**Plasma ghrelin levels appeared to be elevated in patients with medium-chain acyl-CoA dehydrogenase deficiency and glutaric aciduria type II: evidence for that acyl-CoA is the substrate for ghrelin acylation**

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Ghrelin requires a fatty acid modification for binding to the GH secretagogue receptor. Octanoylation of the Ser3 residue of ghrelin is essential for

ghrelin-mediated stimulation of GH secretion and regulation of energy homeostasis via increased food intake and adiposity. Other than octanoylation (C8:0), the hormone is subject to other types of acyl modification, decanoylation (C10:0), and possibly decenoylation (C10:1). The fatty acid substrate that contributes to ghrelin acylation, however, has not been clarified, although the presumed donor is acyl-CoA. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency causes elevated serum octanoylcarnitine levels, reflecting elevated octanoyl-CoA levels. Glutaric aciduria type II (GA2) is characterized by elevated serum acylcarnitine levels, including octanoyl carnitine. We hypothesized that acyl-CoA is the fatty acid substrate for ghrelin acylation. Because serum octanoyl-CoA levels are altered in these disorders, we examined blood levels of acyl (A) and desacyl (D) forms of ghrelin in affected patients. Plasma acyl ghrelin levels and A/D ratios appeared to be elevated in patients with MCAD deficiency or GA2 in comparison to those in normal subjects. Reverse-phase high-performance liquid chromatography confirmed that n-octanoylated ghrelin levels were elevated in these patients. In addition, serum C10-acylcarnitine levels were also elevated in patients with GA2. In conclusion, changing serum medium-chain acylcarnitine levels may affect circulating acyl ghrelin levels, suggesting that acyl-CoA is the substrate for ghrelin acylation. Moreover, acylation of ghrelin may be linked to energy homeostasis and fat metabolism. In other words, ghrelin may play an important role in metabolic balance via its own fatty acid metabolism. Finally, our results may have pathophysiologic implications for these disorders. Alterations of plasma ghrelin levels in these disorders may reflect and/or influence the patient's metabolic status.

Declaration of interest

I fully declare a conflict of interest. Details below.

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## P989

### Ghrelin suppresses angiotensin II-induced tissue damages by reducing oxidative stress

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Objectives

Angiotensin II (AngII) induces renal premature senescence by multiple mechanisms including by increasing oxidative stress. Recent study revealed that growth hormone secretagogue ghrelin exerts anti-senescence effects. In this study, we examined whether ghrelin inhibits AngII-induced renal senescence and damages.

Methods

Renal senescence was induced by infusion of Ang II in C57BL/6 mice with osmotic mini-pump. Ghrelin was administered by the daily intraperitoneal injection. Eight weeks after the treatment, kidneys were removed and utilized in the various experiments. In *in vitro* experiment, cultured human proximal cell line, HK-2 cells were incubated with 1 μM of AngII for 72 h and ghrelin were administered 30 min prior to AngII stimulation.

Results

AngII infusion induces senescence and oxidative stress as accessed by senescence-associated (SA) β-Gal and 4-hydroxy-2-nonenal (4-HNE) staining, respectively. The expressions of markers for senescence, p21 and p53 as well as senescence-associated cytokines, TGF-β and PAI-1 in the kidney were increased by AngII. AngII infusion also induced renal damages as assayed by the urinary excretion of renal tubular marker, *N*-acetyl-glucosaminide (NAG) and neutrophil gelatinase-associated lipocalin (NGAL) as well as by urinary protein excretion. Finally, AngII infusion provoked interstitial fibrosis as evaluated by Masson-trichrome staining. These changes were attenuated by the treatment with Ghrelin. In HK-2 cells, the receptors for both Ghrelin and AngII were expressed. SA β-Gal assay revealed tubular cell senescence by AngII. AngII also increased the expression of p21 and p53 as well as TGF-β and PAI-1. These changes were attenuated by pretreatment with Ghrelin. In addition, AngII reduced the mitochondria (Mt) number, which was restored by Ghrelin as measured by staining cells with Mt-specific fluorescent dye. Ghrelin treatment increased the expression of Mit-specific uncoupling protein UCP-2 and master regulator of Mit biogenesis, PGC-1α expression. Finally, in HK-2 celsl, Ghrelin reducued Mit-derived ROS levels and Mit membrane potential.

Conclusions

Our data indicated that ghrelin suppressed AngII-induced renal premature senescence and AngII-induced renal damages presumably through reducing the mitochondrial membrane potential and resultant ROS production.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P990

### The effect of ghrelin on the mitochondrial dysfunction in muscle and physical disability associated with aging

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Aim

We investigated the influence of mitochondrial dysfunction associated with aging on physical performance in mice, and the effect of hormone replacement on physical performance.

Method

Using 8, 20, 50 and 100 week-old C57bl6 mice, we evaluated skeletal muscle mass, mitochondrial activity, glucose tolerance and physical performance (muscle power and exercise endurance). In addition, we treated 50-week-old mice with ghrelin or insulin like growth factor 1 (IGF1), which are representative hormones that are decreased associated with aging, and investigated the effect on physical performance.

Result

Skeletal muscle mass and mitochondrial activity was decreased associated with aging. Muscle mass was decreased drastically between 20- and 50-week-old, and the time-course was paralleled to decrease in muscle power. Mitochondrial activity was diminished continuously from 8- to 100-week-old, and this change was paralleled to decrease in exercise endurance. As regulatory factors of muscle mass and the mitochondrial function, we focused on mammalian target of rapamycin complex1 (TORC1) and AMP-activated protein kinase (AMPK) in muscle, respectively. Activity of TORC1 was diminished with aging in parallel to muscle mass and power, and that of AMPK was paralleled to muscle mitochondrial activity and exercise endurance. The treatment with IGF1 in 50-week-old mice recovered muscle mass and power; however, it did not improved mitochondrial activity or exercise endurance. The treatment with ghrelin recovered not only muscle mass and muscle power; but also mitochondrial activity and exercise endurance, which were deteriorated in aging mice. The ghrelin-induced recovery was associated with dual activation of TORC1 and AMPK in muscle.

Conclusion

Muscle mitochondrial dysfunction was closely associated with decrease in exercise endurance. Ghrelin replacement would be a hopeful strategy for the decrease in physical performance associated with aging.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P991

### Health related quality of life and psychological functioning of short statured German children and adolescents: findings form the quality of life in short stature youth study

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Background

About 3% of all children are short statured. Clinically GH deficiency (GHD) or idiopathic short stature (ISS) is frequently diagnosed. GH (GH−) treatment, which is primarily indicated for GHD patients, is provided not only to increase height, but also to improve psychological functioning and raise health related quality of life. The present study examines these psychosocial outcomes in young German patients and their parents, and analyzes differences according to clinical and sociodemographic sample characteristics.

Methods
As part of the international QOLISSY study 143 German patients between 4 and 18 years and their parents filled out the strengths and difficulties questionnaire (SDQ) assessing psychological functioning and a generic health-related quality of life questionnaire (KIDSCREEN 52). Patient self-report and parent reported data were compared with representative German norm data.
Results
In the KIDSCREEN 52, short statured children and adolescents reported a significantly lower total health related quality of life (HrQOL) score compared to a representative German sample ($P<0.01$), and especially regarding their psychological wellbeing ($P=0.04$). No differences within the patient sample were found regarding gender, age, diagnosis and treatment status or actual height. Height also had no significant impact on psychological problems or psychosomatic complaints. Analysing the SDQ data no significant differences ($P=0.83$) were found between this sample and a representive sample.
Parent and patient perspectives, however, differed: parents rated the HrQOL of their children significantly higher than their children did ($P<0.01$), and patients self-report of psychological difficulties was higher than the report from the parental perspective ($P<0.01$).
Conclusion
This cross-sectional study found impairments in self-reported psychological functioning and HRQOL of short statured children as compared to norms, but no differences between patient subgroups. Differences between patient and parent perspectives, however, suggest conflicting viewpoints and specific problems for children which need to be addressed in respective psychosocial interventions.
Declaration of interest
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## P992

### C677T methylenetetrahydrofolate reductase gene polymorphism and colorectal neoplasms in acromegalic patients

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Introduction
Acromegalic patients have an increased risk of developing colorectal neoplasms (CN). A common polymorphism (C677T) in gene coding for methylenetetrahydrofolate reductase (MTHFR), a key enzyme in folate metabolism and in DNA synthesis, reparation and methylation, has been associated with CN risk in general population, but its role in acromegalic patients has never been explored yet.
Aim
To investigate the contribution of MTHFR C677T polymorphism, folate status, and other lifestyle, nutritional and biochemical parameters on CN risk in acromegalic patients.
Patients and design
MTHFR C677T genotype was analysed by restriction fragment length polymorphism (RFLP) in 115 acromegalic patients (40 men; mean age 59.1 $\pm$1.6 years). In all cases, colonoscopy was performed at diagnosis and/or during follow-up. Hormone (GH, IGF1 and insulin) and metabolic parameters (HOMAIR, Hb1AC) were measured in all patients. Fasting homocysteine (tHcy), folate and vitamin B12 levels and lifestyle (alcohol consumption and smoking habit) data were also collected.
Results
The distribution of MTHFR C677T was in the Hardy–Weinberg equilibrium and was similar in patients with or without CN. CN were detected in 51 patients (adenocarcinoma in three male T allele-carriers patients). Occurrence of CN was significantly correlated with TT genotype ($P=0.03$). There was a significant interaction between folate levels and MTHFR genotype on CN risk ($P=0.037$). In the low folate levels subgroup, TT patients had a 2.4 higher OR for CN than C allele-carriers (95%CI: 0.484–11.891; p NS). At multivariate analysis, smoking habits ($P=0.007$), HbA1c levels ($P=0.021$), dyslipidaemia ($P=0.05$) and uncontrolled acromegaly ($P=0.05$) were independently associated with CN.
Conclusions
In this large cohort of acromegalic patients, risk for CN seems increased, despite not significantly, in patients with MTHFR 677TT and low folate levels. HbA1c, dyslipidaemia, smoking, and disease activity were associated with increased CN risk.
Declaration of interest
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## P993

### Clinical characteristics and outcome of acromegaly induced by ectopic secretion of GHRH: a French nationwide series of 21 cases

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Context
Ectopic GHRH secretion is a rare cause of acromegaly described mainly in isolated case reports.
Setting
From the registry of the sole laboratory performing plasma GHRH assays in France, we identified cases of ectopic GHRH secretion presenting with acromegaly between 1983 and 2008.
Patients
In total, 21 patients aged 14–77 years, from 12 French hospitals. Median GHRH was 548 ng/l (270–9779).
Main Outcome Measures
Description of tumor features and outcome, relation between plasma GHRH values and tumor site, size and spread.
Results
The primary neuroendocrine tumor was identified for 20/21 patients (12 pancreatic, seven bronchial, one appendicular). Tumors were large (10–80 mm), identified on CT scan in 18 cases and by endoscopic ultrasound and somatostatin receptor scintigraphy (SRS) in two cases. SRS had a similar sensitivity to CT scan (81 vs 86%). Tumors were all well-differentiated; 47.6% had metastasized at the time of diagnosis of acromegaly. After a median follow-up of 5 years, 85% of patients were alive. About 91 percent of patients whose tumor was completely removed were considered in remission and most had normalized plasma GHRH. Remaining patients were treated with somatostatin analogs: IGF1 normalized except for one patient who required pegvisomant, but GHRH levels remained elevated. No correlations were found between GHRH levels and tumor site, tumor size nor the existence of metastases. Determination of plasma GHRH during follow-up was an accurate indicator of recurrences.
Conclusions
The prognosis of endocrine tumors responsible for GHRH secretion appears relatively good. Plasma GHRH assay is an accurate tool for diagnosis and follow-up.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P994

### Comparison of intuitiveness, ease of use and preference in two disposable GH injection devices

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Aim
GH is used to treat short stature in children with GH deficiency (GHD), Turner syndrome (TS) and born small for gestational age (SGA). As GH is injected daily, treatment adherence may be problematic. To maximise adherence, improvements in injection devices have been targeted. This report compares intuitiveness and ease of use of two disposable GH devices: Norditropin NordiFlex (NF; Novo Nordisk A/S, Bagsværd, Denmark) and GoQuick (GQ, Pfizer, Inc., New York, USA).

Methods
Children with GHD, TS or SGA, treated with GH (≥6 months) were randomised to intuitiveness (Int, $n=32$; mean (S.D.) age, 13.1 (2.1) years) or instruction (Ins, $n=32$; age, 13.4 (2.0) years) groups. All patients participated in a usability test involving dose setting and injection into an Eppendorf tube for both devices. The Int group was given brief instruction on device use. The Ins group received full instructions according to the patient user guide. Time taken for dose delivery and handling errors was assessed. Following the usability test, participants assessed intuitiveness of use, ease of use and overall preference by questionnaire.
Results
In both groups, fewer errors were made by participants using NF than GQ (Int, 3 vs 19; Inst, 2 vs 5). Time for dose delivery was lower with NF than GQ (Int, 43s vs 66s; $P<0.001$; Inst, 43s vs 48s, NS (Student's *t*-test)). Both groups scored NF higher than GQ in 9/13 categories including: easiest to use, most intuitive, and device of overall preference. In the Int group, 30/32 (94%) participants reported that they would feel safe operating NF without instruction, compared with 10/32 (31%) for GQ.
Conclusions
NF was rated as more intuitive to use; the majority of patients felt safe using it without instruction. The majority of patients preferred NF to GQ, ranking it as easier and more convenient to use.
Declaration of interest
The authors declare that there is a potential conflict of interest.
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## P995

**Diabetic retinopathy and GH the case of acromegaly**
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Although GH has been implicated in the pathogenesis of retinopathy in diabetic subjects, the frequency of retinopathy in diabetic acromegalic subjects is deemed to be rare. The aim of our study is to evaluate the prevalence of retinopathy in hypersomatotropic patients.
Subjects and methods
In total, 40 acromegalic diabetic patients (21 M, 19 F, mean age=50 years) underwent funduscopy to search for diabetic retinopathy
Results
Among 40 patients, only five have retinopathy (prevalence=12.5%), it was at an early stage in three cases (background retinopathy) and at a more advanced stage in two cases (proliferative retinopathy). The mean age was 44.6±15.12 years in patients with retinopathy vs 51.31±13.13 years in patients without retinopathy. There was no correlation with GH levels: 56.8±42.87 ng/ml in patients with retinopathy vs 65.15±70.49 ng/ml in patients without retinopathy, but there was correlation with glycemic control: all patients with retinopathy had bad glycemic control whereas 50% of those without retinopathy had bad glycemic control.
Conclusion
We conclude from this study that diabetic retinopathy is rare in acromegaly. Among different factors only glycemic control seems to be implicated in its pathogenesis
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P996

**Assessing the quality of life of children and adolescents with short stature: development and psychometric testing of the QOLISSY: instrument**
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In children whose height is substantially below norms, behavioural and emotional problems can be experienced. When evaluating the outcomes of treatments or making treatment decisions, health related quality of life (HrQoL) should be taken into consideration. At present, no standardised HrQoL instrument for short stature youth exists. Through consensus building in five countries, the objective of this study was to develop and psychometrically test a growth-related HrQoL instrument for use in clinical and epidemiologic research.

The target population consists of short stature children (height $<-2$ SDS) with a diagnosis of GHD/ISS, treated/untreated with GH. Children (8–12) and adolescents (13–18) were included as well as their parents and parents of children between 4 and 7 years. The project followed international instrument development guidelines and a patient-centred methodology. Focus groups eliciting statements about HrQoL, cognitive debriefing, pilot testing and a field test with a retest of the eventual questionnaire were conducted simultaneously in all five countries. Both qualitative and quantitative analyses were carried out.

Following item generation through focus groups with a total of 196 participants, 124 items for children and 156 for parents were included in a pilot test with cognitive debriefing. Statistical analysis of the pilot test results identified the psychometric properties of the instrument. A total of 336 families participated in the field test. Of these, 162 completed a re-test questionnaire. Further item reduction was performed using differential item functioning and structural equation modelling. The final questionnaire consists of a 3-domain core structure with 21 items, the full questionnaire being 49 items for children in six domains and 65 items for parents across eight domains. The questionnaire demonstrated good criterion and construct validity. In addition the questionnaire has good face validity and has been shown to provide reliable results over time.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P997

**Factors affecting IGF1 response to GH during transition**
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Background
GH replacement during the childhood-adult transition period is important for somatic maturation. The aim of the study was to explore the factors influencing IGF1 response to GH during transition.
Methods
KIMS (Pfizer International Metabolic Study) database in UK was interrogated, and 98 patients (55 male, median age 20.7 years (15.7–25.8)) with childhood-onset GH deficiency (Co-GHD), who were started on adult dose of GH during transition (age <26 years) were identified. IGF1 SDS were estimated using age and gender specific normative data. 'IGF1 response' was calculated from baseline IGF1 levels prior to GH therapy, mean IGF1 levels and the corresponding GH doses from 6 to 24 months of starting treatment, using the formula: ΔIGF1 SDS/GH dose (mg/surface area).
Results
During GH treatment, mean IGF1 SDS ±S.D. of the patients increased from -2.90±1.96 to 0.054±1.79 after a median period of 12 months. However, the IGF1 levels were lower than the recommended ranges (0–2 SDS) in 43 patients (44%). In women, baseline IGF1 levels were lower ($P=0.006$), and was associated with a higher GH dose ($P<0.001$) and reduced IGF1 response ($P=0.021$) (Table). IGF1 response was also reduced in younger patients ($r=0.29$, $P=0.006$). Among women, oral oestrogen therapy ($n=27$) was associated with a higher GH dose (0.34±0.12 vs 0.24±0.09 mg/m$^2$, $P=0.007$), but lower IGF1 levels (−0.84±1.70 vs 0.69±1.42, $P=0.004$) and thus a lower IGF1 response (10.0±4.7 vs 13.8±6.7 mg/m$^2$, $P=0.001$). In contrast, testosterone therapy in men ($n=30$) was related to a higher IGF1 response (16.6±8.2 vs 9.9±5.8 mg/m$^2$, $P=0.016$). The number or type of other hormonal deficiencies did not affect the response to GH.
Conclusions
In this study, IGF1 response to GH during transition was evaluated in a large group of patients for the first time, and associations with age, gender and sex hormone replacement therapy were observed. Further studies are required to explore the effects of these factors on other endpoints of GH replacement during this period of somatic development.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1**

| | **Women** ($n=43$) | **Men** ($n=55$) | ***P*** |
|---|---|---|---|
| Age (years) | 20.7 (2.6) | 21.4 (2.3) | 0.17 |
| Baseline IGF1 SDS | -3.50 (2.13) | -2.42 (1.7) | 0.006 |
| IGF1 SDS on GH | -0.15 (1.8) | 0.23 (1.7) | 0.26 |
| GH dose (mg/m2) | 0.30 (0.12) | 0.21 (0.09) | $<0.0001$ |
| IGF1 response (SDS/ mg/m2) | 11.4 (5.7) | 13.9(8.0) | 0.021 |

Means (s.d.).

## P998

### Quality of life in adult patients with GH deficiency and neurosecretory dysfunction at the end of GH therapy

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GH is used primarily to increase adult height in short children with GHD/NSD with little knowledge about the impact of GH on health-related quality of life (HRQoL). Therefore this study was carried out to evaluate HRQoL in adult patients with childhood onset GHD/NSD at the end of GH therapy.

HrQoL was measured by patient self-report using two generic questionnaires, SF36 and NHP. Patient data were compared to representative norm data. Clinical data as well as socio-demographic data was collected.

The patient group comprised 53 men (M = 22.2 years) and 32 women (M = 24.2 years). About, 53% patients were diagnosed with GHD and 46% were diagnosed with NSD. At the beginning of treatment the mean height of male and female patients was 126 cm, with no significant difference between males and females. Patients were treated between 1 and 13 years with GH. Mean age at start of GH therapy was 10.5 years, mean height gain was 41 cm. Subject reported mean final height was 167 cm, males were significantly taller than females (172 vs 158 cm).

In the SF-36 significantly lower scores were found on the scales vitality ($P<0.001$) and social functioning ($P=0.037$) as compared to the reference population. Males reached higher vitality scores than females ($P=0.043$). In the NHP patients reported lower scores in the dimensions energy ($P<0.001$) and social isolation ($P=0.003$) compared to the reference population; no gender differences were found.

Earlier studies reported conflicting results concerning the HrQOL of GHD patients after GH treatment. The present study found impairments in HrQOL compared to the reference population, especially regarding vitality/energy and social functioning/-isolation. However, due to the cross-sectional nature of this study, HrQOL at start of treatment could not be included in the analysis.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P999

### Hypertension and cardiovascular risk factors in GH deficiency

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Studies showed an increase of cardiovascular risk (CVR) and reduction of the life expectancy observed in the anterior pituitary insufficiency (API) subjects. The morbimortality would be related to GH deficiency not substituted.

Aim
To seek the frequency of the hypertension and analyse the predictive factors in subjects with API.

A retrospective study relating 55 (47 women, eight men) subjects with API: interrupted pituitarystalk n:6, Sheehan syndrom n:45, non-functioning pituitary adenoma n:4 followed during 10 years and substituted apart from the GH. The average of PAS and PAD were compared with a reference group (n:60:51 women, nine men).

API subjects with HTA (API+) were compared with non-hypertensive subjects (API−)

Results
The average of PAS and PAD were higher than the witness: 120.86 vs 120.23 mmHg ($P<0.064$); 70.76 vs 70.27 mmHg ($P<0.01$) for men 120.90 vs 120.60 mmHg ($P<0.05$); 70.88 vs 70.30 mm Hg ($P<0.005$) for women.

Hypertension was found in 20% of cases (11/55). Its appeared 8 years after (1–15) diagnosis.

IAPI+ARE OLDER 552.45±3 vs 35.65±2 years $P<0.001$) and have more important hypercholesterolemia than others (CT = 2.27±0.11 vs 1.86±0.08 g/l $P<0.02$).

In the other hand we did not found significant difference for family history, BMI (31.02±2.7 vs 22.2±0.81 kg/m $P<0.106$), triglyceridemia 1.91±0.3 vs 1.39±0.15 g/l $P<0.12$) glycemia (0.81 vs 0.84 g/l $P=0.59$ and GH (1.66±0.68 vs 2.17±0.4 mUI/l $P<0.52$).

Our results joint the literature regarding the frequency of the hypertension, the age and the dyslipidemia as predictive factors, the familiar history, the BMI and the degree of GHD do not seems to be CVR. Its necessary to substituted with growth hormone and to treat the others CVR.

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## P1000

### Anti-androgen flutamide alters gene expression in the porcine fetal ovary

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In pigs, the assembly of primordial follicles and their subsequent transition to the primary stage occur in the late gestational and neonatal period. Previously, we reported the presence of androgen receptors in the porcine fetal ovary on different days of gestation, which suggests a fetus capacity to respond to androgens. Since testosterone was detected in the plasma of porcine female fetuses during the second half of pregnancy, it is possible that androgens are involved in prenatal folliculogenesis. Hence, we hypothesize that androgen deficiency during critical prenatal windows may alter expression of genes associated with ovarian development. Therefore, pregnant sows were injected with anti-androgen flutamide (50 mg/kg bw, seven times, every day) starting at day 43, 83 and 101 of gestation. Fetal ovaries were obtained on days 50, 90 and 108 of gestation, fixed in Bouin's fixative for histology and immunohistochemistry or frozen in liquid nitrogen for real-time PCR analysis.

Immunohistochemistry was performed using a mouse monoclonal anti-Ki67 antibody (cell proliferation marker; dilution 1:75, Dako) or a goat polyclonal anti-GATA4 antibody (transcription factor involved in gonadal gene regulation; dilution 1:2000, Santa Cruz). Afterwards, to show Ki67 and GATA4 mRNA in the fetal porcine ovaries real-time PCR was carried out.

Immunohistochemical reaction revealed a higher number of proliferating cells in the fetal ovaries of flutamide-treated animals. Moreover, GATA4 expression has changed after flutamide exposure. Interestingly, prenatal antiandrogen administration resulted in GATA4 mRNA and protein up-regulation in the ovaries from days 50 and 108 of gestation, and their down-regulation on day 90 of gestation.

Our preliminary results suggest that diminished androgens action via blocking of androgen receptors leads to changes in the expression of genes crucial for ovarian development. It seems that androgens exhibit diverse biological actions depending on the gestational period.

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## P1001

### Relationship between GH activity and reproductive hormones: a randomized cross-over study in healthy male volunteers treated with GH and a GH receptor antagonist for three weeks

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Introduction
The GH/insulin-like growth factor 1 (IGF1) system may modulate the pituitary-gonadal axis in males. Direct pituitary and/or testicular effects might be involved, as well as indirect effects on sex hormone binding globulin (SHBG) and aromatase activity.
Methods/design
Nine healthy male volunteers (mean age 37, range 29–49 years) were treated in random order with increasing doses of GH for three weeks (1st week 0.01, 2nd week 0.02, 3rd 0.03 mg/kg per day) or a GH receptor antagonist (pegvisomant; 1st week 10, last 2 weeks 15 mg/day), separated by eight weeks of wash-out. Before and after three weeks of GH treatment or GH receptor blockage circulating levels of testosterone, estradiol, LH and SHBG were measured.
Results
During GH treatment IGF1 increased ((mean $\pm$ 1S.D.) $140 \pm 41$ vs $448 \pm 97$ μg/l, $P < 0.001$) together with estradiol ($77 \pm 22$ vs $107 \pm 30$ pmol/l, $P = 0.015$) and the estradiol/testosterone ratio ($P = 0.001$). By contrast LH tended to decrease ($4.3 \pm 1.9$ vs $3.6 \pm 1.7$ U/l, $P = 0.053$). The opposite was found during pegvisomant treatment where IGF1 ($151 \pm 35$ vs $103 \pm 29$ μg/l, $P = 0.001$) and estradiol ($86 \pm 27$ vs $79 \pm 23$ pmol/l, $P = 0.038$) decreased. No changes in testosterone, SHBG or calculated free testosterone (testosterone $\times$ 100/SHBG) occurred during the two treatment regimens.
Conclusions
High GH/IGF1 activity was positively associated with serum estradiol. The study supports that GH/IGF1 action stimulates aromatase activity *in vivo* with subsequent increased conversion of testosterone to estradiol during GH treatment and visa versa during GH receptor blockage. The decrease in LH during GH treatment may support increased oestrogen activity with increased negative feedback at the pituitary gland. High levels of endogenous GH/IGF1 during puberty might chance the estradiol/testosterone-ratio thereby contributing to development of pubertal gynaecomastia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1002

### Baseline characteristics and differences in short- and long-term response to rhGH between GHD adults with craniopharyngioma and nonfunctioning pituitary adenoma

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Patients with craniopharyngioma (CP) are more often operated by transcranial route than patients with nonfunctioning pituitary adenoma (NFPA), have higher prevalence of pituitary deficiencies, are more obese and dyslipidemic and have a higher mortality rate. A previous study in a large group of GHD subjects, showed that the effects of 2-year rhGH replacement are similar in patients operated for CP and in patients operated for NFPA, except for less reduction in fat mass in CP patients. Aim of the study was to compare both short- (12 months) and long-term (mean follow-up 8 years, range 3–14 years) effects of rhGH in 26 GHD adults patients, 12 operated for CP and 14 for NFPA. Serum IGF-I, lipid profile, glucose metabolism, insulin resistance (HOMA-IR), anthropometric parameters and body composition (BF%) were evaluated. At baseline, no difference between the two groups was observed. After 12 months, SDS IGF-I normalized and BF% significantly decreased in both groups. Concerning long-term effects, increase in IGF-I levels was maintained, while persistent decrease in BF% was observed only in NFPA group. Comparing the data between groups at each follow-up, CP group had a lower ΔBF% than NFPA group ($-3.4 \pm 2$ vs $-10.1 \pm 10$, at short-term, $P < 0.04$ and $-0.7 \pm 11$ vs $-11 \pm 13.7$, at long-term, $P < 0.05$, respectively), as well as higher serum insulin levels ($19 \pm 15$ vs $9.4 \pm 6.5$, $P < 0.04$ and $16.2 \pm 10.4$ vs $9 \pm 4$ uIU/mL, $P < 0.03$ respectively) and higher HOMA-IR ($4.1 \pm 3.2$ vs $2 \pm 1.3$, $P < 0.04$ and $4.4 \pm 4.5$ vs $1.9 \pm 0.9$, $P < 0.05$, respectively). In conclusion, we confirm in the long term study the minor BF% reduction in CP versus NFPA patients. Moreover, patients with CP were more prone to develop insulin resistance than NFPA patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1003

### A new somavert (pegvisomant) 30 mg/ml formulation compared to the currently marketed 15 mg/ml formulation: pharmacokinetics, safety, and tolerability in healthy subjects

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Introduction
Pegvisomant is indicated for acromegaly. Doses $>20$ mg are not commercially available, although daily doses up to 30 mg are approved.
Objectives
1. To estimate the relative bioavailability (relBA) of single-dose pegvisomant s.c. administrations, one injection of 30 mg/ml ($1 \times 30$) vs two injections of 15 mg/ml ($2 \times 15$), and 2. To evaluate their pharmacokinetics (PK), safety and tolerability.
Methods
This was a 2-period, single-dose, crossover study in 14 healthy male and female subjects. All subjects received both doses, $1 \times 30$ and $2 \times 15$, during two treatment periods separated by two weeks. The injection instructions were given in great details and closely followed to reduce variability. Intensive serum samples were collected up to 16 days post injection and were assayed by a validated ELISA for pegvisomant. PK parameters including AUC and Cmax were derived by noncompartmental analyses. Mixed effects model was used to obtain bioavailability estimates. Safety and tolerability were assessed by clinical monitoring, including adverse events, laboratory assessments and injection site reactions.
Results
All subjects completed the study. The relBA of $1 \times 30$ relative to $2 \times 15$ was 123.89% with a 90% CI (112.91–135.93%). The PK parameters are summarized in Table 1. Adjusted for the difference between actual and nominal pegvisomant amounts in both formulations the difference in dose-adjusted AUC reduced to 13%. Different injection solution concentrations and different number of injections might also have a role. No laboratory abnormalities, vital signs, ECG, or injection site reactions of clinical concern were observed in either treatment.
Conclusions
Comparable BA, safety and tolerability of the new 30 mg/ml strength to the currently marketed 15 mg/ml strength were established in this study.
Declaration of interest
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**Table 1** Pegvisomant PK Parameters

| Parameter | $2 \times 15$ mg/ml | $1 \times 30$ mg/ml |
|---|---|---|
| AUCinf (μg.h/ml) | 153.9 (53) | 190.6 (54) |
| AUClast (μg.h/ml) | 147.1 (56) | 184.4 (56) |
| Cmax (μg/ml) | 1.567 (57) | 1.652 (56) |
| Tmax (hr) | 42.0 (30.0–84.0) | 54.1 (34.0–84.0) |
| $t_{1/2}$ (hr) | 64.06 (30) | 59.84 (22) |

Geometric mean (%CV) for all except median (range) for Tmax and arithmetic mean (%CV) for $t_{1/2}$.

## P1004

**Thyroid function during long-term GH treatment in children affected by idiopathic GH deficiency**

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Alterations in the thyroid axis have been reported following GH therapy both in adults and children with GH deficiency (GHD), even if the clinical significance of these changes, in small and short-term studies, remains uncertain. To evaluate the impact of GH replacement on thyroid status in a large selected cohort of prepubertal children with idiopathic GHD during a 3-years follow-up. Study outcome considered FT3, FT4 and TSH modifications (delta) during therapy and their correlations with IGF-1, biochemical and auxological data. Data of 105 children (82 M, 23 F; mean age 11.13) were retrospectively analysed. At diagnosis, the areas under the curve of GH (AUCGH) were calculated during GHRH-Arg test and ITT. At baseline and yearly up to 36 months during GH treatment, we measured height-velocity, BMI, IGF-1, FT3, FT4, TSH. A significant FT3 delta ($P<0.001$) was documented in 89/105 (84.7%) patients at 12 months after starting GH treatment, without any further change at 24 and 36 months. No significant FT4 and TSH delta were observed during the follow-up. Grouping all patients according to FT3 delta at 12 months in those with lower (No 80, 76%) or greater value than 75 percentile (No 25, 24%), the latter showed significantly lower AUCGH during GHRH-Arg ($P=0.018$) and ITT ($P=0.023$). These children also showed lower FT3 levels at baseline ($P<0.001$), without difference in FT4 and TSH. No significant differences in auxological parameters were detected between the two groups. In GHD children, GH treatment is associated with a significant increase in FT3 levels in the first 12 months. However, the lack of significant effects of these changes in relation to the auxological parameters does not suggest the routinely monitoring of thyroid function in initially euthyroid patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1**

| | baseline | 12 months | 24 months | 36 months |
|---|---|---|---|---|
| Height (S.D.) | −2.11± 0.89 | −1.73± 0.75 | −1.44± 0.76 | −1.03±0.85 |
| IGF-1 (μg/l) | 98.83± 47.94 | 314.68± 193.25 | 330.30± 172.95 | 349.90±145.88 |
| FT3 (pg/ml) | 3.10±0.94 | 4.13±0.53 | 4.12±0.53 | 4.21±0.53 |
| FT4 (ng/dl) | 1.25±0.24 | 1.25±0.28 | 1.19±0.25 | 1.19±0.23 |
| TSH (μU/ml) | 1.98±0.84 | 1.92±0.97 | 1.95±1.03 | 1.51±0.54 |
| Δ FT3 | – | 1.02± 1.08* | 0.02±0.73 | 0.07±0.44 |
| Δ FT4 | – | 0.01±0.04 | 0.06±0.01 | 0.01±0.02 |
| Δ TSH | – | 0.06±0.13 | 0.03±0.06 | 0.44±0.59 |
| Δ IGF-1 | – | 217.6± 181.5* | 26.2± 153.3* | 44.3±98.5* |

* $P<0.005$.

## P1005

**Variations of IGF1 in GH deficiency and Algerian health children**

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IGF1 represent an important key player in several physiologic process, so it can be implicated in different pathologies among delays of growth. A comparative study was realized comparing a group of healthy Algerian children of normal size (*n*: 266) and a group presenting a GH deficiency (GHD *n*: 107)to a group of healthy westerners children of normal size(Serie of ROSENFIELD).

The results are as follows

Before the age of 04 years, there is a great overlapping in the results of IGF1 between children who suffer from GHD and those of the comparative group.

After this age, results of IGF1 are significantly low in GHD subjects, compared to the Algerian reference group.

The values of IGF1 found in the Algerian healthy subjects are significantly lower than those observed in the western healthy children. So this parameter must be carefully interpreted when investigating a delay of growth in Algerian and must take into account the clinical and biological context of the patient. It is necessary to establish Algerian standards for IGF1. The introduction of the IGFBP3 not yet available would be more interesting in our context.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1006

**Association Turner's syndrome and GH deficiency**

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One of the most clinical characteristic of Turner's syndrome is the final short stature.

In order to cure this handicap, several teams were interested to treat these patients by the GH.

We report two cases of Turner syndrome associated with GH deficiency.

In the first, a 17 years old girl, having a delayed growth lower than −4 S.D. associated with delayed puberty and dysmorphic syndrome. A karyotype made confirmed the diagnosis of Turner's syndrome. In front of the short stature lower than that usually found in the Turner syndrome, GH was measured during insulin induced hypoglycaemia test and L-dopa test. The two tests revealed a GH deficiency.

The second observation is about a 21 years old girl having a delayed growth between −2 and −3 S.D. associated with delayed puberty and dysmorphic syndrome.

The karyotype finds a chromosomal formula with 45×0 confirming the Turner's syndrome.

GH was measured under stimulation test, showing a GH deficiency.

The dosage of the GH in Turner's syndrome appears interesting and must be of current practice especially when the short stature is lower than that usually found in Turner syndrome.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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# Male Reproduction

## P1007

**The homologous hormones lutropin and choriogonadotropin are interacting differently with the LH/CG receptor**

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Activation of the human LH/CG receptor (LH/CGR) by lutropin (LH) and choriogonadotropin (CG) is essential in the human reproduction. Deletion of the Exon10 (LH/CGR-delExon10) resulting in a lack of 27 amino acids within the extracellular hinge-region of LH/CGR causes Leydig cell hypoplasia type II. To clarify why this deletion impairs LH but not CG action, we investigated the molecular determinants of LH/CGR activation elucidating the different behaviour of both hormones.

It is reported that the LH/CGR exist as oligomers in a unitary signalling unit when activated with CG, since individual binding- and signalling-defective mutants can be rescued by combining both. We performed a similar strategy including also LH/CGR-delExon10 as signalling defective mutant. However, in contrast to CG,

the functional rescue was not possible by stimulation with LH, which fails to bypass the lack of Exon10 by trans-activating the neighbouring protomer. Our results indicate different activation mechanisms for LH and CG at LH/CGR-delExon10. Moreover, similar results were also achieved with a complete hinge-region but different signalling disturbing positions. Fluorescence correlation spectroscopy indicates no disturbance in oligomerization of the LH/CGR-delExon10. Homology modelling suggest a helical entity for exon10 region. Substitution of block wise alanine mutations showed no effect on both LH and CG activation.
We conclude that residues comprising Exon10 form a structural interface with helical portion, which might be necessary for cis-interaction of LH and LH/CGR. Thus the LH related dysfunction of LH/CGR-delExon10 is likely caused by disturbed native cis-interaction between LH and receptor in which each protomer bind and signal on its own, rather than by an interrupted LH related trans-activation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1008

**Differential expression of pseudoautosomal region genes in patients with Klinefelter syndrome**

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Klinefelter syndrome (KS) was first described in 1942 and the cause for the syndrome was identified as a supernumerary X chromosome resulting in the karyotype 47,XXY. 80–90% of KS cases bear this karyotype, whereas the remaining exhibit (in decreasing frequency) varying mosaicism (e.g. 47,XXY/46,XY), carry additional sex chromosomes (48,XXXY; 48,XXYY; 49,XXXXY) or structurally abnormal X chromosomes. The prevalence of KS is up to 1 in 500 boys, and it is the most common chromosomal aberration in men (0.1–0.2% of the male population). Although KS has been extensively studied in the last decades, the pathophysiology, i.e. the link between the supernumerary X and the phenotype, largely remains unclear and the variability unexplained. Apart from normal interindividual genetic variation, several genetic mechanisms may be involved in the variability of the phenotype: in principal, the parental origin of the X chromosome and gene-dosage effects (GDE) in conjunction with (possibly skewed) X chromosome inactivation. In order to clarify the role of GDE in KS, we analyzed 63 patients with non-mosaic karyotype by Quantitative Real Time PCR, comparing the results of expression of 12 genes (ASMTL, CSF2RA, DHRSXY, DXYS155E, MIC2, P2RY8, PGPL, PR48, SLC25A6, SPRY3, SYBL1, ZBED1) located in the pseudoautosomal region1 and 2 (PAR1 and 2) of X-chromosome with the results obtained from 38 XY fertile male, 35 XX fertile female and 4 non-mosaic Turner syndrome patients. The obtained results showed a differential expression of PAR1 and 2 genes between KS and controls (male and female), and for many of these genes this is the first description of their expression behaviour in male and KS. Finally, a genotype–phenotype correlation for the genes whose function is known was attempted, disclosing appealing pathogenetic hypothesis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1009

**Rab-like 2 (RABL2) function in male fertility, intra-flagella transport and sperm tail assembly**

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Infertility affects 1 in 20 Australia men of reproductive age, and for the majority, the causes remain unknown. In an effort to identify addition key pathways and proteins involved in male fertility we undertook a random mouse mutagenesis screen. In the process we identified the Mot1 line which carries a point mutation in the *Rabl2* gene. Mot1 homozygous male are sterile, have a reduced daily sperm output, short sperm tails and drastically reduced progressive sperm motility.
RABL2 is an uncharacterised member of the RAS GTPase superfamily. Many RAS proteins are involved in membrane trafficking and cell signaling. Some small GTPases are capable of switching between an inactive and active state and of binding to a specific set of effector proteins that they transport around the cell. Our data shows that RABL2 is expressed highly in male haploid germ cells and other ciliated tissues and interacts with a key pathway involved in flagella development. Specifically RABL2 binds to intra-flagella transport (IFT) complex B particles. Further, GTP-bound RABL2 binds to a specific set of effector proteins, including key components in glycolytic pathway that it delivers into the growing sperm tail.
Further, molecular modeling shows the Mot1 mutation is located within a β-sheet required for protein–protein interactions. The Mot1 mutation leads to a delayed conversion of RABL2 from the inactive GDP-bound state into the active GTP-bound state. Ultimately this leads to decreased RABL2-effector protein binding and a failure of effector proteins delivery into the tail and sterility. The above results demonstrate the function of previously uncharacterised protein, RABL2, as an essential regulator of sperm flagellum formation and function and raise the possibility that mutations in RABL2 may underlie cases of human infertility.
Declaration of interest
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## P1010

**Prevalence of olfactory and other developmental anomalies in patients with central hypogonadotropic hypogonadism**

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Introduction
Hypogonadotropic hypogonadism (HH) is a heterogenous disease caused by mutations in several genes. Based on the presence of hyposmia/anosmia it is distinguished into Kallmann syndrome and isolated HH. The prevalence of other developmental anomalies is not well established.
Methods
We studied patients with HH (29 males, 3 females, mean age 41.5), 9 with familial and 23 sporadic HH (29 congenital, 3 adult-onset), by physical examination, smell test (BSIT Sensonics), audiometry, renal ultrasound, and magnetic resonance imaging of the olfactory structures.
Results
Based on the smell test, patients were classified as normosmic ($n=19$, 59.4%) and hypo/anosmic ($n=13$, 40.6%). Hypoplasy/agenesis of olfactory bulbs was found in 38% of patients (8/21), (80% hypo/anosmic, 8.3% normosmic, $P< 0.05$, $X^2$-test). Remarkably, olfactory structures were normal in 2 anosmic patients, while 1 normosmic patient presented a monolateral hypoplastic bulb. 9 of 22 patients (40.9%) presented neurosensorial hearing loss of various degrees (36.3% hypo/anosmic, 41.6% normosmic, $P=$NS). Renal ultrasound revealed 28.1% of cases with renal anomalies (30.7% hypo/anosmic, 26.3% normosmic, $P=$NS). At least one midline defects was found in 48.3% of patients (58.3% hypo/anosmic, 42.1% normosmic, $P=$NS): 13/31 abnormal palate (41.6% hypo/anosmic, 42.1% normosmic, $P=$NS), 6/31 agenesis of one or more teeth (25% hypo/anosmic, 15.7% normosmic, $P=$NS), 2/31 pectus excavatum (8.3% hypo/anosmic, 5.2% normosmic, $P=$NS), 2/31 bimanual synkinesis (8.3% hypo/anosmic, 5.2% normosmic, $P=$NS), 2/31 iris coloboma and absent nasal cartilage (15.3% hypo/anosmic, 0% normosmic, $P=$NS). Anamnestically 4/26 patients reported cryptorchidism (30% hypo/anosmic, 6.2% normosmic, $P=$NS).
Conclusions
Hypo-anosmia is significantly related to anatomical anomalies of the olfactory bulbs/tracts but the prevalence of other developmental anomalies, especially hearing loss, is high both in HH and Kallmann syndrome and independent of the presence of anosmia/hyposmia. From the clinical standpoint Kallmann syndrome and isolated HH should be considered as the same complex, developmental disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1011

Abstract withdrawn.

## P1012

**Total Testosterone levels and predisposing criteria for metabolic syndrome in healthy young men**

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Object of the study
To evaluate total testosterone (TT) levels in healthy young men and determine its possible influence on metabolic syndrome risk factors.
Materials and methods
One hundred and twenty-six practically healthy young men, mean age 20.16 ± 1.42 years old (18–29) were included in the study. Individuals with any clinical manifestations suspicious for hypogonadism, chronic liver and kidney diseases in the anamnesis or receiving any kind of long-term medical therapy were excluded from the study. Evaluation of every patient consisted of general examination, anthropometric data, blood pressure, blood samples for serum glucose, lipid spectrum, total testosterone and LH.
Results please see Table 1.
During the correlation analyses of total testosterone and its dependence of following indicators of metabolic syndrome: BMI, WC, systolic and diastolic blood pressure, TG and HDLP, positive correlation with serum HDLP level ($r=0.2016$, $P<0.05$) has been established. Despite the fact that, obesity and overweight indicators - BMI and WC are not connected with the level of total testosterone in healthy young men, we managed to establish significant correlation between total testosterone level and % of VF ($r=0.2014$, $P<0.05$)
Conclusion
In young men without metabolic syndrome manifestation has been established significant correlation between total testosterone level and % of visceral fat and HDLP level.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1**

| Parameters | Mean | M ± s.d. | |
|---|---|---|---|
| BMI, body mass index | 22.77 | 20.427; 25.116 | kg/m$^2$ |
| WC, waist circumference | 75.5 | 70.0687; 80.9967 | Cm |
| TF, total fat | 22.11 | 16.8495; 27.3595 | % |
| VF, visceral fat | 5.03 | 2.5481; 8.7181 | % |
| SBP, systolic blood pressure | 119.9 | 108.85; 131.163 | MmHg |
| DBP, diastolic blood pressure | 75.53 | 66.328; 80.7461 | mmHg |
| TT (IFA), total testosterone | 6.78 | 5.0424; 8.527 | nmol/l |
| LH (IFA), luteinize hormone | 5.05 | 3.0839; 4.0837 | nmol/l |
| TC, total cholesterol | 3.56 | 3.0388; 4.0837 | mmol/l |
| TG, triglyceride | 0.889 | 0.4753; 1.3027 | mmol/l |
| HDLP, high density lipoprotein | 1.18 | 0.925; 1.4332 | mmol/l |
| LDLP, low density lipoprotein | 1.937 | 1.4608; 2.4147 | mmol/l |

## P1013

**Functional status of pituitary–gonads axis in males with obesity**

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Aim
– to study functional status of pituitary–gonads (PG) axis in males with obesity.
Materials and methods
We evaluated 15 males with obesity without other associated pathology. Mean age of patients was 28.3 years old. Control group constituted by 20 healthy men with different age.
All patients underwent clinical and biochemical evaluations including endocrine check, lipids profile, hormonal profile (LH, FSH, prolactin, sex steroid binding globulin, free testosterone), genitalia ultrasonography, height, body mass, waist circumference (WC), hip circumference (HC), waist-hip ratio, questioning and other studies.
Results
Hormonal profile showed normogonadotrphemia in nine patients (60%) (mean LH ranged 8.7 ± 1, 2 mIU/l, FSH 6.4 ± 1.5 mIU/l) and significantly low free testosterone levels (mean 5.6 ± 0.3 ng/ml). Most of the patients had central obesity with BMI > 35 kg/m$^2$. WC was in normal range 104.3 ± 7, 4 cm, HC = 85, 6 ± 5.3 cm, whereas waist-hip ratio > 1, 22. Blood tests showed dyslipidemia in all patients (100%).
Conclusion
i) Most fertile men with obesity (60%) have normogonadotrophemia with partial decrease of free testosterone in all patients. ii) To discover the pathogenesis of PG axis disorders in men with obesity further researches are needed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1014

**Deletion of FSTL3 increases testicular size and delays testicular regression**

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Follistatin-like 3 (FSTL3) is a secreted glycoprotein known to act as an inhibitor of TGFβ ligands such as activin and myostatin. Like activin, FSTL3 is expressed strongly in the testis but the role FSTL3 plays in testis development and physiology is not clearly understood. Here we show that FSTL3 is a key regulator of testicular size and a promoter of age-dependent testicular regression. FSTL3 deletion in mice leads to the development of enlarged testes that show delayed age-related regression. Importantly, testosterone production and spermatogenesis are not altered in FSTL3 KO mice. Increased testicular size in FSTL3 KO mice was associated with increased Sertoli cells and germ cell numbers although Leydig cell numbers were not affected. To identify and investigate molecular pathways contributing to these phenotypes we undertook a quantitative proteomics approach. We find increased AKT signalling, a promoter of cell proliferation, and increased expression of Sirt1, an anti-ageing protein, in FSTL3 KO testis. Survival and maintenance of species depend on timed testicular development and regression, testicular regression being particularly relevant in seasonal breeders. We therefore demonstrate that FSTL3 is a protein that normally blocks inappropriate cellular proliferation in the testis and regulates the maintenance of testicular function.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1015

### High prevalence and interaction of hypogonadism and the metabolic syndrome in long-term survivors with germ cell tumours

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Introduction and objectives
Testicular cancer survivors (TCS) are at a putative risk to develop premature testosterone deficiency (TD). The clinical and biochemical components of TD and the metabolic syndrome have not been thoroughly described in such patients.
Material and methods
Prospective clinical trial: 163 TCS took part in the study, clinical parameters, sex hormones and biochemical values were assessed in fasting blood samples, MS was defined according to the harmonized criteria of 2009 (IDF&NCEP). Intima-media thickness of carotid arteries was measured. Validated German Questionnaires of AMS scale and IIEF 15 were administered.
Results
Mean age at presentation was $40.3 \pm 8.4$ years and median follow-up was 7 years. 102/163 pts. (62,6%) had received polychemotherapy, 49 (30%) radiotherapy. 15 pts. had undergone bilateral orchiectomy and are under T substitution, 24 pts. with unilateral tumour were at the time of the study already under T substitution. 39/163 pts. (23.9%) had a total T level of $<12$ nmol/l. 57/163 pts. (35.0%) fulfilled the criteria for the MS. In multivariate models, waist circumference, high triglyceride levels and decreased insulin sensitivity (QUICKI) were markedly associated with low testosterone levels ($P=0.006$, $P=0.005$ and $P=0.002$ respectively). TD was predictive for overall presence of MS ($P=0.01$) and the number of fulfilled criteria ($P=0.01$). Tumour characteristics and type of chemotherapy were not associated with a increased risk to develop TD.
Conclusions
Data confirm the high prevalence of TD and MS in long-term TCS. Pivotal clinical and biochemical components of TD as well as the MS are strongly exhibited in TCS. TCS should be closely monitored beyond a 10-years-follow-up-period and according to risk-prediction models encompassing pertinent markers for both clinical entities, TD and MS. This could help to identify patients who could benefit from an early T replacement, thus improving their quality of life and probably reducing their cardiovascular risk.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1016

### Mutation analysis of *NR5A1* gene encoding steroidogenic factor 1 in cryptorchidism and male infertility

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The gene *NR5A1*, which encodes steroidogenic factor 1 (SF-1), is a pivotal transcriptional regulator of genes involved in adrenal and gonadal function, including several steroidogenic enzymes and key genes necessary for male sex determination and differentiation, testicular descent and reproduction (such as SOX9, AMH, INSL3, and AR). The most severe phenotypes associated with NR5A1 mutations include gonadal dysgenesis, disorders of sex development (DSD) and adrenal insufficiency. However, recent studies are revealing that the spectrum of phenotypes caused by variations in this gene might be broader and include partial gonadal dysgenesis, hypospadias, microphallus with anorchia, and primary ovarian insufficiency. Preliminary studies also suggested that male infertility and cryptorchidism might be linked to mutations in NR5A1, but the data are not conclusive and replication studies in different populations have not been performed.
To clarify the role of NR5A1 variations in these conditions we sequenced the gene in 404 cryptorchid subjects (279 infants with cryptorchidism and 125 men with history of orchidopexy), 237 idiopathic infertile men with azoo-oligozoospermia, and 187 fertile normozoospermic men (controls).
No variations in *NR5A1* gene was found in controls. A previously described polymorphism (*P*.G146A) was significantly associated with cryptorchidism (6.7% of the cryptorchid population, $P=0.002$) and to a lesser extent with spermatogenic impairment (2.1% of the azoo-oligozoospermic population, $P=0.046$). Furthermore, seven novel predicted damaging missense mutations were found in heterozygous condition in 5/404 (1.2%) cryptorchid subjects and 3/237 (1.3%) infertile subjects.
This study strongly suggests that variations in *NR5A1* gene might be associated also with 'mild' phenotypes represented by spermatogenic impairment and especially cryptorchidism. Although none of our patients have evidence of adrenal insufficiency, long-term follow up of infants with cryptorchidism is needed to verify its possible development, particularly in cases with one of the 7 novel missense mutations identified in this study.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1017

### The Muenster EXAKT project: cardiovascular risk factors in Klinefelter patients and healthy controls

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Background and Aim
Klinefelter syndrome (47,XXY; KS) is a very common chromosomal disorder, affecting 1:600 men. Klinefelter men have been described to exhibit clinically relevant metabolic patterns related to a pro-inflammatory status, resulting in a high prevalence of insulin resistance and cardiovascular impairment. Testosterone deficiency in form of primary hypogonadism is a common feature in these men.
EXAKT (epigenetics, X-chromosomal features and clinical applications in Klinefelter syndrome trial) is a Muenster-based prospective project involving Klinefelter patients ($n=130$), and their parents assessing a wide area of cardiovascular, inflammatory and metabolic factors as well as sex steroids and questionnaires in comparison to age-matched healthy male and female controls ($2 \times n=50$). A broad range of genetic and epigenetic investigations completes the approach.
Here, we present first and novel clinical data comparing Klinefelter patients to healthy male controls with regard to cardiovascular and metabolic parameters.
Results
KS patients had a higher waist circumference and Body Mass Index in comparison to controls. Further on, decreased insulin sensitivity, higher levels of triglycerides and lipoprotein type a as well as lower concentrations of HDL-cholesterol were found in patients. Levels of high-resolution C-reactive protein were elevated in Klinefelter patients. Consequently, the prevalence of the metabolic syndrome according the Harmonized Criteria was markedly higher in Klinefelter men than in controls (52/130 vs 5/50). Corroboratingly, carotid artery intima-media thickness was increased and flow mediated dilatation of the brachial artery was decreased in patients vs controls. These differences were statistically significant. Metabolic disadvantages of patients were further enhanced by low testosterone concentrations and already present in the sub-cohort younger than 40 years.
Conclusion
The EXAKT project revealed an unfavorable pattern of cardiovascular risk factors in KS in comparison to healthy male controls. This picture is already present in younger patients and enforced by testosterone deficiency.
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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1018

**Cellular distribution, regulated expression and functional role of the anorexigenic peptide, NUCB2/nesfatin-1, in the testis**

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Nesfatin-1, product of the precursor NEFA/nucleobindin 2 (NUCB2), was initially identified as anorectic hypothalamic neuropeptide, acting in a leptin-independent manner. In addition to its central role in the control of energy homeostasis, evidence has mounted recently that nesfatin-1 is also produced in peripheral metabolic tissues, such as pancreas, adipose and gut. Moreover, nesfatin-1 has been shown to participate in the control of body functions gated by whole-body energy homeostasis, including puberty onset. Yet, whether, as is the case for other metabolic neuropeptides, NUCB2/nesfatin-1 participates in the direct control of gonadal function remains unexplored. We document here for the first time the expression of NUCB2 mRNA in rat, mouse and human testes, where NUCB2/nesfatin-1 protein was identified in interstitial mature Leydig cells. Yet, in rats NUCB2/nesfatin-1 became expressed in Sertoli cells upon Leydig cell elimination, and was also detected in Leydig cell progenitors. While NUCB2 mRNA levels did not overtly change in rat testis during pubertal maturation and after short-term fasting, NUCB2/nesfatin-1 content significantly increased along puberty-to-adult transition and was markedly suppressed following fasting. In addition, testicular NUCB2/nesfatin-1 expression was up-regulated by pituitary LH, since hypophysectomy decreased, whereas human choriogonadotropin (hCG; super-agonist of LH receptors) replacement enhanced, NUCB2/nesfatin-1 mRNA and peptide levels. Finally, nesfatin-1 increased hCG-stimulated testosterone secretion by rat testicular explants *ex vivo*. Our data are the first to disclose the presence and functional role of NUCB2/nesfatin-1 in the testis, where its expression is regulated by developmental, metabolic and hormonal cues, as well as by Leydig cell-derived factors. Our observations expand the reproductive dimension of nesfatin-1, which may operate directly at the testicular level to link energy homeostasis, puberty onset and gonadal function.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1019

**Neurocognitive phenotype and personality profile in men with Klinefelter syndrome and their vulnerability to psychiatric symptoms**

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Background

Klinefelter syndrome (KS) is associated with increased risk of psychiatric disease and behavioral problems, as well as social problems. The background for these risks is not known.

Aim

The aim was to describe the cognitive function, personality traits and the vulnerability to psychiatric symptoms in patients with KS.

Methods

Fourty one KS patients and 41 age- and educational-matched control subjects participated in the study. 30 (73%) KS patients received testosterone treatment. All participants were tested with standardized neuropsychological tests and 4 questionnaires investigating psychological problems.

Result

KS patients scored significantly lower in processing speed, working memory, verbal abilities and showed a selective deficit in executive function compared to control subjects, whereas visual cognitive abilities and cognitive response inhibition was preserved. The KS patients displayed significantly higher levels of cognitive failures, emotional distress and autism traits as reported in questionnaires. Furthermore symptoms of anxiety were also significantly higher among KS patients, whereas there were no difference in depressive symptoms between KS patients and control subjects. On the NEO PI-R personality test KS patients scored high on the neuroticism scale, low on the extraversion scale and low on the conscientiousness scale. We could not discriminate between those KS receiving testosterone supplementation or those who did not, which is likely due to a small sample size.

Conclusion

Men with KS have deficits in several cognitive domains and have an altered personality phenotype. Furthermore our results suggest that KS may be associated with an increased vulnerability to psychiatric symptoms. In future analyses, we are going to assess the neuroanatomical, neurofunctional, endocrine and genetic basis for the cognitive deficits, altered personality phenotype and increased psychiatric symptoms seen in KS patients. Whether testosterone therapy or other interventions can alleviate these deficits remain to be proven.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1020

**FSHB promotor polymorphism influences male reproductive parameters**

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Introduction

Recently, a single nucleotide polymorphism (SNP) in the FSH (FSH)-β gene (*FSHB* −211G>T, rs10835638) that leads to reduced mRNA transcription was associated with serum FSH levels in an Estonian cohort of young men (Grigorova *et al., Hum Reprod* 2008). Further investigations showed an increased frequency of the T-allele in patients with oligozoospermia compared to men with normozoospermia (Grigorova *et al., JCEM* 2010). Another Baltic study (Grigorova *et al., JCEM* 2011) revealed significant associations with additional reproductive parameters (e.g. testicular volume). Since the three previous studies are limited to men from the Baltic area, a comparable approach was undertaken with patients from the Centre of Reproductive Medicine in Münster.

Methods

A large number of 1213 patients visiting for infertility workup (615 with normal and 598 with reduced sperm concentration, cut-off 20 Mill./ml) were retrospectively selected. Patients with known causes for male infertility (e.g. cryptorchidism, infections, chromosomal aberrations) were excluded. The SNP in the *FSHB* gene was analysed by TaqMan assay.

Results

The T-allele frequency was higher in the oligozoospermic group compared with men with normal sperm concentration (18.1 vs 14.7%, $P=0.023$). The T-allele showed significant dosage effects (each $P<0.05$) for FSH (−0.56 U/l per T-allele), LH (0.27 U/l) and bi-testicular volume (−5.0 ml). Fitting trends were found for associations with sperm concentration (−7.5 Mill./ml, $P=0.057$) and total sperm count (−24.1 Mill./ml, $P=0.088$). Meta-analysis of all published studies – comprising 3017 men in total – confirmed highly significant associations for FSH, LH and testicular volume. In addition, a significantly different distribution of genotypes between normo- and oligozoospermic men was found in the pooled analysis for the first time ($P=0.0052$).

Conclusions

The associations between *FSHB* genotype and serum FSH as well as testicular volume were confirmed in our study population and in the meta-analysis and further substantiate the *FSHB* −211G>T SNP as a novel risk factor for male infertility. Whether T-allele carriers benefit from FSH treatment remains to be elucidated.

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1021

### Side effect profile of long-term treatment of hypogonadal men with testosterone undecanoate

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Introduction
Testosterone therapy for hypogonadal men has been used for decades. However, there are still concerns regarding the safety of this treatment, particularly in elderly men.
Methods
Prospective registry study of 252 men (mean age 60.6±8.0 years), with testosterone levels between ≤3.5 ng/ml. They received parenteral testosterone undecanoate 1000 mg at day 1, week 6 and every 12 weeks thereafter for up to 66 months.
Results
After 60 months the following changes were observed.
Erythropoiesis
Haemoglobin increased from 14.44±0.72 to 14.99±0.45 g/dl ($P<0.0001$ vs baseline). Haematocrit increased from 43.18±2.83 to 48.78±1.7% ($P<0.0001$ vs baseline). Four patients had haematocrit levels >52% which resolved without intervention.
Prostate
PSA increased from 1.76±0.96 to 1.82±0.96 ng/ml ($P<0.0001$ vs baseline) with a plateau after 24 months. 3/255 patients were diagnosed with prostate cancer following elevated PSA at 18 weeks of treatment. Tumour grade was T2 in all three and Gleason score 3+3 in two and 3+2 in one patient, resp. They all underwent radical prostatectomy and were excluded from the analysis. Prostate volume increased from 28.3±11.12 to 30.23±12.4 ml ($P<0.0001$ vs baseline). The International Prostate Symptom score (IPSS) improved from 6.7±4.22 to 2.83±1.25. This was statistically significant vs baseline ($P<0.0001$) and the decrease remained statistically significant compared to the previous year over the first 48 months.
Liver enzymes
Aspartate transaminase (AST) decreased from 43.34±17.31 to 20.16±3.21 U/l, alanine transaminase (ALT) from 44.04±18.16 to 20.54±3.92 U/l ($P<0.0001$ vs baseline with a plateau after 24 months).
Conclusions
The incidence of 3/255 patients with prostate cancer is similar to that in screening programmes. Long-term treatment with testosterone undecanoate with monitoring according to the guidelines is acceptably safe.
Declaration of interest
I fully declare a conflict of interest. Details below.
Funding
This work was supported, however funding details unavailable.

## P1022

### Effect of testosterone on seminal proteome in male hypogonadism

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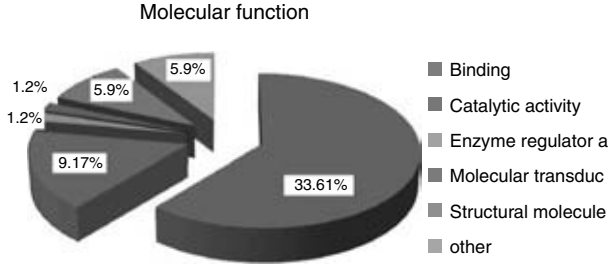
Seminal plasma (SP) contains proteins secreted by testis, epididymis and male accessory glands, involved in the successful fertilization of the oocyte. The function of epididymis, prostate and seminal vesicles are dependent upon the presence of androgenic stimuli.

To investigate the role of testosterone in the modulation of the proteomic pattern in SP, we analyzed human SP proteome comparing the panel of common seminal proteins in five fertile normogonadal males with the proteome by five patients with severe hypogonadism. In patients and control hormonal assays and standard semen analysis were performed; proteomic analysis was performed by an Ultimate 3000 Nano/micro-HPLC apparatus equipped with an FLM-3000-Flow manager module, and coupled with an LTQ Orbitrap XL hybrid mass spectrometer. In addition, GO annotation analysis was performed in the panel of androgen-dependent proteins.

88 to 1529 unique proteins were identified per individual subject sample. 83 proteins were present in all samples of normogonadal men, while 50 of these 83 proteins were absent in all hypogonadal patients. Some of these proteins may be involved in male fertility, such as ubiquitin carboxyl-terminal hydrolases 36, the olfactory receptor 5R1, the human cathelicidin antimicrobial peptide (hCAP18), complement factor H and spindlin1. GO annotation analysis (Figure 1: molecular function) provided further information to clarify which molecular function and biological process are mainly affected by androgen deficiency.




This is the first study that used a proteomic approach to offer a complete description of the proteic panel in severe male hypogonadism, indicating possible physiological targets for testosterone action, which might represent indicators of androgen-mediated protein synthesis at cellular level. Furhermore, the finding of the absence of seminal proteins involved in fertility in hypogonadal patients may explain the association between male hypogonadism and infertility.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1023

### Comparing effects of weight loss on sexual, urinary and endothelial function, insulin resistance and quality of life in obese men with and without erectile dysfunction

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Introduction
Abdominal obesity and insulin resistance are risk factors for erectile dysfunction (ED). ED is associated with hypoandrogenism, endothelial dysfunction, lower urinary tract symptoms (LUTS), and reduced quality of life (QoL). We aimed to compare effects of lifestyle modification-induced weight loss on insulin resistance, endothelial and sexual function, LUTS and QoL in obese non-diabetic men with and without ED.
Methods
Seventy abdominally obese Asian (body mass index ≥30 kg/m$^2$, waist circumference (WC) ≥90 cm) men (mean age 43.1 years, range 30–61) were tested with International Index of Erectile Function 5-item (IIEF-5) questionnaire. 68.5% ($n=48$) had ED (IIEF ≥21). Weight loss was induced using caloric restriction (500 kcal/day below basal metabolic rate) and moderate-intensity exercise (2000 kcal/week). IIEF-5, Sexual Desire Inventory (SDI), International Prostate Symptom (IPSS) and 36-item Short Form Survey Instrument (SF-36) scores, plasma sex-hormone binding globulin (SHBG), total testosterone (TT), insulin and glucose, endothelial function (by reactive hyperaemia index (RHI) using finger plethysmography on EndoPAT), were measured at baseline and 12 weeks later. Homeostasis model assessment (HOMA) was used to estimate insulin resistance.
Results
At baseline, men with ED had lower IIEF-5 and SDI scores, TT, SHBG and calculated free testosterone (FT), and higher WC, HOMA, and IPSS score. Men with ED had significantly greater increases in IIEF-5 (2.83 vs 0.05), SDI (8.27 vs 2.45) scores and decrease in IPSS score (1.90 vs 0.82) and HOMA (2.03 vs 0.65). Improvements in weight, WC, RHI, TT, SHBG, and SF-36 scores were similar (Table 1). IIEF-5 score normalized in 12 (25%) men.
Conclusions
Weight loss through diet and exercise in obese men reverses ED and improves sexual desire, endothelial function, LUTS, insulin resistance and quality of life, producing significantly greater benefits on insulin resistance, sexual and urinary function in men with ED.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.
Table 1

**Table 1** Changes in anthropometry, sexual function, sex hormones, LUTS, sexual and endothelial function and LUTS after 12 weeks of lifestyle modifcation.

| | **ED** ($n=48$) Mean±S.D. | **No ED** ($n=22$) Mean ±S.D. | ***P* value** |
|---|---|---|---|
| Baseline age (years) | 44.3±8.5 | 40.5±7.4 | 0.07 |
| Baseline BMI (kg/m$^2$) | 32.5±3.5 | 31.7±3.4 | 0.12 |
| Baseline weight (kg) | 96.9±10.6 | 94.2±10.8 | 0.32 |
| Baseline WC (cm) | 106.9±7.1 | 101.6±6.6 | 0.004 |
| Baseline IIEF-5 | 15.9±4.6 | 23.7±1.2 | <0.001 |
| Baseline SDI | 47.2±20.0 | 57.2±16.2 | 0.03 |
| Baseline IPSS | 6.1±4.2 | 2.6±2.3 | 0.001 |
| Baseline TT (nmol/l) | 12.26±3.97 | 12.71±4.74 | 0.68 |
| Baseline SHBG (nmol/L) | 25.88±9.01 | 24.58±10.12 | 0.59 |
| Baseline FT (pmol/l) | 287±97 | 298±87 | 0.67 |
| Baseline RHI | 1.89±0.56 | 1.92±0.61 | 0.83 |
| Baseline HOMA | 6.55±4.34 | 4.51±2.29 | 0.01 |
| Baseline SF-36 (physical component) | 45.3±7.8 | 49.6±6.4 | 0.02 |
| Baseline SF-36 (mental component) | 49.0±7.9 | 49.2±8.0 | 0.94 |
| Δweight (kg) | -4.1±3.4 | -3.4±2.1 | 0.32 |
| Δ WC (cm) | -3.7±3.1 | -3.3±2.1 | 0.62 |
| Δ IIEF-5 | 2.8±2.5 | 0.1±1.6 | <0.001 |
| Δ SDI | 8.3±10.3 | 2.5±9.1 | 0.02 |
| Δ IPSS | -1.9±2.7 | -0.8±1.6 | 0.04 |
| Δ TT (nmol/l) | 1.66±2.70 | 1.43±2.43 | 0.73 |
| Δ SHBG (nmol/l) | 2.58±4.36 | 2.43±3.22 | 0.88 |
| Δ FT (pmol/l) | 27±58 | 26±61 | 0.94 |
| Δ RHI | 0.36±0.50 | 0.41±0.63 | 0.70 |
| Δ HOMA | -2.03±2.88 | -0.65±1.19 | 0.01 |
| Δ SF-36 (physical component) | 5.1±6.6 | 3.2±4.9 | 0.24 |
| Δ SF-36 (mental component) | 3.7±4.8 | 5.2±6.8 | 0.35 |

## P1024

### Inhibitors of 5α-reductase-related side effects in patients seeking medical care for sexual dysfunction

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Introduction
Despite their efficacy in the treatment of benign prostatic hyperplasia (BPH) the popularity of inhibitors of 5α-reductase (5ARIs) is limited by their association with adverse sexual side effects. However, the real impact of 5ARIs on sex hormones and sexual function is controversial.
Aim
The aim of this study is to investigate the role of 5ARIs therapy on hormonal parameters and sexual function in men already complaining of sexual problems.
Methods
A consecutive series of 3837 men (mean age 63.5±12.8 years) attending our outpatient clinic for sexual dysfunction was retrospectively studied.
Main outcome measures: Several clinical, biochemical and instrumental (penile color doppler ultrasound; PCDU) factors were evaluated.
Results
Among the patients studied, 78.7% reported erectile dysfunction, 51.1% hypoactive sexual desire (HSD), 86.7% perceived reduced sleep-related erections (PR-SREs) and 19.1% premature ejaculation. The use of 5ARIs was associated with an increased risk of HSD and PR-SRs whereas no relationship was found with erectile dysfunction and ejaculation disturbances. Subjects using 5ARIs also more frequently had gynaecomastia along with reduced SHBG and higher calculated free testosterone levels. All these associations were confirmed in a case-control study comparing 5ARIs users with age-body mass index-smoking status and total testosterone matched controls.
Conclusions
Our data indicates that use of 5ARIs in men with sexual dysfunction does not significantly exacerbate pre-existing ejaculatory or erectile difficulties, but can further impair their sexual life by reducing sexual drive and spontaneous erection.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1025

### Multiplex ligation dependent probe amplification analysis of KAL1, GnRH1, GnRHR, PROK2 and PROKR2 in male patients with idiopathic hypogonadotropic hypogonadism

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Introduction
To date, several mutations have been identified as the underlying cause of hypogonadotropic hypogonadism. However, they account for a small minority of cases, suggesting that other genes play a significant role in the pathogenesis. The aim of the present study was to examine the prevalence of KAL1, GnRH1, GnRHR, PROK2 and PROKR2 mutations. Because it has several advantages over traditional screening methods, multiplex ligation dependent probe amplification (MLPA) was used to identify genomic rearrangements.
Description of methods/results
Eighty six hypogonadal young male patients (76 with nIHH and 10 with KS) and 95 age-matched, healthy control subjects participated in our study. Following DNA denaturation, hybridization of MLPA probes, ligation reaction, PCR reaction and separation of amplification products by electrophoresis data were evaluated using Genotyper 2.0 Software. Peak areas for each exon were converted into an Excel file and the relative DNA copy number ratios of each fragment were compared to the same fragments from 2 to 3 healthy subjects.
Result
Using MLPA for the described genes, seven mutations were present in 6 of the 86 patients with IHH. three patients with KS had heterozygous deletions in exon 9 of the KAL1 gene, one of whom also carried a duplication in exon 11 of the same gene, and 3 patients with nIHH possessed a duplication in exon 3 of the PROK2 gene, a heterozygous deletion in exon 1 and a duplication in exon 2 of the GnRHR gene, respectively. No abnormalities were identified in the healthy control individuals.
Conclusion
Defining the genetic basis of disease is essential to improve our understanding of this complex disorder, and can be useful for genetic counseling and for directing therapy. For detecting genomic deletions and duplications, MLPA is advantageous over the traditional methods because of its simplicity, relatively low cost and efficiency.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1026

### Cimetidine induces testosterone reduction and impairs leydig cell-microvasculature paracrine interaction in adult rats

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Cimetidine, an antagonist of histamine H2-receptors used for treatment of gastric ulcers, exerts antiandrogenic effect. In testes, cimetidine causes histopathological disorders in the seminiferous tubules and impairs spermatogenesis. Additionally to testosterone, Leydig cells (LC) produce several paracrine factors, including EG–VEGF (endocrine gland-derived vascular endothelial growth factor) which participates in the testicular microvasculature control. Regarding the importance of histamine and androgens for the blood vessels maintenance, we purposed to evaluate whether cimetidine impairs testicular microvasculature and/or structural and functional integrity of LC. Adult rats received 100 mg/kg BW of cimetidine (CMTG) and saline solution (CG) for 50 days. The testes were fixed in buffered 4% formaldehyde and embedded in historesin and paraffin. Some testicular fragments were embedded in Araldite for analysis under transmission electron microscopy. The serum levels of testosterone were also evaluated. In the PAS-stained historesin sections, the microvascular density (MVD) was obtained. Paraffin sections were submitted to the following reactions: TUNEL method; 17β-HSD6 (for LC quantification); 17β-HSD6+TUNEL (for detection of LC undergoing cell death); and Prokineticin-1 (for detection of EG–VEGF expression). The semiquantitative score (1+, 2+, 3+) of EG–VEGF-immunolabeled LC was obtained. Statistical analyses were performed ($P \leq 0.05$). In CMTG, a significant decrease (17%) in the MVD was observed. The presence of 17β-HSD6+TUNEL positive LC and the typical ultrastructural features of apoptosis confirm the harmful effect of cimetidine in LC. This is reinforced by the significant reduction in the number of LC and in the serum

levels of testosterone (42%). The immunoexpression of EG–VEGF in LC was also reduced in CMTG. The results indicate that cimetidine induces LC apoptosis. As vascular cells express ARs and VEGF receptors, the testicular microvasculature impairment may be caused by the interference of cimetidine treatment in the Leydig cell-microvasculature paracrine control due to LC death.

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1027

### Gonadal status in adult male survivors of childhood cancer

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Introduction

Survival rates in childhood cancer survivors (CCS) have enormously increased in the last 40 years. However, this improvement has been achieved at the expense of serious late effects, including the potential for severe gonadal damage. Here we report the effects of anticancer therapies on gonadal function in male long-term survivors of childhood cancer.

Methods

We estimated, at the last follow up available, the prevalence of primary hypogonadism, central hypogonadism and spermatogenesis damage in 151 male CCS mainly treated for hematologic malignancies (72%), brain tumors (13%) or sarcomas (7%). Since CCS are often reluctant to provide semen sample, in the absence of semen analysis we considered to have spermatogenesis damage all male patients with FSH values $>10$ IU/l and/or inhibin B $<100$ pg/ml. Statistical analysis was performed comparing the prevalence of gonadal dysfunction by treatments and some patient characteristics, using the $\chi^2$ test.

Result

Mean age at the last follow up was 25.6 years (S.D. = 5.1). Median follow-up time was 16.0 years. At the last follow-up visit, 69 patients (46%, CI = 38–54) showed gonadal dysfunction. Central hypogonadism was found in four brain tumor and in one acute lymphoblastic leukemia survivors who underwent cranial irradiation. Primary hypogonadism was found in 14 patients, mainly cured for hematologic malignancies (71%) or medulloblastoma (14%), all of them treated with radiation therapy involving the testis. Spermatogenesis damage was found in 50 CCS (33%). The main risk factors for gonadal dysfunction were radiation therapy involving the testis, busulfan, platinum-based chemotherapy agents, cyclophosphamide and hematopoietic cell transplantation (Fig. 1).

Conclusion

Male CCS have a very high risk to develop impaired spermatogenesis and, to a lower extent, primary or central hypogonadism. Semen cryopreservation is mandatory in post-pubertal patients. Moreover, new reliable techniques of fertility preservation in pre-pubertal males are needed.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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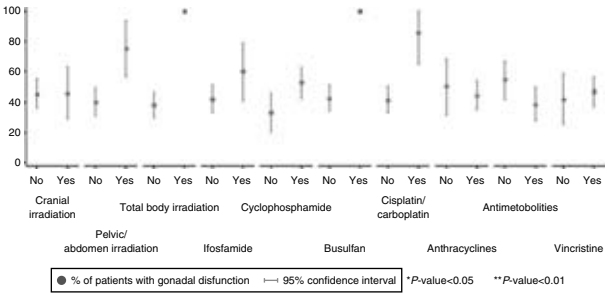


## P1028

### Cyclooxygenase-1 (COX-1) and COX-2, differently expressed in human sperm from normal and pathological patients, might be considered molecular markers in the diagnosis of male infertility disorders

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Varicocele and diabetes mellitus (DM) have long been recognized to impair male fertility by interfering with both sperm parameters and production, however the molecular mechanism by which these effects occur remains largely unknown. Cyclooxygenases are important in spermatogenesis, but their ultrastructural localization in sperm cells and their role in male infertility have not yet established in humans. In the present study we presented the evidence of constitutive (COX-1) and inducible (COX-2) cyclooxygenase expression in healthy human sperm as well as their expression pattern associated with varicocele and DM. By Western blot analysis the comparison between normal and pathologic samples, showed a constitutive expression of both COX isoforms in the spermatozoa from healthy donors, a significant increase in COX-1 and COX-2 protein levels in both 'varicocele' and 'diabetic' semen samples. Our transmission electron microscopic (TEM) data with immunogold analysis provide the first morphological evidence of COX isoforms particular distribution in both healthy and pathological sperm, intriguingly revealing modifications in the amount of these enzymes. Taken together, our finding suggest that varicocele and DM may lead to male factor infertility by a mechanism involving an increased COXs expression in human spermatozoa evidencing that a detrimental effect at the molecular level exists, thus going beyond the abnormal sperm morphology described to date. In conclusion COX isoforms might play an important role in the pathogenesis and/or manteinance of infertility states and might represent a potential therapeutic targets in future strategies designed for the treatment of fertility disorders.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1029

### Activation of selective human sperm functions by EGF and TGF-β3 is mediated by an ERK-depended mechanism

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Introduction

The signaling involved in ligand-stimulated spermatozoa is not yet clarified. Moreover, the main question in sperm biology is the identification of the sperm ligands. Analysis of the role of MAPK in mammalian sperm physiology is a prerequisite for assigning a role for MAPK in mammalian sperm functions. The unique opportunity to study ERK1/2 in human spermatozoa, which are differentiated cells lacking active transcriptional machinery, will enable to correlate it to differentiated responses such as: capacitation, motility, acrosome reaction and fertilization. We examined the effect of EGF and TGF-β3, as potential sperm ligands upon ERK1/2 activation and the role of ERK1/2 in forward and hyperactivated motility, capacitation and acrosome reaction.

Methods

Human semen was obtained from healthy donors with normal sperm concentration, motility and morphology according to WHO guidelines (WHO, 2010), or from males attending the Male Infertility Unit, Sheba medical center, Tel-Ha'shomer hospital, Israel.

Results

We have shown that ERK1/2 is activated during EGF and TGF-β3 activation of sperm after 5–15 min of incubation. Both ligands elevated total motility after 15 min of incubation and this stimulatory effect was abolished by adding, U0126 a selective inhibitor of MEK1/2. EGF also induced hyperactivated motility after 180 min, while TGF-β3 did not seem to induce hyperactivation. Both ligands elevated the percentage of acrosome reacted sperm cells after capacitation in both healthy donors, and OTA patients. This effect was also abolished after adding U0126, a selective inhibitor of MEK1/2. Regarding capacitation, we have identified several

proteins that were phosphorylated on tyrosine residues during capacitation, the effect was markedly reduced in the presence of the MEK1/2 inhibitor.
Conclusions
We conclude that EGF and TGF-β3 activation of selective sperm functions are mediated by an ERK-depended mechanism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1030

### Leydig and sertoli cell failure in myotonic dystrophy

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Introduction
Hypogonadism occurs in myotonic dystrophy (DM) type 1 and 2, a multisystemic autosomal dominant disorder with insulin resistance and visceral obesity. DM patients provide a model to investigate the impact of metabolic alterations on hypogonadism.
Methods
We assessed Leydig and Sertoli cell functions and metabolic features in 32 DM1 (44 + 11 years),13 DM2 patients (54 + 9 years) and 32 age and BMI-matched controls.
Results
Overt primary hypogonadism (sexual dysfunction, total T < 320 ng/dl) occurred in 26% of DM1 and 38% of DM2 patients. Increased LH was detected in 68% of DM1 and in all DM2. INSL3, marker of Leydig cell function, was lower in hypogonadal than in eugonadal patients and in controls. Anamnestic recall suggested reduced fertility in 34% of DM patients. AMH and inhibin-B, markers of Sertoli cell function, were dramatically reduced in DM than controls. FSH levels were elevated in 68% DM1 and in all DM2. DM patients showed an increased visceral body fat: waist/hip and body fat mass were higher than in controls; hepatosteatosis and increased epicardial fat thickness occurred in > 50% of cases. Dyslipidemia also was frequent (62%). Diabetes mellitus and insulin resistance were higher in DM2 (38 and 61%) than in DM1 (3 and 21%). Both HOMA-IR and body fat mass negatively correlated with freeT levels in DM patients. In DM1 patients AMH levels correlated positively with muscle strength, muscle mass, resting energy expenditure and negatively with clinical score. Furthermore, AMH negatively correlated with fat mass and waist/hip. In DM2 patients AMH was positively related with muscle strength, while it is not influenced by metabolic features and fat mass.
Conclusion
Hypogonadism is very frequent in DM patients and linked with muscle impairment. This study firstly demonstrates that hypogonadism in DM is associated with reduced levels of INSL-3, AMH and inhibin B and is negatively influenced by visceral adiposity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1031

### Decreased osteoprotegerin levels during testosterone therapy in ageing men were associated with changed distribution of regional fat

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Objective
The cardiovascular effects of testosterone treatment are debated. Osteoprotegerin (OPG) is an independent marker of cardiovascular risk. We investigated the effect of testosterone therapy on OPG levels in ageing men with low normal bioavailable testosterone levels.
Design
A randomized, double-blinded, placebo-controlled study of six months testosterone therapy (gel) in 38 men aged 60–78 years with bioavailable testosterone < 7.3 nmol/l and waist circumference > 94 cm.
Methods
Clinical evaluation, OPG, and C-reactive protein measurements. Bone mineral density (BMD), lean body mass (LBM) and total fat mass were established by dual X-ray absorptiometry and visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured by magnetic resonance imaging. Power calculation was based on an increase in LBM during testosterone therapy and responders were defined as testosterone treated patients with increased LBM (ΔLBM positive), $n = 14$. Data were presented as median (interquartile range).
Results
Testosterone therapy decreased total fat mass and SAT, whereas VAT was unchanged ($n = 38$). OPG levels decreased during testosterone therapy (from 2.0 (1.9–2.5) to 1.9 (1.6–2.2) ng/ml, $P < 0.05$ vs placebo), whereas CRP levels were unchanged. In responders for testosterone therapy ($n = 14$), ΔOPG levels were inversely associated with ΔSAT ($r = -0.60$, $P = 0.03$) and positively associated with ΔVAT ($r = 0.56$, $P = 0.04$). BMD levels were unchanged during testosterone therapy.
Conclusion
OPG levels decreased during testosterone therapy suggesting decreased cardiovascular risk. Decreased OPG levels were associated with changes in regional fat distribution and future studies are needed to further evaluate the association between OPG and regional fat mass distribution.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1032

### New insights into sperm DNA methylation: intra-and inter individual stability and a comparative analysis versus somatic cells

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Data about the entire sperm DNA methylome are limited to two sperm donors whereas studies dealing with a greater number of subjects focused only on a few genes or were based on low resolution arrays. This implies that information about what we can consider as a normal sperm DNA methylome and whether it is stable among different normozoospermic individuals is still missing. The definition of the DNA methylation profile of normozoospermic men, the entity of inter-individual variability and the epigenetic characterization of quality-fractioned sperm subpopulations in the same subject (intra-individual variability) are fundamental issues for a better understanding of pathological conditions. We addressed these questions by using the high resolution Infinium 450K methylation array and compared normal sperm DNA methylomes against somatic and cancer cells. Our study, based on the largest number of subjects ($n = 8$) ever considered for such a conspicuous number of CpGs ($n = 487{,}517$), provided clear evidence for i) a highly conserved DNA methylation profile among normozoospemic subjects; ii) a stable sperm DNA methylation pattern in different quality-fractioned sperm populations of the same individual. In addition, our analysis provided both confirmatory and novel data concerning the sperm DNA methylome, including its peculiar features in respect to somatic and cancer cells. Our description about a highly polarized sperm DNA methylation profile, the clearly distinct genomic and functional organization of hypo- versus hypermethylated loci as well as the association of histone-enriched hypomethylated loci with embryonic development provides solid basis for future basic and clinical research.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1033

### Complete reversal of isolated idiopathic hypogonadothropic hypogonadism in men

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Idiopathic hypogonadotropic hypogonadism (IHH) is caused by an isolated defect in GnRH release or action and classified as the Kallmann syndrome with associated anosmia or normosmic IHH in the presence of a normal sense of smell. Patients with IHH have absent or incomplete sexual maturation and infertility. Usually long term GnRH or gonadotropin therapy in these patients can induce spermatogenesis and puberty and is followed by testosterone therapy to maintain normal virilization. But in some patients with IHH brief discontinuation of testosterone replacement therapy probably results in reversal of IHH.
Material and methods
We observed prospectively 16 men with delayed of puberty and IHH (two had anosmia). At initial evaluation, all men had absent puberty and all had abnormal secretion of GnRH-induced LH and FSH levels and mean testosterone levels $0.81 \pm 0.14$ ng/ml ($2.75 \pm 0.61$ nmol/l). All men had received testosterone replacement therapy to induce virilization and after 9–12 months the treatment was followed by 8 weeks of treatment discontinuation with next evaluation. Reversal IHH was defined as testosterone levels above 2.5 ng/ml.
Results
In two men serum levels of endogenous testosterone increased from 0.82 $\pm 0.19$ ng/ml ($2.78 \pm 0.64$ nmol/l) to $3.69 \pm 0.81$ ng/dl ($12.5 \pm 2.75$ nmol/l, $P < 0.001$), LH and FSH levels also increased ($2.3 \pm 1.8$ to $7.9 \pm 2.9$ IU/l ($P < 0.001$) and $2.7 \pm 1.9$ to $8.9 \pm 9.6$ IU/l ($P < 0.01$) respectively). The mean testicular volume increased from $7 \pm 4$ to $15 \pm 4$ ml ($P < 0.001$) and spermatogenesis was initiated. In 14 men we did not observed statistical significant changes of LH and FSH levels after discontinuatopn of testosterone replacement therapy.
Conclusions
We documented reversal IHH in men with delay of puberty after relative short-time testosterone replacement therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1034

### G protein-coupled estrogen receptor 1- and estrogen receptor α-dependent pathways regulate spermatocytes apoptosis

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In mammals, spontaneous apoptosis is observed particularly in differentiating spermatogonia and spermatocytes. We have previously demonstrated that 17β-estradiol ($E_2$) in primary rat pachytene spermatocytes (PS) binds ESR1 and GPER1 to activate EGFR/ERK/c-Jun pathway leading to up regulation of bax, a pro-apoptotic factor. Aim of this study was to clarify the effector pathway(s) controlling spermatocytes apoptosis using as model GC-2 cells, an immortalized mouse pachytene spermatocytes cell line, reproducing primary cells responses to $E_2$. Like in primary PS, ESR1 and GPER1 activation in GC-2 cells caused rapid ERK and c-Jun phosphorylation, bax up-regulation associated with apoptosis. We further demonstrated that apoptosis is induced by $E_2$, as well as by specific ESR1 and GPER1 agonists, through sustained ERK, c-Jun and P38 phosphorylation, cytochrome *c* release, caspases 3 and endogenous substrate poly (ADP-ribose) polymerase (PARP) activation and increased expression of cell cycle inhibitor P21. When ESR1 or GPER1 expression were individually silenced, $E_2$ was still able to decrease cell proliferation, only the concomitant silencing abolished $E_2$ effect. These results indicate that GC-2 cells are a valid cell model to investigate the effects of $E_2$ on spermatocytes apoptosis and show that $E_2$, activating both ESR1 and GPER1, is able to induce an ERK1/2-, c-Jun- and P38-dependent mitochondrion apoptotic pathway in this type of cells. The definition of the molecular components of germ cells apoptosis will help us to better understand the consequences of the exposure to environmental estrogens, to provide new potential targets for the development of a non-androgen male contraceptive or for the development of novel therapeutic regimens to control germ cell tumors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1035

### Single nucleotide polymorphism (SNP) of endothelial nitric oxide synthase (eNOS) gene (Glu298Asp variant) in infertile men with asthenozoospermia

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Objective(S)
Single nucleotide polymorphism (SNP) in endothelial nitric oxide synthase (eNOS) G894T may affect the oxidative stress response. Our objective was to examine G894T SNP eNOS genotype frequencies and its potential role with sperm motility in infertile men. Through this prospective controlled study in the Andrology Unit, we have enrolled infertile ($n = 70$) and healthy ($n = 60$) men. Sperm motion kinetics assessed by computer assisted semen analysis (CASA), and allele-specific polymerase chain reaction (PCR-RFLP) to investigate the frequency of guanine (G) Thymine (T) at position -894 within exon 7 of the eNOS gene. Finding(S)
An increased frequency of the G894T eNOS (T) allele observed in asthenozoospermic patients ($P = 0.02$). In asthenozoospermic men, homozygotes eNOS (TT) genotyping showed low percentages of rapid motile sperm (a + b) compared to wild-type eNOS (GG) ($P = 0.02$) or heterozygotes eNOS (GT) genotyping ($P = 0.01$). In Fertile men, wild-type eNOS showed high percentages of rapid motile sperm (a + b) compared to eNOS (TT) ($P = 0.03$) or eNOS (GT) genotyping ($P = 0.04$).
Conclusion(S)
Our findings suggest that the T allele, encoding for aspartic acid, of the eNOS (Glu298Asp) gene may play a role with low sperm motility.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1036

### Severe depressive symptoms and cardiovascular risk in subjects with erectile dysfunction

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Introduction
Although penile blood flow (PBF) has been recommended as an additional diagnostic test in identifying erectile dysfunction (ED) patients at risk for latent cardiovascular disease, no study has ever assessed the possible association of PBF and the relational component of sexual function with incident major cardiovascular events (MACE).
Aim
The aim of this study is to investigate whether severity of ED, PBF and other factors related to a couple's relationship predict incident MACE.
Methods
A consecutive series of 1687 patients was studied. Different clinical, biochemical and instrumental (penile flow at color doppler ultrasound: PCDU) parameters were evaluated.
Main outcomes measures
Information on MACE was obtained through the City of Florence Registry Office.
Results
During a mean follow-up of $4.3 \pm 2.6$ years, 139 MACE, of which were fatal, were observed. Cox regression analysis, after adjustment for age and Chronic Disease Score, showed that severe ED predicted MACE (hazard ratio (HR) 1.75; 95% confidence interval (CI) 1.10–2.78; $P < 0.05$). In addition, lower PBF, evaluated both in flaccid (before) and dynamic (after PGE1 stimulation) conditions, was associated with an increased risk of MACE (HR $= 2.67$ (1.42–5.04) and 1.57 (1.01–2.47) respectively for flaccid ($< 13$ cm/s) and dynamic ($< 25$ mc/s) peak systolic velocity; both $P < 0.05$).
Reported high sexual interest in the partner and low sexual interest in the patient proved to have a protective effect against MACE.

Conclusions
the investigation of male sexuality, and in particular penile blood flow and sexual desire, could provide insights not only into present cardiovascular status but also into prospective risk.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1037

### The protective role of ginger against Di-(2-ethylhexyl) phthalate-induced reproductive toxicity and oxidative stress in male rabbits

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**Abstract**
The experiment was designed to study toxic effects of Di-(2-ethylhexyl) phthalate (DEHP) on semen characteristics, testosterone levels, testicular lipid peroxidation and testicular antioxidants in male New-Zealand white rabbits for 12 weeks. Ginger has been reported to be an important antioxidant. Therefore, the protective effect of ginger against DEHP-induced reproductive toxicity and oxidative stress was studied. Rabbits were orally administered the doses of ginger, DEHP and ginger plus DEHP every day for 12 weeks. Results obtained showed that DEHP significantly ($P<0.05$) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While, initial hydrogen ion concentration (pH), and dead and abnormal sperm were increased ($P<0.05$). Also, testosterone levels, body weight (BW), relative weights of testes (RWT) and epididymis (RWE) were decreased. Thiobarbituric acid-reactive substances and lactate dehydrogenase were increased, while glutathione S-transferase, transaminases and phosphatases were decreased in seminal plasma of rabbits treated with DEHP compared to control. Ginger alone significantly increased testosterone levels, BW, RTW, REW, semen characteristics and seminal plasma enzymes, and decreased the levels of free radicals and lactate dehydrogenase. Furthermore, the presence of propolis with DEHP alleviates its toxic effects. From the present study, it can be concluded that ginger can be effective in the protection of DEHP-induced reproductive toxicity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1038

### Mutations and polymorphism of FSH and LH receptors and sperm quality in men with hypergonadotropic hypogonadism without obvious testicular noxa

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Introduction
Inactivating mutations in LH or FSH receptors (R) are known causes of gonadotrophins resistance.
Aim
This study aims at evaluating the presence of mutations and polymorphisms of FSHR and LHR and sperm quality in men with hypergonadotropic hypogonadism without obvious testicular noxa.
Patients and methods
We selected 8 males with elevated levels of FSH and/or LH and normal levels of testosterone. Sperm parameters were analysed following WHO (1999) guidelines and by transmission electron microscopy (TEM). Elaborated TEM data provided the percentage of sperm pathologies and the number of structurally normal sperm (fertility index). The PCR technique, followed by DHPLC analysis and direct sequencing, enabled to investigate FSHR and LHR gene mutations.
Results
For LHR, 2/8 patients were heterozygous for Ile374Thr mutation and 1/8 for Glu354Lys substitution. Regarding FSHR, 4/8 were heterozygous for Ala419Thr mutation and 2/8 were homozygous for Ala307Thr mutation. We also found in one patient a new mutation (Cys338Arg) localized in the extracellular domain of FSHR. Minor allele frequencies of 43.75% for polymorphism rs6708637 and of 37.5% for polymorphism rs1922464 were observed, both similar to those reported in the general population. A minor allele frequency of 37.5% of polymorphism rs2091787, higher than that reported for the general population (24%), was observed. Two patients were azoospermic, 4/6 patients showed reduced sperm concentration, 6/6 reduced sperm motility, 4/6 high percentage of apoptosis and immaturity, 5/6 increased percentage of necrosis. The fertility index was reduced in all patients.
Conclusions
The relationship between the different mutations (included the new found mutation Cys338Arg in FSHR) and altered semen parameters deserves attention and further studies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1039

### Poor response to alprostadil ici test is associated with arteriogenic erectile dysfunction and higher risk of major adverse cardiovascular events

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Introduction
Intracavernous alprostadil injection (ICI) test has been considered useless in assessing the vascular status of subjects with erectile dysfunction (ED).
Aim
To analyze the clinical correlates of ICI test in patients with ED and to verify the value of this test in predicting major adverse cardiovascular events (MACE).
Methods
A consecutive series of 2,396 men (mean age $55.9 \pm 11.9$ years) attending our outpatient clinic for sexual dysfunction was retrospectively studied. A subset of this sample ($n=1,687$) was enrolled in a longitudinal study.
Main outcome measures
Several clinical, biochemical, and instrumental (penile color Doppler ultrasound; PCDU) factors were evaluated. All patients underwent an ICI test, and responses were recorded on a four-point scale ranging from 1= no response to 4= full erection.
Results
Among the patients studied, 16.4, 41.2, 40.2 and 2.2% showed grade 4, 3, 2, and 1 ICI test response, respectively. After adjusting for confounders, subjects with grade 1 ICI test response showed reduced perceived sleep-related, masturbation-related, and sexual-related erections when compared with the rest of the sample. In addition, a worse response to ICI test was associated with a higher prevalence of hypogonadism-related symptoms and signs along with lower testosterone levels. The prevalence of both diabetes mellitus and metabolic syndrome was inversely related to ICI test response. Accordingly, dynamic and basal peak systolic velocity (PSV), as well as acceleration at PCDU, decreased as a function of ICI test response. In the longitudinal study, after adjusting for confounders, grade 1 response was independently associated with a higher incidence of MACE (hazard ratio $=2.745$ (1.200–6.277); $P<0.05$). These data were confirmed even when only subjects with normal PSV ($>25$ cm/s) were considered.
Conclusions
Our results demonstrate that poor ICI test response is associated with several metabolic disturbances and higher incidence of MACE. We strongly recommend performing ICI test with alprostadil in all ED subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1040

### Hormonal associations and sexual dysfunctions in patients with impaired fasting glucose

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Introduction
The category of impaired fasting glucose (IFG) denotes a state of non-diabetic hyperglycemia, considered a risk factor of the further development of diabetes mellitus (DM) and cardiovascular (CV) diseases.
Aim
The aim of the present study is to evaluate the impact of IFG on sexual health in men. In addition its effect on CV morbidity and mortality will also be addressed.
Methods
A consecutive series of 3451 men (mean age $57.3 \pm 10.1$ years) attending our outpatient clinic for sexual dysfunction was retrospectively studied. A subset of this sample ($n = 1687$) was enrolled in a longitudinal study.
Main outcome measures: Several clinical, biochemical (including testosterone) and instrumental (penile color doppler ultrasound; PCDU) factors were evaluated. IFG was defined by fasting glucose concentrations between 5.6 and 6.9 mmol/l (100–125 mg/dl). A higher threshold (6.1–6.9 mmol/l, 110–125 mg/dl) was also considered.
Results
Among the patients studied, 747 (21.7%) had DM. In addition, 659 (19.1%) subjects were classified as IFG. Patients with IFG, however defined, more often had severe ED, reduced penile blood flow and overt hypogonadism when compared to patients with normal glucose levels. In addition, men with ED and IFG showed poorer blood pressure and lipid profile with an overall increase in CV risk. Unadjusted incidence of major adverse CV events was significantly associated with baseline DM whereas there was a trend toward higher risk also for IFG, but this did not reach statistical significance. Conversely, both IFG and DM were significantly associated with a higher risk of fatal and non fatal cerebral events.
Conclusions
Checking glucose and testosterone levels is mandatory in subjects with ED, because testosterone substitution in impotent IFG subjects might not only ameliorate their sexual life but also their overall health.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1041

### The relationship between total testosterone, cognitive function, depressive behavior and sleep quality in chronic renal failure patients not on dialysis

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Purpose
Studies have shown that testosterone levels were associated with cognitive function, depression and sleep quality in general population. However these relationships between testosterone, cognitive function, depressive behavior, and sleep quality in chronic renal failure (CRF) patients not on dialysis were not evaluated before.
Methods
All patients underwent history taking, physical examination, blood pressure measurement, routine urine and biochemical analysis, 24-h urine collection to measure urinary protein excretion and creatinine clearance, evaluation of cognitive function, depressive behavior and sleep quality.
Results
In total 109 CRF patients were enrolled. The total testosterone levels in stage 3, 4 and 5 CRF patients were $8.32 \pm 4.35$, $6.71 \pm 3.12$, and $4.22 \pm 1.28$, ng/ml respectively ($P < 0.0001$). Post hoc analysis revealed that total testosterone levels were different between stage 3 and stage 5 CRF patients ($P < 0.0001$), between stage 4 and stage 5 CRF patients ($P < 0.0001$) but not different between stage 3 and stage 4 patients ($P$:0.094). Standardized Mini Mental State Examination (SMMSE) Score, Pitssburg Sleep Quality Index Score, Beck Depression Inventory (BDI) Score of the patients were $26.2 \pm 1.9$, $7.1 \pm 3.4$ and $8.6 \pm 6.4$ respectively. In linear regression analysis total testosterone levels were found to be independently associated with SMMSE score (b: 0.170, Confidence Interval: 0.047–0.293, $P$: 0.008) and BDI score (b: $-0.750$, Confidence Interval: $-1.283$–($-0.216$), $P$: 0.006) but not with sleep quality.
Conclusion
Serum total testosterone levels were independently associated with cognitive function and depressive behavior but not with sleep disorders in stage 3 to 5 CRF patients not on dialysis
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1042

### Premature ejaculation and erectile dysfunction in a cohort of infertility men: clinical and ultrasonographic correlations

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Introduction
Premature ejaculation (PE) and erectile dysfunction (ED) are considered pre-testicular causes of male subfertility. No previous study systematically evaluated the prevalence of ED and PE along with their possible correlations with clinical and colour-Doppler ultrasound (CDU) features in infertile men.
Methods
Ejaculatory status, erectile function, prostatitis symptoms and psychopathological traits of 244 men complaining of couple infertility were evaluated by Premature Ejaculation Diagnostic Tool (PEDT), International Index of Erectile Function-15 (IIEF-15), National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) and Middlesex Hospital Questionnaire (MHQ), respectively. PEDT score $\leq 8$ indicates no-PE. IIEF-15 erectile function domain (IIEF-15-EFD) score $< 26$ indicates erectile dysfunction. All patients underwent simultaneous hormone evaluation, seminal analysis and interleukin 8 (sIL8), a reliable surrogate marker of prostatitis, along with penile, scrotal and transrectal CDU, before and after ejaculation.
Results
PE was found in 38 (15.6%) and ED in 43 (17.8%) subjects. After adjusting for age, a positive association between PEDT score and prostatitis symptoms (NIH-CPSI score) and signs (sIL8) was observed. In addition, subjects with a higher PEDT score more often had CDU features suggestive of prostate inflammation, including hypoechoic texture, hyperaemia and a higher arterial peak systolic velocity (APSV). Finally, they showed a higher prevalence of psychopathological traits (MHQ score). In a multivariate model including age and calculated free testosterone (also positively related to PEDT score), APSV, NIH-CPSI and MHQ score were independently positively associated with PEDT score. IIEF-15-EFD score was positively associated with mean arterial blood flow acceleration at basal penile CDU, while a negative association with NIH-CPSI and MHQ score was observed. No correlation between IIEF-15-EFD or PEDT score and semen parameters was observed.
Conclusions
PE and ED should be ruled out in infertile patients. However, they have no impact on semen parameters. PEDT score is positively associated with prostatitis symptoms and signs including CDU features.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1043

### Maternal malnutrition alters the leptin expression in the rats epididymis

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Several papers show that maternal malnutrition can affect the reproductive capability of the offspring. Leptin is an important regulator of the reproductive system, however little is known about its role in the epididymis. The goal of this

study was to evaluate whether the maternal malnutrition during lactation can alter the expression of leptin and its receptors in the epididymis of rats. Six rat dams were randomly assigned to the control group (C), with free access to a standard laboratory diet containing 23% protein; and to a protein-energy-restricted group (PER), with free access to an isoenergy and protein-restricted diet containing 8% protein. After weaning, four male pups of each group had free access to the standard laboratory diet until 90 days of age, when they were killed. The gene expression of leptin and leptin receptors, ObRa and ObRb, were evaluated by qPCR, while the protein expression was evaluated by immunohistochemistry in the caput, corpus and cauda of epididymis. Values were expressed as mean $\pm$S.E.M. Data were analysed using Student's *t*-test and the level of significance was set as $P<0.05$. Maternal malnutrition increased the gene expression in the caput (ObRa-C $=0.5\pm0.1$, PER $=2.1\pm0.6$, $P<0.04$; ObRb-C $=0.2\pm0.1$, PER $=0.8\pm0.2$, $P<0.02$; leptin – C $=0.7\pm0.3$, PER $=2.2\pm0.3$, $P<0.04$) decreased in the cauda (ObRa-C $=1.4\pm0.1$, PER $=0.8\pm0.1$, $P<0.04$; ObRb-C $=0.02\pm0.003$, PER $=0.006\pm0.005$, $P<0.007$; leptin – C $=1.2\pm0.2$, PER $=0.4\pm0.1$, $P<0.02$) while there was no statistical difference in the corpus of epididymis. Imunohistochemistry show positive stain for leptin and leptin receptor in all epididymis regions. The epididymis role in the maturation of spermatozoa is well established. The fact that maternal malnutrition during lactation altered leptin and its receptors expression in a region specific way raise the possibility that this hormone could have an important role in the molecular mechanisms that govern the epididymis function that are still poorly defined.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1044

### Scrotal and transrectal ultrasound correlates of prostatitis-like symptoms in a cohort of infertility patients

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Introduction

Prostatitis-like symptoms (PLS) may originate from the prostate or from pelvic or scrotal organs. The National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) is considered the gold-standard instrument to assess PLS severity. Although several studies previously investigated the impact of prostatitis, vesiculitis or epididymitis on semen parameters, only a few studies evaluated the correlations between PLS and scrotal or transrectal colour-Doppler ultrasound (CDU) characteristics. We here evaluate these correlations, along with PLS and semen parameters possible associations, in infertile men.

Methods

PLS of 400 men complaining of couple infertility were assessed by NIH-CPSI. Index pain score $\geq 8$ has been previously defined as indicative of 'moderate-severe' symptoms. All patients underwent, during the same CDU session, seminal analysis and interleukin 8 (sIL8), a reliable surrogate marker of prostatitis, urino and seminal cultures, along with scrotal and transrectal CDU, before and after ejaculation.

Results

After adjusting for age, a positive association between NIH-CPSI total score and positive urino and/or seminal cultures, leukocytospermia and sIL8 levels was observed. Conversely, no correlation with semen parameters was found. Subjects with a higher NIH-CPSI total score more often had CDU features suggestive of inflammation of the prostate (including a higher arterial peak systolic velocity, APSV), seminal vesicles and epididymis. As assessed by ROC curve, at 10.4 cm/s APSV discriminates subjects with an index pain score $\geq 8$ with a sensitivity and specificity of 76%. When subjects with an index pain score $\geq 8$ were compared to the rest of the sample, a higher prevalence of prostate-vesicular and epididymal CDU abnormalities, positive cultures, leukocytospermia and higher sIL8 levels were observed, but no differences in semen parameters.

Conclusions

PLS are positively associated with positive cultures, leukocytospermia and sIL8 levels, along with prostate-vesicular and epididymal CDU abnormalities suggestive of inflammation. We suggest a 10.4 cm/s APSV cut-off to predict moderate-severe symptoms.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1045

### Elevated body mass index correlates with higher seminal interleukin 8 levels and ultrasound prostate abnormalities in infertile men

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Introduction

It is general acknowledgement that obesity is associated with a systemic, low-grade inflammatory state. Although the relationship between obesity and semen parameters or prostate diseases has been previously investigated, the association between body mass index (BMI), prostate inflammatory diseases and colour-Doppler ultrasound (CDU) features of the male genital tract (MGT) has been poorly studied. This study was aimed at evaluating the association between BMI and CDU features of the MGT, signs and symptoms of prostate inflammation, along with semen parameters.

Methods

We studied 222 men seeking medical care for couple infertility. According to WHO classification, subjects were divided into 3 groups: normal weight ($n=131$, BMI $=18.5$–$24.9$ kg/m$^2$), overweight ($n=71$, BMI $=25.0$–$29.9$ kg/m$^2$), obese ($n=20$, BMI $\geq 30.0$ kg/m$^2$). All patients underwent simultaneous testosterone evaluation and seminal analysis, including interleukin 8 (sIL8), along with scrotal and transrectal CDU, before and after ejaculation. Prostatitis symptoms were evaluated by National Institutes of Health-Chronic Prostatitis Symptom Index questionnaire.

Results

After adjusting for age and testosterone levels, higher BMI was significantly related to higher prostate volume and several CDU features of the prostate, including macro-calcifications, inhomogeneity, higher arterial peak systolic velocity (the latter adjusted also for blood pressure), but not with abnormalities of testis, epididymis, seminal vesicles. Furthermore, higher BMI and BMI class were significantly related to higher sIL8, a reliable surrogate marker of prostate inflammatory diseases, even after adjustment for age. Conversely, no associations among BMI, clinical symptoms of prostatitis or semen parameters were observed.

Conclusions

Subjects with higher BMI might develop CDU and biochemical signs suggestive of prostate inflammation, although not clinically overt.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1046

### Ultrasonographic and clinical correlates of seminal plasma interleukin 8 levels in patients attending an Andrology Clinic for infertility

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Introduction

Interleukin 8 (IL8) is a proinflammatory cytokine highly expressed in seminal plasma. Seminal IL8 (sIL8) has been proposed as a reliable surrogate marker of prostatitis and as a potential marker of male accessory gland infection (MAGI). So far, no study systematically evaluated the correlations of sIL8 with colour-Doppler ultrasound (CDU) features of the male genital tract. Hence, we investigated these possible correlations in infertile patients with MAGI.

Methods

Out of 250 subjects seeking medical care for couple infertility, 79 (mean age $36.4\pm7.5$ years) met the criteria of MAGI and scored higher than the rest of the sample on the National Institutes of Health-Chronic Prostatitis Symptom Index score. All patients underwent, during the same CDU session, hormone evaluation and seminal analysis (including sIL8), along with scrotal and transrectal CDU, before and after ejaculation.

Results

After adjusting for age, sIL8 in patients with MAGI was significantly related to several abnormal semen and CDU parameters. In particular, leukocytospermia was closely associated with sIL8. Ejaculate volume, unlike other semen or hormonal parameters, was negatively associated with sIL8. When transrectal CDU was performed, sIL8 was positively related to prostate abnormalities including calcifications, inhomogeneous or hypo-echoic texture, hyperaemia and

high arterial blood flow. In addition, an association between sIL8 and ejaculatory ducts dilatation and calcifications was observed. Finally, an association with seminal vesicles hyper-echoic texture was found. When scrotal CDU was performed, sIL8 was positively related to epididymal abnormalities including inhomogeneous, hypo- or hyper-echoic texture, calcifications, hyperaemia and an increased size of the tail. Conversely, no association was found with testis parameters.
Conclusions
sIL8 levels in patients with MAGI are associated with leukocytospermia and CDU abnormalities of the prostate, seminal vesicles and epididymis, but not of the testis. Furthermore, sIL8 correlates with signs suggestive of ejaculatory duct inflammatory subobstruction.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1047

**Visceral adiposity index and the effect of testosterone treatment in young men with congenital hypogonadotropic hypogonadism**

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Introduction
Visceral adipose tissue (VAT) is closely related to metabolic derangement, such as dyslipidemia insulin resistance and type 2 diabetes. Also, there is an increase in deposition of visceral adipose tissue in hypogonadal subjects. The visceral adiposity index (VAI) is a sex-specific mathematical index, based on waist circumference (WC), body mass index (BMI), triglycerides (TG) and HDL cholesterol (HDL) levels, indirectly expressing visceral adipose function. We investigated the VAI in treatment-naive, young men with congenital hypogonadal hypogonadism (CHH) and searched for the effect of testosterone replacement on the VAI of this specific patient group.
Description of methods/design
A total of 190 patients (age 21.9±2.25 years) were enrolled. The control group included 200 age and BMI matched healthy young men (age 21.7±1.5 years). Standard regimen of testosterone esters (250 mg/3 weeks) was given to patients.
Results
The levels of VAI was higher in CHH ($P<0.0001$) according to healthy controls. The patients had higher WC, TG, HDL cholesterol ($P<0.001$ for all) levels (Table 1). After 5.52±2.5 months of testosterone treatment WC ($P<0.0001$), TG ($P<0.019$), VAI ($P<0.002$) levels were increased and HDL cholesterol levels were decreased ($P<0.0001$; Table 1).

**Table 1** The metabolic profiles of the patients with hypogonadism and healthy volunteers

| | Patients (*n*: 190) | | Controls (*n*: 200) | p1 | p2 |
|---|---|---|---|---|---|
| | Before treatment | After Treatment | | | |
| Age (years) | 21.9±2.25 | – | 21.7±1.5 | 0.23 | – |
| BMI (kg/m$^2$) | 22.0±3.74 | 22.9±3.3 | 21.9±1.1 | 0.179 | 0.0001 |
| WC (cm) | 82.8±10.8 | 85.6±9.9 | 76±4.7 | 0.0001 | 0.0001 |
| SBP (mmHg) | 112.4±9.6 | 116.1±10.5 | 107.5±10.7 | 0.0001 | 0.001 |
| DBP (mmHg) | 71.9±8.0 | 72.4±8.6 | 67.7±9.0 | 0.0001 | 0.23 |
| TG (mg/dl) | 102.5±46.8 | 109.2±43.5 | 81.4±42.9 | 0.0001 | 0.019 |
| HDL (mg/dl) | 46.7±11.4 | 42.6±10.6 | 53.1±9.8 | 0.0001 | 0.0001 |
| VAI | 3.1±1.8 | 3.6±1.6 | 2.0±1.4 | 0.0001 | 0.002 |

p1, patients vs controls; p2, before treatment vs after treatment; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, trigliseride; HDL, high density lipoprotein; VAI, visceral adiposity index.

Conclusion
This study shows that VAI levels are increased in treatment-naive, young men with CHH and after the testosterone replacement, the VAI levels are increased further.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1048

**Prevalence of erectile dysfunction is greater in young middle-aged HIV-infected men than in hiv-uninfected men**

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Introduction
Erectile dysfunction (ED) is common among elderly men and patients suffering from chronic diseases, the latter probably including also HIV infection. No studies, however, compared the prevalence of ED in HIV-infected and -uninfected individuals using the IIEF-15.
Aim
To compare ED prevalence in young-middle aged men with and without HIV infection using the International Index of Erectile Function (IIEF-15) questionnaire.
Methods
We conducted a cross-sectional, observational, controlled study on 444 HIV-infected and 71 -uninfected men. The IIEF-15 questionnaire was used to assess ED. A cutoff score of <25 of the erectile domain was used to diagnose ED. Serum testosterone, demographic and anthropometric (weight, height, BMI) characteristics were obtained from all participants. Statistics included the T-test, the Fisher's test, univariable and multivariable logistic regression and univariate and multivariate Spearman's correlation analysis.
Results
HIV-uninfected group was significantly younger than HIV-infected and presented a higher BMI ($P<0.001$). The prevalence of mild, moderate, and severe ED was higher in HIV-infected than in HIV-uninfected men of all decades of age (Fig. 1). In univariate analysis, HIV infection was associated with ED (OR=34.19, $P<0.001$). In multivariable logistic regression analysis, HIV infection remained the strongest predictors of ED (OR=42.26, $P<0.001$) followed by hypogonadism, after adjusting for age and BMI.
Conclusions
This study demonstrates a clear association between ED and HIV infection, after adjusting for age and BMI. Other than HIV infection, hypogonadism was associated with ED. In addition, the prevalence of ED was higher in HIV-infected than in HIV-uninfected men, in all decades of age. The early onset of ED in HIV-infected men could be considered a peculiar clinical hallmark of HIV and confirms precocious aging in these patients. ED should be of concern to clinicians when managing HIV-infected men even if the latter are young or middle aged.

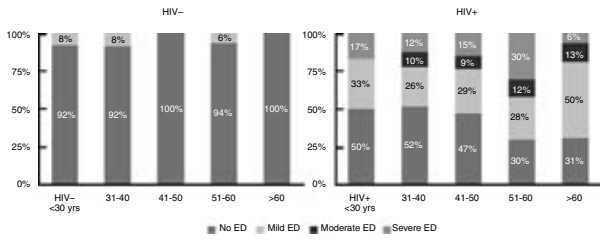


**Figure 1** Prevalence of erectile dysfunction in HIV-infected and HIV-uninfected patients in all decades of age.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1049

**Effectiveness of gonadotropin treatment for spermatogenesis induction in hypogonadotropic hypogonadism: a possible role of androgen receptor CAG repeat polymorphism**

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Prepuberal-onset HH (PRHH) and postpuberal-onset HH (PSHH) men required GnTh by means of administration of both the β human chorionic gonodadotropin (ηHCG) and the FSH to induce a complete spermatogenesis. However, the response to GnTh is always unpredictable concerning either the effectiveness or the duration of the therapy. Consequently, different studies have been carried out to identify clinical and biochemical markers that can be useful to predict the effectiveness of GnTh. As testosterone plays an essential role in inducing and maintaining spermatogenesis, we measured the AR CAG in HH men, in order to examine whether the AR-CAG extensions could co-regulate the GnTh effectiveness. Twenty-three HH subjects subdivided according to the age at onset (pre- and postpubertal), were treated with the same scheme and doses of GnTh up to 30 months. Thirty-five healthy and fertile men served as a control group (CG).Twelve HH men (three PRHH and nine PSHH), who reached complete spermatogenesis within 12 months, showed AR CAG repeat number (20 (19–23) = median (interquartile range 25th – 75th percentile)) not statistically different from our CG (20 (19–22)), while CAG repeat number (23 (20–25)) of 11 HH patients (nine PRHH and two PSHH) who reached complete spermatogenesis beyond a year to within 30 months, was significantly higher. Our results suggest that AR CAG length might affect the response to GnTh in HH men, especially in PRHH patients.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1050

**Phosphodiesterase type 5 expression in human and rat lower urinary tract tissues and the effect of tadalafil on prostate gland oxygenation in spontaneously hypertensive rats**

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Introduction
In humans, prostate PDE5 expression was prominently localized in the endothelial and smooth muscle cells of the vascular bed, suggesting a possible action of PDE5 inhibitors on prostate blood flow.

Aim
To investigate PDE5 expression in human and rat lower urinary tract (LUT) tissues and determine the effects of PDE5 inhibition with tadalafil on prostatic blood perfusion.

Main outcome measures
Human vesicular-deferential arteries (which originate from the inferior vesical artery) were analyzed for PDE5 expression and activity. The effects of tadalafil on prostate oxygenation were studied in spontaneously hypertensive rats (SHR), characterized by ischemia/hypoxia of the genitourinary tract.

Methods
PDE5 expression was evaluated by quantitative reverse transcription-polymerase chain reaction and immunohistochemistry. SHR were treated with tadalafil (2 mg/kg per day) for 1, 7, or 28 days and compared with untreated SHR and the unaffected counterpart Wistar–Kyoto (WKY) rats. Prostate oxygenation was detected by hypoxyprobe-1 and hypoxia markers (hypoxia-inducible factor-1α (HIF-1α) and endothelin-1 type B (ETB)) immunostaining.

Results
Human vesicular-deferential artery expressed high levels of PDE5, similar to corpora cavernosa, immunolocalized in the endothelial and smooth muscle layer. In these arteries, tadalafil inhibited cGMP breakdown (half maximal inhibitory concentration in the low nanomolar range, as in corpora cavernosa) and increased the relaxant response to sodium nitroprusside. SHR prostate resulted markedly hypoxic (hypoxyprobe immunopositivity) and positive for HIF-1α and ETB, while tadalafil treatment restored oxygenation to WKY level at each time point. The mRNA expression of the HIF-1α target gene, BCL2/adenovirus E1B 19 kDa interacting protein 3, was significantly increased in SHR prostate and partially restored to WKY level by tadalafil.

Conclusion
Human vesicular-deferential artery is characterized by a high expression and activity of PDE5, which was inhibited by tadalafil in vitro. In SHR, tadalafil increases prostate tissue oxygenation, thus suggesting a possible mechanism through which PDE5i exert beneficial effects on LUT symptoms.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1051

**Ten years of EAA/EMQN quality control scheme for microdeletions of the Y chromosome**

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Introduction
Y-chromosomal azoospermia factor (AZF) microdeletions are one of the few well-established genetic causes of male infertility, specifically azoospermia or severe oligozoospermia. Since the introduction of a PCR-based method to easily detect the distinct deletion patterns, the screening of infertile men for Y microdeletions has become a very common genetic test performed frequently by a large number of laboratories. An external quality control scheme was established in 1997 upon initiative of the Institute of Reproductive Medicine, University of Münster, Germany, shortly thereafter continued in cooperation with the European Academy of Andrology (EAA), and from 2004 onward within the framework of the European Molecular Quality Network (EMQN). Best practice guidelines describing methodology and interpretation of results are also available (Simoni *et al., Int J Androl*. 2004 **27** 240–9).

Methods
In most of the annual quality control schemes, three validated DNA samples with mock clinical case descriptions were distributed to participating labs. We retrospectively analysed the final results of each year.

Results
Between 2000 and 2010 the number of participating laboratories almost tripled from 57 to 144. The diagnostic error rate (an incorrect genotype that would lead to a misdiagnosis), decreased steeply during the first five years from almost 8% and now fluctuates at around 2%. While also variable, an assessment of the quality of diagnostic report content shows that around 50% of analyses in the last four years have scored full marks which is also an improvement on the previous early time of the scheme. Recurrent interpretation problems still arise due to laboratories using an unnecessary high number of markers, which are specifically included in commercially available kits.

Conclusions
This established external quality control scheme is a successful tool to improve the performance of participating labs and has demonstrated an improvement on reporting practice and decreasing diagnostic error rates.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1052

### SIEDY scale 3, a new instrument to detect intrapsychic component in subjects with erectile dysfunction

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Introduction
We previously developed and validated a structured interview (SIEDY) dealing with the organic (scale 1), relational (scale 2) and intrapsychic (scale 3) components of erectile dysfunction (ED). The aim of the present study is to identify a pathological threshold for SIEDY scale 3 and to analyze scale 3 score with biological and psychological correlates in subjects with sexual dysfunction.
Method
A pathological threshold of SIEDY scale 3 score in predicting subjects with a medicals history of psychopathology and using psychiatric drugs was identified through receiver operating characteristic (ROC) curve analysis, in a sample of 484 patients (sample A). Sensitivity and specificity, along with possible interactions with biological and psychological (Middlesex Hospital Questionnaire, MHQ-score) correlates were verified in a further sample of 1275 patients (sample B).
Results
In sample A, 39 (8%) and 60 (12.4%) subjects reported a positive medical history for psychiatric disturbances or for the use of psychotropic medication respectively. The association with both conditions was present in 28 (5.8%) subjects. ROC curve showed that SIEDY scale 3 score predicts psychopathology with an accuracy of $69.5 \pm 5.9\%$ ($P < 0.002$), when a threshold of 3 was chosen. When the same threshold was applied in Sample B, it identified a higher ranking in MHQ-A (free-floating anxiety), MHQ-S (somatized anxiety) and MHQ-D (depressive symptoms) subscales, even after adjustment for age and $\Sigma$-MHQ (a broader index of general psychopathology). In the same sample, we also confirmed that pathological scale 3 score was related to a higher risk of psychopathology at medical history or to the use of psychotropic drugs as well as with risky lifestyle behaviours, including smoking and alcohol abuse, and elevated BMI.
Conclusions
SIEDY represents an easy tool for the identification of patients with a relevant intra-psychic component who should be considered for psychological/psychiatric treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1053

### Immunogold localization of testosterone and immunohistochemical detection of AR, SHBG, CYP1A1, ERβ and PCNA indicate a participation of steroid hormones in primordial germ cells of adult male bullfrogs

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In vertebrates, ARs have been detected in spermatogonia and also fetal gonocytes, confirming the importance of testosterone for the initial process of spermatogenesis. During seasonal spermatogenesis of adult bullfrogs (Lithobates catesbeianus), primordial germ (PG) cells show testosterone immunoexpression in winter; however, a weak or none testosterone immunoexpression was detected in summer. With the aim to confirm the role of testosterone in these cells, the immunogold localization of testosterone and the immunohistochemical (IHC) detection of AR and SHBG in PG cells were performed. Regarding the important role of estrogen for spermatogenesis, the immunoexpression of aromatase (CYP1A1), ERβ and PCNA was also evaluated in PG cells according to seasonality. The bullfrog testes were collected in winter and summer and were embedded in glycol methacrylate, for PG cells quantitative analysis; or in paraffin, for detection of stem cell markers (alkaline phosphatase activity and GFRα1), AR, SHBG, CYP1A1, ERβ and PCNA. The mitotic index of PG cells and the semiquantitative score (HSCORE) of ERβ-immunolabeled PG cells were obtained. Some fragments were processed for transmission electron microscopy for testosterone immunocytochemistry. PG cells were positive to stem cell markers. Immunogold-labeling of testosterone was usually observed in the cytoplasmic regions next to mitochondria. Moreover, cytoplasm immunoexpression of ARs and SHBG was also detected in the winter PG cells, confirming the participation of testosterone in these cells. In summer, the cytoplasm immunoexpression of CYP1A1 and the high ERβ HSCORE in PG cells confirm that testosterone is converted into estrogen. At winter, the number of PG cells was significantly higher ($P \leq 0.05$) than summer; however, a high number of PCNA-positive PG cells was coincident to the high ERβ HSCORE at summer. Therefore, the results indicate that testosterone and estrogen seems to play a role in the control of the seasonal mitotic activity of PG cells during spermatogenesis of bullfrogs.
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Declaration of interest
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## P1054

### The high prevalence of testosterone deficiency in population of Polish men over 65 years with erectile dysfunction

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About 8–10% of erectile dysfunction (ED) is caused by hormonal causes, but in men over 65 years testosterone deficiency due to aging seems to play important role. In population of Polish men with relative poor health status, the prevalence of testosterone deficiency in elderly men with ED is probably higher. We evaluated the prevalence of low and low-normal testosterone levels in men with ED in population of Polish men over 65 years.
Material and methods
286 men (mean age 72.2 years) wit ED completed a sexual activity questionnaire (International Index of Erectile Function - IIEF-5). Possible scores on the IIEF-5 are 1 to 25 and erectile dysfunction was classified into 5 categories namely severe - 1 to 7, moderate - 8 to 11, mild to moderate - 12 to 16, mild - 17 to 21 and none - 22 to 25 points. Serum total testosterone was measured in all men.
Results
The prevalence of testosterone deficiency was 17, 33, 42 and 57% for testosterone levels of $<200$, $<250$, $<300$, and $<350$ ng/dl respectively. The degree of ED was significant higher in men with lowest testosterone levels ($P < 0.002$). The degree of ED in all group was mild in 39.5% of cases, mild to moderate in 26.2%, moderate in 18.2% and severe in 16%. We observed a statistically significant inverse relationship between age and total testosterone levels ($r = -0.3328$, $P < 0.05$), similar to IIEF-5 score and total testosterone ($r = -0.3149$, $P < 0,05$) and IIEF-5 score and age ($r = -0.3463$, $P < 0.05$).
Conclusions
Testosterone deficiency was very common in population of Polish men with ED and testosterone levels correlated negatively with age and IIEF-5 score.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1055

**Production of nitric oxide and peroxynitrite by human spermatozoa and their possible role on sperm motility**

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Spermatozoa generate small amounts of O2°- and NO°. Under physiological condition these compounds exist at very low concentration and the formation of peroxynitrite (ONOO−) appears likely. Peroxynitrite reacts rapidly with proteins, lipids and DNA. Moreover, tyrosine nitration is a widely used marker of peroxynitrite. The nitration of protein residues gives rise to 3-nitrotyrosine which represents a protein modification specific for ONOO− formation *in vivo*. We determined NO and ONOO− production in semen and their potential role with sperm motility in infertile men, and we set out to determine whether protein tyrosine nitration takes place in sperm cells motility. Semen samples from 25 normal fertile donors and 40 infertile patients were analyzed. NO was measured by Griess Reaction while peroxynitrite concentration through the fluorescence of the DCFDA probe. Protein tyrosine nitration was determined by Western Immuno Blot as well as the presence of eNOS and iNOS. The controls exhibited both NO and ONOO− productions that was significantly lower than asthenozoospermic patients and were inverse correlated with the motility parameters. Moreover, the western immuno blots showed an increase in the nitration of the tyrosine residues in the asthenozoospermic samples compared to controls and the presence of both iNOS and eNOS proteins. The same pattern was confirmed by means of immunohistochemistry. The present data suggest a critical negative effect of NO and peroxynitrite on sperm motility when spermatozoa concentration is normal. Thus, a possible pathogenic role in infertile men when asthenozoospermia is the main critical problem may be suggested.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1056

**Two infertile patients with 45X/46XY mosaicism and structural rearrangements of chromosome Y present with different phenotypes**

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Introduction
Within the high prevalence of male infertility there is the rare 45,X/46,XY mosaicism, associated or not with hypogonadism. The phenotypes of this genetical disorder present with a wide variability ranging from Turner stigmata to apparent normal male.
Case report
Two men who presented with azoospermia in the context of couple infertility were subsequently found to be affected by 45,X/46,XY mosaicism. Physical examination revealed a complete different phenotype; case 1 presented with normal weight and short stature, whereas case 2 presented with abdominal obesity and normal height. Case 2 had also bilateral gynecomastia, micropenis and a history of monolateral gonadectomy for criptorchidism at puberty. Both cases presented with small and soft testes, decreased bone mineral density and perceptive hearing loss at audiometric testing. No cardiorenal alterations were found. Assessment of gonadal function showed an increase of LH and FSH with normal testosterone levels (3.5 ng/ml) in case 1 and low testosterone levels in case 2 (2.2 ng/ml). A state of glucose intolerance with normal insulin levels at baseline (4.0 μU/ml) and after glucose load (peak: 37 μU/ml) was found in case 1, whereas case 2 had normal glucose tolerance, basal hyperinsulinemia (19 μU/ml) but normal insulin response to glucose load (peak: 77 μU/ml). The lipid profile was normal with unexpected high HDL-cholesterol levels (case 1: 78 mg/dl; case 2: 61 mg/dl). Cytogenetic examination revealed a different prevalence of 46,XY cells between case 1 and 2. Structural Y chromosome abnormalities were found in both: 45,X/46,X,idic(Y) (pter->q12::q12->pter) in case 1; 45,X/46,Xinv(Y)(q.11.2q12) in case 2.
Conclusion
Assessment of karyotype should be a key part of the initial evaluation of male patients with azoospemia regardless of the phenotype. The prevalence of 46,XY cells and the presence of Y rearrangements should always be evaluated for the potential explanation of different phenotypes, including comorbidities.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1057

Abstract withdrawn.

## P1058

**Seminal vesicles ultrasound features in a cohort of infertility patients**

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Introduction
Previous studies concerning ultrasound evaluation of the seminal vesicles (SV) have been performed on a limited series of subjects, taking into consideration few parameters, often only before ejaculation and without assessing sexual abstinence of the patients. The aim of this study is to evaluate the volume and the emptying characteristics of the SV and their possible correlations with scrotal and transrectal ultrasound features.
Methods
The SV of 368 men seeking medical care for couple infertility have been evaluated by ultrasound. All patients underwent simultaneous seminal analysis, along with scrotal and transrectal ultrasound, before and after ejaculation. A new parameter, SV ejection fraction, calculated as ((SV volume before ejaculation − SV volume after ejaculation)/SV volume before ejaculation)×100, was evaluated.
Results
After adjusting for sexual abstinence and age, both pre-ejaculatory SV volume and SV ejection fraction were positively associated with ejaculate volume. As assessed by receiver operating characteristic (ROC) curve, at 21.6% SV ejection fraction discriminates subjects with normal ejaculate volume ($\geq$ 1.5 ml) and pH ($\geq$7.2 ml) with a sensitivity and specificity of 75%. Subjects with reduced SV ejection fraction ($<$21.6%) more often had higher post-ejaculatory SV volume and ejaculatory duct abnormalities. Furthermore, a higher post-ejaculatory SV volume was associated with a higher prostate volume and SV abnormalities. Higher epididymal and deferential diameters were also detected in subjects with a higher post-ejaculatory SV volume or reduced SV ejection fraction. No association between SV and testis ultrasound features or sperm parameters was observed. Associations with SV ejection fraction were confirmed in nested 1:1 case–control analysis.
Conclusions
SV mainly contribute to ejaculate volume. A new parameter, SV ejection fraction, has been investigated, and could be useful in assessing SV emptying. Reduced SV ejection fraction ($<$21.6%) is associated with prostate-vesicular and epididymal ultrasound abnormalities.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1059

**Nitric oxide synthase and tyrosine nitration in idiopathic asthenozoospermia: an immunohistochemical study**

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It has been previously shown that human spermatozoa produce nitric oxide (NO), a short-lived free radical synthesised by three isoforms of NO synthase (NOS): endothelial (eNOS), neuronal (*n*NOS), and inducible (iNOS), which are responsible for the conversion of L-arginine to L-citrulline and NO. Spermatozoa of fertile men express both eNOS and nNOS. The specific roles of NOS isoforms in gametes are not well understood, even if iNOS seems to negatively affect sperm function through the production of large amounts of NO. NO plays a relevant role in sperm physiology, although at high concentrations it could combine with superoxide to produce peroxynitrite, which can rapidly react with proteins, lipids, and nucleic acids. In the present study we aimed to assess the immunohistochemical expression of NOS isoforms in human spermatozoa isolated from normospermic fertile donors and infertile subjects affected by idiopathic asthenozoospermia. In addition we evaluated the immunoexpression of citrulline, to achieve the microscopic visualisation of NOS catalytic activity, and nitrotyrosine, since ample evidence supports the tyrosine nitration of proteins *in vivo* in different pathological conditions.
Our results show that constitutive NOS expression was greater in spermatozoa isolated from normospermic fertile donors. On the contrary, the immunohistochemical expression of inducible iNOS and nitrotyrosine was higher in asthenozoospermic samples. Our data concerning citrulline indicated an enhanced NOS activity in sperms from idiopathic asthenozoospermic patients.
In conclusion, our study strongly support the hypothesis that an increased NOS activity, and consequently an excess of tyrosine nitration are involved in the pathogenesis of idiopathic asthenozoospermia that causes male infertility, furthering the development of new therapeutic strategies in the near future.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1060

**Testosterone replacement therapy: A comparison of testosterone mixed esters, extended release testosterone implants and testosterone undecanoate depot**

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Introduction
Male hypogonadism is one of the most common endocrine disorders affecting 5.6% of men aged between 30–79 years. Testosterone deficiency (TD) is an independent risk factor for multiple cardiovascular risk factors including obesity, diabetes mellitus, hypertension, dyslipidaemia and endothelial dysfunction. The aims of testosterone replacement treatment (TRT) in hypogonadism are to improve or ameliorate the clinical features and to restore the serum T to physiological levels. Treatment with TRT for hypogonadism is typically lifelong so needs to be safe, effective, convenient and minimise impact on lifestyle. Therefore the frequency of administration, mode of delivery, efficacy and side-effect profile are important.
Methods/design
Records of 155 hypogonadal males treated at the Waikato Endocrine Unit from 1998–2011 were examined to compare use of testosterone mixed esters, extended-release testosterone pellet implants and testosterone undecanoate depot (TUD). Efficacy of types of TRT in terms of achievement of physiological levels of T over two years, and changes in known risk factors such as Hb & Hct changes, PSA, and lipid profile changes were examined retrospectively.
Results
Mean T levels of men on Sustanon ($n=22$) ranged 1.6 to 54 nmol/l. Normal serum T trough levels were found in 53% of men using T implants ($n=134$) compared with 75.5% while using TUD ($n=155$), $P<.0001$. Mean Hct levels were higher on TUD than implants, $P<.0001$, with elevations in Hct managed by dose alterations. PSA elevations were no different on each TRT. HDL levels were sig lower using TUD than T implants, $P<.0001$, but total cholesterol, triglycerides and LDL were not significantly different by TRT. Only 4.5% of men elected to stop TUD or return to another TRT.
Conclusion
Testosterone undecanoate depot therapy maintained serum T at more physiological levels and was more acceptable to men as compared to other TRT options.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1061

Abstract withdrawn

## P1062

**Reduced fatigue and improved sexual function in hypogonadal men treated with TESTIM in routine daily practice**

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Introduction
Hypogonadal men frequently suffer from fatigue and reduced sexual function. International guidelines recommend testosterone replacement therapy (TRT) for symptomatic hypogonadal men. Our aim was to document testosterone changes and the impact of TRT with TESTIM on quality of life in routine daily practice.
Methods
In this non-interventional-study (NIS) data from 117 hypogonadal patients from 43 office-based-study-sites were evaluated. Data were collected at baseline, 3 and 6 months of TRT. Laboratory values, BMI and relevant clinical symptoms (loss of libido, obesity, erectile dysfunction and others; scale 0–4) were recorded. Patients were asked to fill in quality of life questionnaires: aging males symptoms scale (AMS), multidimensional fatigue inventory (MFI) and international index of erectile function (IIEF-5). Common pharmacovigilance monitoring was conducted.
Results
Testosterone increased significantly within 6 months from a median of 2.38 to 4.99 ng/ml. All recorded clinical symptoms improved significantly under TESTIM therapy. The most prominent clinical symptoms at baseline improved by 1.23, 1.53 and 0.78 points for erectile dysfunction, loss of libido and obesity respectively ($P<0.0001$).
Quality of life (AMS, MFI and IIEF-5) improved significantly under TESTIM therapy by 32.4, 28.6 and 48.3%, respectively ($P<0.0001$). We found significant associations of higher testosterone levels with freedom of symptoms from hypogonadism in AMS and IIEF-5 after 6 months of therapy ($rs=0.279$, $P=0.0045$).
Frequency of adverse events was 5.1%, with only 1.7% attributed to therapy. The most frequent adverse event was nausea (1.7%).
Conclusion
This NIS in hypogonadal men substantiates the clinical efficacy of TESTIM. Overall quality of life as well as sexual function improved significantly within 6 months of therapy, and fatigue was significantly reduced. A good or excellent efficacy and safety was demonstrated in the vast majority of patients in routine daily practice.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P1063

**The mitochondrial citrate carrier regulates insulin secretion by human male gamete**

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Sperm energy management is poorly studied and understood. The present study provides biochemical and morphological evidence that ejaculated human sperm

express the mitochondrial citrate carrier (CIC) which is restricted to the midpiece. CIC inhibition by the specific substrate analogue 1,2,3-benzenetricarboxylate (BTA) reduced cholesterol efflux, proteins tyrosine phosphorylation, *P*-AKT, *P*-*P*60src addressing to its functional role in sperm acquisition of fertilizing ability. Worthy, CIC inhibition reduced sperm insulin secretion, glucose-stimulated insulin secretion (GSIS) and glucose-6-phosphate dehydrogenase (G6PDH) activity, supporting its role in the regulation on insulin secretion. In this study, we discovered a novel sperm factor involved in the regulation of metabolism and acquisition of sperm fertilizing ability. It may be taken into account as molecular target in the therapies designed for male infertility disorders.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1064

**Mild androgen insensitivity syndrome due to novel mutation in androgen receptor gene**

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We describe a clinical case of the patient with the syndrome of partial resistance to androgens. The patient, a man 25 years old, complained of a barren marriage. Lab and clinical findings included high gonadotropin and testosterone levels, hypogonadal appearance, coronal hypospadias, left-sided varicocele, oligoasthenoteratozoospermia. The results of cytogenetic analysis showed a normal male karyotype (46, XY). Molecular genetic studies identified mutation in exon 1 of androgen receptor gene – s.731_736delCGGTGT, which allowed a precise diagnosis – Reifenstein syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1065

**Semen quality in patients with newly diagnosed Hodgkin's disease**

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Objective
To analyse the semen quality in patients with newly diagnosed Hodgkin's disease before starting the treatment.
Materials and methods
We evaluated semen quality in 67 patients with Hodgkin's disease who underwent sperm banking in our clinic over a 10-year period. Semen samples were collected by masturbation after 2–7 days of sexual abstinence. Age at banking, semen volume, sperm concentration, and total and progressive sperm motility were recorded. Semen parameters were compared to established World Health Organisation (WHO) reference values (WHO Laboratory Manual for the Examination and Processing of Human Semen, Fifth Edition, 2010).
Results
The median age of the patients was 27 years (range 17–43); median semen volume was 2.0 ml (range 0.2–6.5); median sperm concentration was 57.9 $\times$106 per ml (range 0–413); median total and progressive sperm motility were 42 (range 0–79) and 24% (range 0–69) respectively. According to the reference values of the WHO 18 of 67 patients (26.9%) in this series had a semen quality within the normal range, and 49 of 67 patients (73.1%) had abnormal semen quality. In 2 patients (3%) the semen samples were not frozen because of azoospermia (no spermatozoa in the ejaculate). 35 patients (52.2%) had single damages (oligozoospermia or asthenozoospermia) and 12 patients (17.9%) had combined damages (oligoasthenozoospermia).
Conclusion
Patients with Hodgkin's disease have an increased risk for inadequate semen quality before any treatment. 73.1% of these men in our study had abnormal semen parameters according to the WHO. Generally, all patients with newly diagnosed Hodgkin's disease need counselling about their reproductive function and semen cryopreservation should be offered before undergoing gonadotoxic treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1066

**Subcutaneous gonadotropin therapy for male infertility by hypogonadotropic hypogonadism**

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Objetive
Evaluate the efficacy of subcutaneous HCG with or without FSH in male infertility associated to hypogonadotropic hypogonadism (HH).
Patients and methods
Descriptive study of patients with HH treated with HCG s.c. with or without FSH, for fertility objective (2004–2011). The Treatment was initiated with HCG 500 UI/72 h s.c., periodic monitoring of testosterone and semen analysis was realized, adjusting doses up to 2500 UI/72 h. If no answer obtained after 1 year, combined treatment with FSH s.c. was started. Analyzed data: cause of HH, age, testosterone levels, semen, dose of HCG and FSH treatment. The result has been assessed to the achievement of pregnancy.
Results
Ten patients. 30.6$\pm$2.9 years old. Cause of HH: 50% idiopathic, 20% nonfunctioning pituitary adenoma, 10% prolactinoma, 10% acromegaly and 10% craniopharyngioma. Fertility 60%, 22.6$\pm$13.5 months treatment time. Only one patient discontinued treatment without success. No presence of side effects.
Subgroup result:
Conclusions
Subcutaneous gonadotropin therapy is effective in infertility HH. Treatment with subcuataneous HCG alone is successful. No presence of side effects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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Table 1

**Table 1** Subgroup results.

| | Less than 24 months ($n$=6) | More than 24 months ($n$=4) |
|---|---|---|
| Patients (%) | 60 | 40 |
| Doses HCG (UI/72 h) | 1250$\pm$612.4 | 2125$\pm$750 |
| FSH (%) | 30 | 50 |
| Testosterone (mcg/l) | 7.6$\pm$3.9 | 7.3$\pm$5.5 |
| Time (months) | 13.2$\pm$7.5 | 36.7$\pm$3.4 |
| Fertility (%) | 50 | 75 |

## P1067

**Translation of EMAS sexual functions questionnaire from English to Lithuanian**

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EMAS - SFQ is recently available instrument, validated and prooved valid in multicentral European study of more than 3000 eldery men (O'Connor DB,

Corona G & Forti G *et al*. 2008). This questionnaire assess male sexual functions rather than only erection disturbances.
The aim of this study was to perform translation of EMAS - SFQ from English to Lithuanian for assessing elderly Lithuanian men suffering of prostate cancer. Permission for translation was kindly offered by I Huhtaniemi and J Finn as well as detailed procedure when having the questionnaire translated to Lithuanian.
The procedure consisted of translation – backtranslation of two pairs of persons, resulting in two versions of Lithuanian EMAS – SFQ followed by expert evaluation, resulting in the only final version. Instructions for translators were: <no deadline, no hurry, but quality>. Quality of translation was considered text to be close to English original and Lithuanian language and understanding.
Permission for translation - 6th October, Final version pair 1st–26th October. Final version pair 2nd–6th December. Final version of EMAS – SFQ – 10th December.
1st pair – psychiatrist–endocrinologist/ andrologist, 2nd pair – endocrinologist–psychologist, expert evaluation–endocrinologist/andrologist/sexologist and endocrinologist/sexologist/English philologist.
197 positions for translation were found in EMAS – SFQ.
63 positions (32%) were evaluated by experts as the same in sense and grammar between 1st and 2nd pairs.
57 positions (28.95%) were evaluated by experts in favour of the 1-st pair translation, and 27 positions (13.7%) – in favour of the 2-nd pair translation.
In 21 position (10.65%) text was finally modified by experts.
Final version of EMAS - SFQ was approoved by experts.
The final version was composed of positions with the same sense and grammar in both pairs of translators, favoured positions of 1-st or 2-nd pair of translators and all the positions with modified text.
Proposed procedure of translation proves that the only translation - back-translation may be insufficient for optimal result.
Model of translation using two pairs of translators – backtranslators plus expert evaluation elucidates coincidences and differences in translation and permits the emission of final optimal version.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1068

**A surprising diagnosis in a man with high testosterone levels**
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Introduction
Marked elevation of testosterone levels can be caused by tumors with hormonal hypersecretion. However, we should be aware of the relationship between thyroid and sexual hormones, including changes of SHBG.
Case report
A 48-year-old man was recommended by an urologist to endocrinological examination due to high levels of total testosterone.
CT scan of abdomen, pelvis and the pituitary did not show any abnormalities.
The patient did not complain of any problems, but after inquiry he admitted a weight loss of 15 kg during last year which he related to his effort of eating less because of obesity. Apart of that we did not find any deviations from physical normal status including thyroid palpation, skin temperature and heart rate. Laboratory tests revealed the following results: $fT_4$ 48 pmol/l, $fT_3$ 16 pmol/l, TSH 0.005 mIU/l, total testosterone 59 nmol/l and SHBG 190 nmol/l (34–66). Free androgen index was still within the normal range 31 (30–152).
Within four months of therapy by antithyroid drugs, both the levels of thyroid hormones and total testosterone returned to norm.
Conclusions
Hyperthyroidism may lead to an elevation of total testosterone in males due to an increase of hepatic synthesis of SHBG. The metabolic clearance of testosterone is also decreased and the peripheral conversion of androstendion to testosterone is increased. Despite this, the levels of free testosterone are usually normal.
The restoration of all these parameters is in most cases achieved after euthyroidism is established.
This report shows the need of judging the levels of sexual hormones in connection with the levels of thyroid hormones (and SHBG) with respect to the fact that thyroid disorders may be clinically silent.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1069

**Recapture of the study on the reproductive function of Estonian, Latvian and Lithuanian Young Men after 8 years**
S. Nikulina[1], S.. Abramavicius[1], R. Preiksa[1], V. Matulevicius[1], V. Adomaitiene[1], R. Linkevicius[1], R. Verkauskiene[1], N. Sabeckiene[1], B. Zilaitiene[1], J. Pundzius[2] & I. Saukiene[3]
[1]Lithuanian University of Health Sciences, Kaunas, Lithuania; [2]Lithuanian University of Health Sciences Kaunas Clinics, Kaunas, Lithuania; [3]Delfi.lt, Vilnius, Lithuania.

In 2003–2004 year a study named The Reproductive Function of Estonian, Latvian and Lithuanian Young Men, 18–25 year aged, was performed (QLK4-CT-1999-01422, NAS: QLK4-CT-2001-02911). Determination of semen quality, physical appearance and testicular size in relation to the level of reproductive hormones and environment, including life style factors was performed.
The aim of our study was to recapture young men, who had participate in the study 7–8 years ago and repeat the same examination also to examine the sexuality of these young men using EMAS- Sexual Functions Questionnaire, translated and approved for using in Lithuania. This group will be one of the groups in which relationship between general health, sexuality and physical activity and fitness will be performed.
Using the contacts (name, surname, address, phone number) we have, but keeping one's confidenciality, searched for everyone, who had participated at the study in 2003– 2004 year. The search was performed by phone, sending a posted letter; inviting through internet news-portal www.Delfi.lt, social network www.Facebook.lt, and internet phone network Skype.
Ninety nine (30.27%) men were recaptured. 86 (86.86%) men were recaptured by phone; 9 (9.09%) men- using internet news portal www.Delfi.lt; 4 (4.04%) men- by internet phone network Skype; 1 (1.01%) man by post; and none by social network www.Facebook.lt. 4 (4.04%) of recaptured men refused to continue the research.
Using given contact data (phone number and address) were recaptured 87 (87.87%) men, and using modern technologies (internet news portal, social networks)- 13 (13.13%) men.
It is known that 7 (2.14%) men live in foreign countries today, and one man was killed few years ago.
According to young age of our participants we believed that majority of them could change their living place, phone number or emigrate. Despite that, most of participants were found using given contact data by phone. And modern technologies didn't approved our expectations and minority was recaptured using internet news portal, phone network or social network.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Neuroendocrinology

## P1070

**SNPs and CNVs genotyping analysis of patients with idiopathic central hypogonadism (ICH). A novel approach to detect new candidate mechanisms**
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Introduction
Idiopathic central hypogonadism (ICH) is a rare and heterogeneous disease due to defects of GnRH secretion or action. ICH could be associated or not with hypo-anosmia respectively identifying the Kallmann's syndrome (KS) or the normosmic ICH (nICH). Even though 14 disease genes have been identified, in 70% of patients no genetic cause could be identified, suggesting additional regulatory genes and still unknown mechanisms. Thus, with the aim to identify new candidate genes, we decided to use a new and genome-wide approach on a group of ICH patients that resulted negative to the previous genetic analysis.

Methods
A total of 32 DNA samples from KS and nICH patients and their unaffected relatives were analyzed together with 1864 negative controls from the normal population. Samples were genotyped using 660W-Quad BeadChip Illumina (660.000 SNPs and 100.000 CNVs). The data obtained were analysed using statistical and bioinformatics tools.
Results and conclusions
Our results showed a statistically significant difference in the number of deletions between cases and controls which might indicate a genetic predisposition to ICH. The SNPs analysis allowed us to identify some candidate loci and in particular one gene with strong brain expression and two different microRNA clusters, thus indicating a possible involvement of microRNA in the pathogenesis of ICH. Loss of Heterozygoisity region analysis showed an enrichment for some pathways/families of protein, such as FGFR pathway, cadherins, brain-expressed GPCR and proteins involved in axon guidance. Furthermore analyzing the chromosomal position we found that 8 of the identified genes mapped in a cluster on chromosome 10 originated by an event of genetic duplication from the primitive block that contains the GNRH1. This may indicate that even though the GNRH1 function in this locus is lost during evolution, it is possible that this chromosome block has maintained or developed new functions in the control of reproduction.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1071

**Cidea as a posible mediator of ghrelin resistance induced by high fat diet**

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Ghrelin is a peptide hormone secreted by the stomach with orexigenic properties that induce food intake trough the activation of hypothalamic AMP activated kinase (AMPK). In rodents fed on normal diet the central administration of ghrelin induces an increase in food intake, however, when fed a high fat diet (HFD) rodents are resistant to the orexigenic effect of ghrelin. Recent data show that Cidea interacts with AMPK β-subunit and promotes AMPK degradation trough ubiquitin-dependent pathway.
The main goal of this work was to study the mechanism mediating ghrelin resistance in the hypothalamus under HFD.
For this purpose male Sprague–Dawley rats were fed either a low fat diet (LFD) or a HFD. These animals were i.c.v. injected with ghrelin (5 μg/μl) or saline. The hypothalamic levels of different proteins were analyzed by western blot using specific antibodies.
Food intake induction by ghrelin administration was blunted in rats fed on HFD when compared with rats fed on LFD, which indicated resistance to ghrelin effect caused by HFD. In rats fed on LFD, ghrelin injection induced an increase in hypothalamic levels of phosphorilated forms of AMPK (pAMPK) and its downstream target acetyl-CoA carboxylase (pACC). However in rats fed under HFD, ghrelin treatment caused no changes in pAMPK and pACC levels. Of note, these animals showed a decrease in AMPK β1 and AMPK β2 levels, which was associated with the increase of the hypothalamic levels of Cidea.
These results may suggest that the mechanism of ghrelin resistance could be associated with dysregulation of Cidea and consequently of AMPK in the hypothalamus.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1072

**Effects of obestatin on proliferation and survival of adult rat hippocampal progenitor cells**

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Obestatin (Ob) is a peptide recently identified as a product of the ghrelin gene. It was claimed to bind to the orphan receptor GPR39, but this finding is still a matter of debate. Ob exerts peripheral effects, for example, it promotes cell survival and has antiapoptotic actions in different cell lines. In addition, Ob has central effects, such as inhibition of thirst, modulation of anxiety, sleep and mnemonic functions. Mnemonic processes involve neurogenesis; in particular, hippocampal neurogenesis, which is fundamental for learning, comprise proliferation and differentiation of progenitor cells. Interestingly, both acylated ghrelin and the synthetic peptidyl GH secretagogue hexarelin have been previously shown to stimulate proliferation of adult rat hippocampal progenitor cells (AHPs). In the present study, we investigated Ob effects on proliferation and apoptosis of AHPs and the underlying signaling pathways. Ob effects were assessed in AHPs cultured in the absence of growth factors, a condition which reduces cell survival and proliferation, and increases apoptosis. Cell survival was measured by MTT assay; cell proliferation by BrdU incorporation and apoptosis through caspase-3 activity and Bcl-2 expression. Western blot analysis was performed to determine Ob-induced activation of neuroprotective pathways. Ob was found to promote cell survival and proliferation and to protect against growth factor deprivation-induced apoptosis. Furthermore, Ob effects involved PI3K/Akt, ERK1/2, Wnt/β-catenin and mTOR, as demonstrated by both activation of these pathways and by use of specific inhibitors. These results indicate that Ob promotes survival and proliferation, and inhibits apoptosis of AHPs through activation of neuroprotective pathways. Moreover, they suggest a possible role of Ob in neuronal precursor cell protection and candidate Ob as potential therapeutic molecule in conditions such as hippocampal damage.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1073

**Normalization of cortisol levels in Cushing's disease after medical pretreatment before surgery: effects on somatostatin receptor subtype expression and *in vitro* response to somatostatin analogs.**

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Introduction
Corticotroph pituitary adenomas that cause Cushing's disease (CD) predominantly express the dopamine 2 receptor (D2) and somatostatin receptor subtype (sst) 5. The expression of sst2 is relatively low because of downregulating effects of high endogenous cortisol levels. This may explain why the sst2-preferring somatostatin analog octreotide is not effective in CD. To assess whether normalization of urinary free cortisol (UFC) excretion modulates the sst expression pattern, sst expression profiles of corticotroph adenomas from patients with normalized or elevated preoperative cortisol levels were compared. Moreover, the effects of pasireotide and octreotide on ACTH production were examined *in vitro*.
Methods
Corticotroph adenoma tissue was examined from patients from group 1 ($n=11$; mean duration of normocortisolism 10.3 weeks) and group 2 ($n=21$; elevated preoperative UFC). RT-PCR and immunohistochemical studies were performed to determine sst expression of these adenomas and to compare them to somatotroph adenomas ($n=10$).
Results
At mRNA level, adenomas from group 1 had tenfold higher sst2 expression levels compared to group 2 ($P<0.01$). Sst2 mRNA expression in group 1 was even comparable to that in somatotroph adenomas. There were no differences in sst5 and D2 mRNA expression levels between groups 1 and 2. At the protein level, no differences were found in sst2, sst5 or D2 receptor expression between group 1 ($n=7$) and 2 ($n=5$). Finally, octreotide was significantly less potent than pasireotide (both 10 nM) with respect to inhibition of ACTH secretion by adenomas of group 1 ($-18.9\pm9.1\%$ ($n=4$) vs. $-36.3\pm11.2\%$ ($n=6$); $P<0.001$).
Conclusion
After 10 weeks of normocortisolism induced by medical therapy, cortisol-mediated sst2-downregulation on corticotroph adenomas appears to be reversible at mRNA, but not yet at protein level. Octreotide remains less potent than pasireotide with respect to *in vitro* inhibition of ACTH secretion. This suggests that a sustained period of normocortisolism induced by medical therapy is required to induce re-expression of sst2 protein in corticotroph adenomas.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1074

**Mechanisms for the protective effects of 17-β-estradiol: relevance to depressive symptoms in Parkinson's disease**

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Background
Parkinson's disease (PD) is a neurodegenerative disease and a movement disorder characterized by loss of dopaminergic neurons in the substantia nigra causing dopamine depletion in the striatum. Neurodegeneration in PD occurs due to multiple pathways including oxidative stress, mitochondrial damage, protein aggregation. These changes increase during menopausal condition in females when the level of estradiol is decreased. Recently, there has been a growing interest in the action and functions of the ovarian steroid hormone estradiol, particularly on whether they are neuroprotective for such age related disease and neurodegenerative conditions like stroke, PD and Alzheimer's disease.
Objective
The objective of this study was to investigate protective potential of 17β estradiol ($E_2$) treatment on the activity of monoamine oxidase, calcium homeostasis, membrane polarization, genomic DNA degradation, 4- hydroxynonenal and protein oxidation levels occurring in brains of female rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of estradiol.
Methods
The aged rats (12 and 24 months old) ($n=8$ for each group) were given subcutaneous injection of 17β-estradiol (0.1 μg/g body weight) daily for 1 month. After 30 days of hormone treatment, experimental animals of all the groups were sacrificed and brains were isolated for further study.
Results
The results obtained in the present work revealed that normal aging was associated with significant increases in the activity of monoamine oxidase, calcium homeostasis, genomic DNA degradation, 4- hydroxynonenal and protein oxidation levels in the brains of aging female rats, and a decrease in membrane polarization. Our data showed that exogenous administration of $E_2$ brought these changes to near normalcy in aging female rats.
Conclusions
It can therefore be concluded that $E_2$'s beneficial effects seemed to arise from its, antioxidant and antilipidperoxidative effects, implying a therapeutic potential drug for age related changes. Based on our studies and others, we conclude that $E_2$ have therapeutic potential for adjunctive therapy along with dopamine replacement in PD.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1075

**Transient inhibition of the resolvin system in the hypothalamus of diet-induced obese mice**

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Inflammation and dysfunction of the hypothalamus is a common feature of experimental diet-induced obesity. As in any inflammatory process, both pro- and anti-inflammatory activity is expected to take place in the hypothalamus of obese animals. Here, we test the hypothesis that consumption of a saturated fatty acid-rich diet can modulate the activity of the important immunomodulatory system of the resolvins in the hypothalamus. Swiss mice were fed for 8 or 16 weeks (w) either on a control chow (CT) containing 4% total fat, or on a high-fat diet (HF) containing 36% fat, mostly from lard. In addition, some mice were initially fed for 8 weeks on HF and then shifted to a high-fat diet containing 9.6% omega-3 oil (W3) for another 8 weeks. At 8 weeks, the hypothalamic expression of the phospholipase A2 (PLA2) was increased. However, 15-lipoxigenase (15LO) and GPR32 (resolvin D1 receptor) expression which catalyze initial steps of the resolvin synthesis and activity, were reduced. At 16 weeks, PLA2, 15LO and GRP32 were increased as compared to control. When mice were fed on W3, significant reduction of markers of inflammation in the hypothalamus was accompanied by reduction of PLA2 and 15LO expression. Finally, a direct acute intracerebroventricular injection of resolvin D1 in the hypothalamus increases the expression of the anti-inflammatory cytokine IL10 while reducing the expression of the p65 subunit of NFkB. Thus, this is the first evidence that during diet-induced inflammation of the hypothalamus the activity of the resolvin system is modulated. This modulation seems to occur in two phases, being initially suppressed and then activated. Because the resolvin system can be activated by nutrients, we believe it may be an interesting potential target for dietary approaches to tackle obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1076

**Predictors of neuropsychiatric side effects of dopamine-agonist therapy in patients with prolactinomas**

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Introduction
Treatment with dopamine agonists in patients with prolactinomas and Parkinson's disease is associated with central side effects. Central side effects may depend on a substance's ability to pass the blood-brain barrier which can be actively controlled by transporter molecules such as the P-glycoprotein encoded by the ABCB1 gene.
Aim of the study
To determine whether cabergoline is transported by the P-glycoprotein and whether polymorphisms of its encoding ABCB1 gene predict central side effects of cabergoline therapy in patients with prolactinomas.
Methods
i) In an experimental mouse model lacking the homologs of the human ABCB1 gene (abcb1ab double-knockout mice), we examined whether cabergoline is a substrate of P-glycoprotein. ii) In a human case–control study, we investigated the association of four selected ABCB1 gene SNPs (rs1045642, rs2032582, rs2032583, rs2235015) with the occurrence of central side effects under cabergoline therapy in 92 prolactinoma patients treated at the Max Planck Institute of Psychiatry in Munich.
Results
i) In the experimental mouse model, we observed that brain concentrations of cabergoline were tenfold higher in the mutant mice compared to their wild-type littlemates implying that cabergoline is indeed a substrate of the transporter P-glycoprotein at the blood–brain barrier level. ii) In humans, we found significant negative associations for the C-carriers and heterozygous CT-individuals of SNP rs1045642 with two central side effects (frequency of fatigue and sleep disorders) and for the G-carriers of SNP rs2032582 with the enhancement of dizziness under cabergoline. For the SNPs rs2235015 and rs2032583, no associations with central side effects under cabergoline were found.
Discussion/conclusion
This is the first study demonstrating that individual ABCB1 gene polymorphisms reflecting a different expression and function of P-glycoprotein could predict the occurrence of central side effects under cabergoline. Our findings can be viewed as a step into personalized therapy in patients with prolactinomas.
Declaration of interest
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## P1077

### Shift work at young age is associated with elevated long-term cortisol levels and body mass index

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Introduction
The incidence of obesity and other features of the metabolic syndrome is increased in shift workers. This may be due to a misalignment between the internal circadian rhythm and the behavioral rhythm. The stress hormone cortisol could play a role in this phenomenon, because it is secreted in a circadian rhythm, and long-term elevated cortisol levels leads to components of the metabolic syndrome. Our aim was to study changes in long-term cortisol levels due to shift work.
Methods
This study consists of two parts. Part 1 (pilot study): hair samples were collected in 33 shift workers and 89 day workers. Height and weight were measured, and BMI was calculated[1]. Part 2: hair samples were collected in 100 males working in shifts or only during the day. Hair samples were collected together with height, weight, hip and waist circumference and questionnaires concerning diet, exercise and perceived stress. Cortisol was extracted from the hair samples with methanol, and cortisol levels were measured using ELISA.
Results
Part 1: Hair cortisol levels were higher in shift workers than in day workers: 47.32 pg/mg hair (95% CI = 38.37–58.21) vs 29.72 pg/mg hair (95% CI = 26.18–33.73; $P < 0.001$). When divided in age groups based on the median age, elevated cortisol levels were present particularly in younger shift workers: 48.53 pg/mg hair (95% CI = 36.56–64.29) vs 26.42 pg/mg hair (95% CI = 22.91–30.55) ($P < 0.001$). BMI was increased in younger shift workers as well: 27.2 (95% CI = 25.5–28.8) vs 23.7 (95% CI = 22.8–24.7) in young day workers ($P = 0.001$). Hair cortisol and BMI were positively correlated ($\beta = 0.262$; $P = 0.005$)[1]. The results of the second part will be presented at the congress.
Conclusion
Shift work at young adult age is associated with elevated long-term cortisol levels and increased BMI. Elevated cortisol levels and BMI may contribute to the increased cardiovascular risk found in shift workers.
(1) Manenschijn *et al*. Shift work at young age is associated with elevated long-term cortisol levels and body mass index. *J Clin Endocrinol Metab*. 2011 Nov;**96** (11) E1862–5.
Declaration of interest
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## P1078

### The limited screening value of insulin-like growth factor-I as a marker for alterations in body composition in very long term adult survivors of childhood cancer

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Introduction
The clinical relevance of low IGF-I levels, caused by cranial radiotherapy, in adult childhood cancer survivors has not been studied extensively. We evaluated whether IGF-I is a useful marker for altered body composition and growth hormone deficiency in this group.
Methods
We analyzed retrospective data from 610 adult childhood cancer survivors, retrieved from the late effects clinic. Median age at diagnosis was 6 years (interquartile range 3–11) and follow-up time was 18 years (13–24). We assessed IGF-I standard deviation scores (SDS), anthropometrical measures, growth hormone stimulation tests in patients with clinical signs of GHD and measures of body composition (assessed by dual X-ray absorptiometry, Lunar Prodigy).
Results
In 58 cranially irradiated acute leukemia survivors (25 Gy (24–25)) and 56 locally irradiated brain tumor survivors (42 Gy (35–54)) we found significantly lower IGF-I SDS ($P < 0.001$), lower height SDS ($P < 0.001$), higher body mass index ($P = 0.01$), higher waist-hip ratio ($P = 0.001$), higher total fat percentage SDS ($P < 0.001$) and lower lean body mass SDS ($P < 0.001$), as compared to 452 not cranially irradiated survivors. IGF-I showed a weak inverse correlation with BMI ($r = -0.12$, $P = 0.04$), waist hip ratio ($r = -0.15$, $P = 0.01$), total fat percentage ($r = -0.14$, $P = 0.02$) and a positive correlation with lean body mass ($r = 0.15$, $P = 0.01$). In patients with low IGF-I levels, IGF-I did not significantly differ between subjects with and without GHD as determined by GH-stimulation testing ($P = 0.39$).
Conclusion
This study shows that IGF-I has limited value as a marker for alterations in body composition in adult childhood cancer survivors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1079

### Timing determines the suppressive effect of sleep loss on testosterone

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Background
Sleep loss in the early morning has been shown to reduce secretory activity of the pituitary–gonadal axis in men, but the determinants of this effect are unknown.
Objective
To assess the effects of sleep restriction on serum concentrations of LH, testosterone , and prolactin (PRL).
Methods
Fifteen young, healthy men were examined in a condition of sleep time restriction to 0245–0700 h for two consecutive nights and in a control condition of regular sleep (2245–0700 h). After the second night, serum concentrations of LH, testosterone, and PRL were monitored over a 15 h period. In addition, these hormones were measured in serum samples obtained in eight healthy men in the morning after one night of total sleep deprivation, of 4.5 h sleep (2230–0300 h), and of regular 7 h sleep.
Results
Serum LH, testosterone, and PRL concentrations showed characteristic diurnal variations across the 15-h period without any differences between the 4 and 8 h sleep conditions. However, total sleep deprivation and 4.5 h of sleep restricted to the first night-half markedly decreased morning testosterone and PRL concentrations (both $P \leq 0.05$).
Conclusion
Collectively, our data indicate that the effect of sleep restriction on pituitary-gonadal secretory activity depends on the timing of sleep restriction. While sleep loss in the early part of the night does not affect testosterone and PRL, early awakening and wakefulness during the second part of the night profoundly reduce circulating testosterone and PRL.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1080

### CT Scan of the anterior skull base in Kallmann syndrome reveals specific ethmoid abnormalities

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Context
Kallmann syndrome (KS) is a developmental disease associating congenital hypogonadotropic hypogonadism (CHH) and sense of smell impairment owing to olfactory structures (OS) aplasia/hypoplasia. Although rhinencephalic MRI allows to detect specific KS OS abnormalities useful to discriminate KS from normosmic CHH (nCHH), this technique is not efficient enough to study anterior skull bone structures.

Objectives
To search for specific anterior skull base abnormalities in KS through CT scan.
Patients
Thirty seven KS patients were compared to matched nCHH ($n=15$) and controls ($n=30$).
Design
Prospective case–control study. All patients and controls underwent a high resolution three-plane CT scan in bone window with axial, coronal and sagittal reconstructions. Olfactory gutter (OG) height, width, surface and foveo-lateral angle were measured. Cribriform plate foramina were counted bilaterally. Rhinencephalic MRI were performed in parallel and OS abnormalities were scored as normal, hypoplasic or aplastic and compared to CT scan findings.
Results
In KS patients OG height, width and surface were all significantly lower than in nCHH and controls ($P<0.0001$). OG height $<3.6$ mm discriminates KS with the higher sensitivity and specificity. A correlation between OG height at CT scan and OS abnormality scores at MRI was found. KS subjects also presented a wider foveo-lateral angle than nCHH and controls ($P<0.0001$). Cribriform plate foramina median number was similar in KS, nCHH and controls (7 vs 8 and 9 respectively).
Conclusions
This study allowed us to discover specific ethmoid abnormalities in KS patients. OG height $<3.6$ mm at CT scan specifically discriminates KS from nCHH, providing a new diagnostic tool. The presence of cribriform plate foramina in KS patients indicates that OS integrity is not mandatory for their formation and maintenance, unraveling original aspects on KS pathophysiology.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1081

### Wrist actigraphy detects sleep disorders in patients with Cushing's syndrome

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Interrelationships between HPA axis and sleep architecture are well documented and increased glucocorticoid levels seem associated with quantitative and qualitative sleep disruptions. The knowledge about sleep parameters in chronic hypercortisolism in humans is scanty. Our aim was to evaluate sleep efficiency in patients with Cushing's syndrome (CS) at time of diagnosis, without ongoing specific therapy, using wrist actigraphy, a methodology that provides good estimations of sleep duration and consolidation. In 12 CS patients with different etiology (eight ACTH-secreting pituitary adenoma, CD, and four cortisol-secreting adrenal adenoma, AA) (11 F, 1 M, 40.0 ± 10.9 year) and in 12 age- and sex-matched healthy control subjects (NS), sleep recording was performed by Actiwatch (Mini Mitter Co, Inc.; Bend, OR, USA) on three consecutive working days under free living conditions. Data were analysed using Actiware-Sleep Software. All the subjects completed questionnaires about sleep habits. In CS patients morning circulating levels of ACTH, cortisol and free urinary cortisol (UFC) levels were measured. Wrist actigraphy revealed no significant difference between CS and NS in terms of time in bed ((mean ± S.D.) 8 h4' ± 0 h55' vs 7 h40' ± 0 h53'), actual sleep time (7 h8' ± 0 h 54' vs 6 h48' ± 0 h44'), sleep efficiency (88.47 ± 2.44 vs 88.9 ± 2.88%), sleep latency (0 h6' ± 0h3' vs 0h8' ± 0h7'). However, some sleep parameters were deranged in CS, such as an higher actual wake time (0h43' ± 0h10' vs 0h33' ± 0h12'), total activity score (8318 ± 4308 vs 4971 ± 2372), mean activity score (8.67 ± 4.23 vs 5.44 ± 2.16), mean score in activity time (104.83 ± 39.23 vs 74.81 ± 23.15), sleep fragmentation (16.17 ± 4.21 vs 13.01 ± 3.6). compared to NS ($P<0,05$). No positive correlation between sleep efficiency parameters and UFC was found. In conclusion, these results, though preliminary, indicate that hypercortisolism is associated with a deranged sleep efficiency, in a dose-independent manner. Further studies in a larger cohort of patients and the use of more accurate instruments, such as in-home polysomnography, are needed to confirm these findings.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1082

### Comparison of Chinese herbal mixture and electro-acupuncture on regulating endocrine disturbances and hypothalamic androgen receptor in rats with DHT-induced PCOS

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting reproductive women. Treatments with Chinese herbal mixtures (HM) and electro-acupuncture (EA) have been proven efficient in clinic. This study evaluated the peripheral and central effects of HM and EA in dihydrotestosterone (DHT)-induced PCOS rats. The female Wistar rats were divided into control (C), control with placebo (CP), DHT-induced PCOS model (PCOS), PCOS model exposed to herbal mixture (PCOS + HM) and EA (PCOS + EA) groups. At age 3 week, DHT or DHT placebo pellets were inserted to slow release. From 10 week of age, PCOS + HM group were continuously intragastric administrated with the herbal mixture (1 ml/100 g BW, twice a day) while PCOS EA rats received 2-Hz EA (30 min, once a day, five times/week), totally 4 weeks. The results showed that PCOS rats presented with increased body weight, disturbed estrus cycles, cysts follicles and disordered level of steroid hormone, whereas these were restored both in HM and EA group. Interestingly, the fat depots and muscles of rats in EA group are significantly reduced, while the HM is mainly effect the weight of uterus and ovaries. Moreover, HM and EA also reduced the elevated hypothalamic androgen receptor (AR) protein expression and GnRH-immunoreactive (GnRH-IR) in medial preoptic area (MPO) and the ventro medial hypothalamus (VMH) compared with PCOS rats by immunohistochemistry. Therefore, we suggested that there might be differentially particular emphasis of HM and EA on endocrinology and metabolism of PCOS rats.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1083

### Endogenous estradiol levels in female acute stroke patients: an independent determinant of stroke severity and early outcome

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Introduction
Data on the role of endogenous sex steroids in cerebrovascular disease is sparse. Our aim was to investigate the clinical relevance of circulating sex steroids in a postmenopausal acute stroke population and search for associations with disease severity and short-term outcome.
Patients and methods
We prospectively studied 302 postmenopausal female patients hospitalized for an acute stroke in two tertiary hospitals, during a time period of 2 years. A detailed medical history and physical examination were performed and risk factors for stroke were recorded. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS). One month after stroke onset, the functional outcome and degree of handicap were evaluated with the modified Rankin Scale (mRS). In all patients, we performed basal biochemical investigation and hormonal testing, measuring circulating estradiol, using a high sensitivity assay, as well as androgen (dehydroepiandrosterone sulfate, Δ4-androstenedione and testosterone) and sex hormone binding globulin levels.
Results
Estradiol levels were the only hormonal parameter significantly related to stroke severity on admission, as expressed by NIHSS, after correcting for confounding factors in the multivariate analysis ($\beta$0.262, $P<0.001$). Estradiol was an independent determinant of 1-month mortality (odds ratio (OR) with 95% confidence intervals (CI): 1.012 (1.005–1.019), $P$ 0.001), along with stroke severity and history of arterial hypertension. Estradiol levels remained an independent predictive factor of the adverse functional outcome (mRS $\geq 4$) in the multivariate analysis (OR with 95% CI: 1.011 (1.000–1.021), $P$ 0.047), along with stroke severity and hemorrhagic stroke type.
Conclusions
We found an independent association of endogenous estradiol levels with stroke severity and short-term mortality and outcome. Circulating estradiol might

represent an adjunctive biomarker to identify patients at increased risk for death and disability.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1084

### Long term evolution of patients with transsphenoidal surgery due to pituitary adenomas

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Aim
To study the results of transsphenoidal surgery in our Neuroendocrinology Unit in last 10 years.
Methods
We reviewed all patients operated in our hospital and followed in our Neuroendocrinology Unit in the last 10 years. We excluded patients with previous surgery or irradiation on this area. We obtained data about type and size of tumor, previous hormonal deficit, local symptoms,complications of surgery and the status after this (remission, persistence or recurrence).
Results
One hundred and six patients, mean age 46.2±16.1 years, 42.4% women. Diagnosis:non-functioning adenomas :57.2%, acromegaly: 23, 6% and others 19.2%. The size: 92.5% macroadenomas. Prior to surgery, 16% had hyperprolactinemia, 22.6% TSH deficiency, 22.6% FSH/LH deficiency, 15.1% ACTH deficiency and 11.6% GH deficiency. Local symptoms (62%): the most frequent symptom was visual disturbance (43.4%) followed by headache (39.6%) and cranial nerve impairment (2.8%). Early complications were: 17.4% diabetes insipidus, 5.4% cerebrospinal fluid fistula, 6.5% both simultaneously, one case of obstructive hydrocephalus and one case of intratumoral bleeding. Late complications: two patients diabetes insipidus, two cerebrospinal fluid fistula and two patients both. 48.9% had postoperative hormone deficiency: FSH/LH deficiency 37%,TSH 37%,ACTH 35.9% and GH deficiency 27.4%. There was local symptoms improvement in 54.4% of patients.After surgery,tumor mass remained in 46.7% (100% of prolactinomas, 50.8% of non-functioning and 48% of acromegaly), 6.5% recurred and 29.3% are in remission (the rest are in postoperative evaluation period). After the first surgery, 16.9% required further medical treatment, 17% were reoperated and 33.7% received radiotherapy. Age over 40 years was associated with an increased likelihood of remnant mass ($P=0.03$) regardless of tumor size. The presence of ACTH deficiency before surgery was significantly associated with the possibility of hormonal deficit after surgery ($P=0.00$) and possibility of remnant mass ($P=0.05$) The presence of local clinic was related to the appearance of early complications after surgery ($P=0.007$).
Conclusions
After transsphenoidal surgery, there is a low incidence of local complications but high incidence of postoperative hormonal deficits (as described in other literature). Age over 40 years, preoperative ACTH deficiency and the presence of local symptoms are related to worse outcomes after surgery.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1085

### Panhypopituitarism treated and untreated with GH: long term evolution of echocardiographic parameters

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Objective
To evaluate the effects of GH on cardiac structure and function of patients with panhypopituitarism.
Material and methods
We performed echocardiographic studies of patients with panhypopituitarism treated and not treated with GH at baseline and after 12 years of follow up. We collected clinical and echocardiographic data (systolic and diastolic ventricular diameter (LVEDD and DTSVI), interventricular septum thickness (IVS), left ventricular posterior wall thickness (PPV), left ventricular mass and diastolic and systolic volume of left ventricle (LVEDV and LVESV), ejection fraction (EF) and size of the waves E, A, E /A ratio, deceleration time (DT) and isovolumic relaxation time (IVRT).
Results
Data were obtained from 30 patients, mean age 48.9±13.7 years, 63.3% men. Etiology of GH deficit: 40% nonfunctioning adenomas, 20% macroprolactinoma, 20% craniopharyngioma, empty sella 13.3 and 6.6% other causes. The years of evolution at the time of first echocardiogram was 8.5 years in treated patients and 4.5 years in untreated patients. There were no differences in other cardiovascular risk factor. There were no differences in the mass or the dimensions of the left ventricle, aorta and left atrium in both groups basal and after reevaluation. The ejection fraction was 63% basal vs 70% in reevaluation in the treated group ($P<0.05$) and 57 vs 66% respectively in untreated group ($P<0.05$). There were differences in treated patients in the E wave (44.11 cm/s in basal ECO vs 73.11 cm/s in reevaluation, $P<0.05$) and E/A ratio (0.73 in vs 1.06 respectively, $P<0.05$), while there were no differences in untreated patients after reevaluation.
Conclusions
In patients treated with GH there was a significant improvement in diastolic function parameters (E wave and E/A).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1086

### Relationship between sex hormones, cognitive performance and substance use

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Hypogonadism is prevalent with opiate-like drug use and might be a factor in cognitive abnormalities. With the increasing substance use (SU) worldwide, it is critical to recognize its impact on cognition, which may decrease adherence to treatment and alter quality of life. We hypothesized that men with SU, by virtue of hypogonadism secondary to HIV and/or drug use, may demonstrate impaired cognitive function.
We recruited men aged 18–50 from a low-income population in Baltimore, Maryland. Details of HIV and SU status, blood levels of total testosterone (TT), free testosterone (FT) and estradiol ($E_2$) were assessed. All subjects were administered ten neuropsychological tests that tested cognition, visuomotor and visuospatial abilities, graphomotor and psychomotor speed, and verbal learning and memory.
Our sample consisted of 68 men (mean age: 43.2 (s.d. 5.8), African Americans: 86.6%). The studied population was primarily uneducated and unemployed. The mean level of TT was 553.9 ng/dl (s.d. 262.0), median 507 ng/dl, the mean level of FT was 69.5 pg/ml (s.d. 34.8), median 70.5 pg/ml, mean $E_2$ was 3.2 pg/ml (s.d. 4.4), median 2.1 pg/ml. We found that 30.9% were hypogonadal (TT<300 ng/dl or FT<50 pg/ml) and it was associated with higher SU.
We observed some relationships between sex hormones and cognitive domains, however, after adjustment for age, drug use category, education, depression, and HIV, there was no statistically significant correlation between cognitive performance and sex hormone levels.
In this cross-sectional study of men with a high prevalence of SU and hypogonadism (30.9%), endogenous levels of TT, FT or $E_2$ were not related to cognitive performance. Future research should identify alternative factors that affect poor cognitive functioning in the setting of SU.
Non-users = no substance use in the past three years Occasional users = cocaine and/or heroin use less than three times per week Heavy users and methadone = methadone, cocaine and/or heroin use more than three times per week or being on methadone maintenance
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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Table 1

**Table 1** Relationship between drug use category and sex hormones levels (adjusted by age and HIV infection status).

| | Mean (standard error) | | | | P (Pair-wise comparison) | | |
|---|---|---|---|---|---|---|---|
| Hormone | (1) Non-users $n=20$ | (2) Occasional users $n=14$ | (3) Heavy users & methadone $n=34$ | P (Cross-group comparison) | (1) vs (2) | (1) vs(3) | (2) vs (3) |
| Total Testosterone | 541.6 (59.4) | 593.9 (73.3) | 544.7 (46) | 0.831 | 0.582 | 0.967 | 0.579 |
| Free Testosterone | 71.1 (7.5) | 92.6 (9.3) | 59.1 (5.8) | 0.014 | 0.077 | 0.21 | 0.004 |
| Estradiol | 2.7 (1.0) | 3.4 (1.2) | 3.4 (0.8) | 0.827 | 0.645 | 0.564 | 0.997 |

## P1087

### The proliferation and apoptosis activity of blood lymphocytes in acromegaly

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Introduction
The number of cells in tissue is governed by two processes – the proliferation and 'programmed cell death' – apoptosis. Both processes in the body are controlled by enabling or inhibiting factors that are expressed on the surface of CD25+ and CD95+-lymphocytes. Somatotropin (GH) and insulin-like growth factor 1 (IGF1) stimulate proliferation, differentiation, and division of immune cells simultaneously inhibits their apoptosis and can cause tumor growth in acromegaly.
Aim
To study the expression of CD25+ and CD95+-lymphocytes level depending on the acromegaly stage.
Methods
The study population consist 47 acromegalic patients (38 with active acromegaly and 9 in disease remission, mean age $50.4 \pm 12.58$, range 27–73 years, the latent period duration of acromegaly was $5.72 \pm 5.02$ years) and 65 age-matched controls. The surface lymphocytes markers expression was assessed by indirect immunofluorescence method using the FITC-marked monoclonal antibodies to CD25 and CD95. The concentrations of GH and IGF-I were measured by ELISA.
Results
Compared to the control both acromegalics – with active phase and disease remission had significantly higher level of CD25+- (respectively; $P<0.001$ and $P<0.01$) and CD95+-lymphocytes ($P<0.01$ and $P<0.05$). However it was found that the expression of apoptotic activation markers was higher in patients with disease remission compared to active acromegaly group respectively: CD25+- (median 66.5 vs 37.0%; $P<0.05$) and CD95+-cells (43.5 vs 18.0%; $P<0.05$).
Conclusion
Stimulating and antiapoptotic effect of GR/IGF1 in acromegaly simultaneously promotes proliferation and expression of apoptotic activation markers of lymphocytes, but causes an accumulation in the body of acromegalics the long-lived immunoreactive cells. Long-term effects of IGF-I in acromegaly mediate the intensity of proliferation and apoptosis in patients with the disease remission. According to these, it can be suggested that increased oncological risk in active acromegaly patients ongoing even in remission period.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1088

### Long term evolution of patients with prolactinoma in a neuroendocrinology unit

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Aims
To know characteristics and long term evolution of patients with prolactinoma in our Neuroendocrinology Unit.
Material and methods
We studied retrospectively all patients with diagnosis of prolactinoma in our Neuroendocrinology Unit in last 12 years.
Results
We studied 126 patients. Predominant sex was female (69.07%), 56.7% were macroadenomas. The time from the onset of symptoms until they are seen by the specialist ranges 12 to 24 months. The clinical presentation and hormones values were: menstrual disorders 56.7%, galactorrea 43.3%, erectile dysfunction 18.55%, headache 41.23% and visual disorders 18.55% (In men,the most frequent symptom was headache and in women menstrual disorders). Dysfunction of other axis: decrease of LH/FSH 25.8%, GH deficiency 6.4%, TSH 8.2% and ACTH 6.4%.
93.8% were pharmacologically treated (95.6% cabergoline). Four patients needed surgery as first treatment. Only one case was familiar: a patient with giant macroadenoma that belonged to a FIPA family with a new AIP mutation non described before in literature. There was total cure(asymptomatic, normal prolactin and disappearance of tumour in RM) in 11.34% (19.5% of microadenomas and only three cases of the macroadenomas) all of them with more than 2 years of medical treatment. The size of the tumour and basal levels of prolactin were inversely related to the possibility of cure.
Conclusion
In our serie, although prolactinomas have a relatively benign course, long-term definitive cure is infrequent.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1089

### Influence of various doses of Cabergoline in dynamics of long-term treatment on the level of blood soluble apoptosis markers in patients with inactive pituitary adenomas

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Aim
Studying of influence of various doses of cabergoline in dynamics of 6, 12 and 24 months of treatment on level of blood serum soluble apoptosis markers *P*53, Bcl-2 and TNF-α in patients with IPA.
Materials and methods
One hundred and twenty-seven patients with IPA (38 men, 89 women) were observed, among them 65 (51.2%) with microadenoma and 62 (48.8%) with macroadenoma/ patients were at the age from 35 till 60 years.Patients with pituitary micro-and macroadenomas were divided into two groups by method of casual sample: the 1st group was made by 34 patients with microadenoma and 31 patients with macroadenoma which in course of treatment were prescribed small doses of Cabergoline by 0.4–0.6 mg/week. The 2nd group was made by 31 patients with microadenoma and 31 with macroadenoma (doses of Cabergoline from 1.0 to 3.0 mg/week).
Results
In patients with IPA with microadenoma before treatment the level of blood serum soluble apoptosis markers exceeded similar indicators in the control group: *P* 53, Bcl-2 and TNF-α in 1st and 2nd groups on the average by 25.4, 26.0 and 38.3% ($P<0.01$), and in patients with macroadenoma by 43.1, 47.2 and 59.5% ($P<0.001$). It is established, that increase of TNF-α was associated with increase of *P* 53 ($r=0.73$; $P<0.01$) and Bcl-2 ($r=0.75$; $P<0.01$), and *P* 53 with Bcl-2 had stronger association - $r=0.86$ ($P<0.001$). Long-term use of Cabergoline in patients with IPA promoted the expressed decrease in all observed apoptosis factors. In patients of the 1st and 2nd groups with microadenoma apoptosis indices *r*53 and Bcl-2 were basically within the control level in 6–12 months of treatment and maintained in such condition up to 24 months of therapy, whereas

TNF-α only by 24 month of Cabergoline therapy. At the same time in patients with macroadenoma restoration of level of apoptosis indices depended on the dose of Cabergoline prescribed.
Conclusion
In blood of 127 patients with IPA with pituitary micro-and macroadenoma high level of apoptosis markers *P* 53, Bcl-2 and TNF-α was revealed. Cabergoline prescribed during 6, 12, 24 months in big doses (from 1.0 to 3.0–mg/week) restored apoptosis markers in blood of patients with IPA with pituitary micro-and macroadenoma more effectively and by 12 months earlier, than at prescription of small doses of the drug (from 0.4 to 0.6 mg/week).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1090

**The blood lymphocytes oxidoreductases activity in patients with active acromegaly**

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Introduction
Somatotropin (GH) and insulin-like growth factor 1 (IGF1) realize their influence on the system of intracellular metabolism and a number of important biochemical lymphocytes reactions through the receptor apparatus. It is allows to use the peripheral blood lymphocytes as the investigations object of intracellular metabolism disorders in acromegaly.
Aim
To study the NAD- and NADP-dependent dehydrogenases activity in blood lymphocytes in patients with active acromegaly.
Methods
The activity of NAD(P)-dependent dehydrogenases in blood lymphocytes was studied in a group of 88 patients (35 men and 53 women) with active acromegaly, mean age $51.0 \pm 12.5$ years. The NAD(P)-dependent dehydrogenases activity was determined by biochemiluminescence method. The concentrations of GH and IGF1 were measured by ELISA.
Results
In active acromegaly were revealed the decreasing activity of all studied oxidoreductases: glucose-6-phosphate dehydrogenase ($P<0.01$), NAD-lactate dehydrogenase (LDH) ($P<0.001$), NADH-LDH ($P<0.001$), NAD-malate dehydrogenase (MDH) ($P<0.001$), NADH-MDH ($P<0.001$), NADP-MDH ($P<0.001$), NAD-glutamate dehydrogenases (GDH) and NADH-GDH ($P<0.001$), NADP-GDH and NADPH-GDH ($P<0.001$), NAD- isocitrate dehydrogenases (IDH) and NADP-IDH ($P<0.01$ and $P<0.001$ respectively), glutathione reductase ($P<0.001$). Our data further observed that decreasing activity of NADP-GDH positively correlated with the basal GH level ($r=+0.23$, $P=0.04$) and NADP-MDH activity with IGF1 level ($r=+0.30$, $P=0.008$). The low NADH-MDH activity negatively correlated to the basal GH concentration ($r=-0.23$, $P=0.04$).
Conclusion
The chronic excess of GH and IGF1 in acromegaly causes a significant depletion of metabolic lymphocytes reserves. The main indicators of functional lymphocytes impairment in acromegaly are: the reduction of intermediates formation for the reactions of macromolecular synthesis and aerobic processes, the low intensity of glycolysis, nitrogen metabolism and inhibition of glutathione complex activity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1091

**Hypercortisolism in patients with diabetes mellitus, adrenal incidentaloma and obesity**

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Some studies reports the high prevalence of unsuspected hypercortisolism in high-risk populations.
Aim
To evaluate the prevalence of undiagnosed hypercortisolism in patients with diabetes mellitus (DM), adrenal incidentaloma and obesity.
Material and methods
We prospectively evaluated 30 DM patients (8 male, 22 female, 53.5 years old (18; 73), HbA1c-7.1% (5.6; 13.8)), 22 patients with adrenal incidentaloma (6 male, 16 female, 53.5 years old (20;73)) and 31 obese patients (21 male, 10 female, 22 years old (18;62)). The overnight 1-mg dexamethasone suppression test (DST-1) was done first. If cortisol level exceeded 50 nmol/l after DST-1 (positive result) the additional evaluation was done (2 mg/day for 48 h dexamethasone suppression test (DST-2), urine free cortisol (UFC), midnight salivary cortisol. If the hypercortisolism confirmed the additional examination prepared to found it's source.
Results
i) DST -1 was positive in 9 of 31 (29%) obese patients. DST-2 reduced the number of positive results to 3 (9.6%). UFC and salivary cortisol were normal in all of them. They did not have clinically apparent hypercortisolism so the DST-2 results interpreted as false-positive. ii) DST-1 was positive in 18 of 30 (58%) patients with DM. After DST-2 the only four (12.9%) of them had positive result. Further investigations revealed Cushing's syndrome and Cushing's disease in two of these four patients. Other two patients are in under our observation. It should be noted that patients with confirmed hypercortisolism had clinical signs of hypercortisolism, but referred to our clinic because of uncontrolled DM. iii) DST-1 was positive in 13 of 22 (59%) patients with adrenal incidentaloma. DST-2 was positive in 7 (31.8%) of them. Six of them have Cushing's syndrome and one is on under observation.
Conclusion
The highest percentage of hypercortisolism revealed in patients with adrenal incidentaloma. High percent of hypercortisolism in DM was due to the fact that their doctor did not suspect overt hypercortisolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1092

**Levothyroxine therapy in patients with secondary hypothyroidism in nonfunctioning pituitary adenomas**

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Aim
To study the effectiveness of levothyroxine therapy in patients with secondary hypothyroidism in nonfunctioning pituitary adenomas (NFPA).
Materials and methods
We followed 35 patients with NFPA. Of them, 17 were females and 18 males. Mean age of patients was 37.2 years old. All patients were evaluated for hormones levels (GH, LH, FSH, TSH, estradiol, progesterone, testosterone, prolactin, cortisole, free thyroxine, etc.), visual fields, pituitary MRI, thyroid ultrasonography, etc. None of the patients had thyroid disorders.
All evaluations were repeated in 6 months after levothyroxine (Euthyrox, Nycomed, Germany) therapy with the dosage of 100 μg a day before meals.
Results
In patients with long disease background we revealed hypopituitarism with GH, LH, FSH, and TSH deficit as well as IGF1, estradiol, testosterone and thyroxine (100%).

All patients had symptoms characteristic for hypothyroidism including sleepiness, loss of memory, dry skin, constipations etc. Blood tests showed dyslipidemia.
Six months therapy with levothyroxine showed significant improvement in well-being of patients such as regression of applicable complaints, laboratory and biochemical values improvement in 25 patients (71.4%).
Conclusion
i) Levothyroxine therapy significantly improves clinical and biochemical values in patients with NFPA ii) it should be recommended to administer levothyroxine therapy to patients with NFPA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1093

**Neuroendocrine abnormalities in adolescents with the Turkish saddle volume formations**

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Aim
To study character of neuroendocrine disorders in children and adolescents with the Turkish saddle volume formations.
Materials and methods
we examined 35 children and adolescents, 17 girls and 18 boys among them with the Turkish saddle volume formations at the neuroendocrine department of the Center for the scientific and clinical study of endocrinology. All examinees underwent general clinical examination as well as biochemical and hormonal investigations (levels of pituitary hormones), Turkish saddle CT and MRI and hand roentgenogram, clinical ultrasound of the thyroid and sex organs, anthropometric examination, having their sexual development stage by Tanner assessed.
Results
By character of pathology the examinees were divided into five groups. The 1st group included 23 patients (63%) with nonfunctional pituitary adenomas, the 2nd group including five patients (14.3%) with Cushing disease comprising the 3rd group, two patients (77.1%) with prolactinomas comprising the 4th group and one patient (2.9%) having germinoma and pinealoma combination was in the 5th group. By character of tumor (by Kadashev B.A., 2007) the patients were distributed as follows: 1st group of endosellar localization comprised i) 27 patients (77.1%) with microadenomas (under 1 cm) and ii) one patient (2.9%) with macroadenoma, 2nd group of endoextrasellar localization comprising (a) five patients (14.3%) with tumors of suprasellar growth and two patients (5.7%) with tumors of supra-parasellar growth.
Examination of 35 patients allowed diagnosing a number of neuroendocrine disorders, such as, growth and puberty delay ($n=9$, 25.7%), physical development delay ($n=6$, 17.1%), dysplastic obesity ($n=5$, 14.3%), precocious puberty development ($n=3$, 8.6%), diabetes insipidus ($n=3$, 8.6%), gigantism ($n=2$, 5.7%) and secondary hypocorticoidism ($n=1$, 2.9%). Delay of puberty and growth more often was founded in patients with nonfunctional pituitary tumours, cranyopharingiomas and prolactinomas – 15 cases from 35 patients (42, 8%); but precocious puberty – only in patients with Cushing disease – three patients from 5 (60%).
Conclusions
i) Growth and puberty disorders are the most frequent Turkish saddle volume formations with growth and puberty retardation predominating (25.7%), ii) the Turkish saddle tumors in children and adolescents result in hypo- and panhypopituitarism ($n=19$, 54.2%).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1094

**Somatostatin analogue treatment of acromegaly**

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Aim
To study complications in patients with acromegaly receiving somatostatin after radiotherapy.
Materials and methods
We examined 16 patients with mean disease duration $8.5\pm4.2$ years (mean age $41.5\pm5.6$ years) receiving distant gamma-therapy in the total dose of 60 Gy. The patients were divided into two groups: eight patients receiving somatostatin in the dose of 0.5 mg thrice a day and symptomatic therapy for 2 months were included into the 1st group, other eight patients received only symptomatic therapy. To establish the disease activity we examined diurnal GH secretion at 8.00, 11.00, 13.00, 15.00 and 18.00
Results and discussion
At first examination (6 months after radiotherapy) GH diurnal secretion showed the presence of active acromegaly. Average daily GH level was $58.6\pm8.2$ and $49.8\pm9.3$ mIU/l in two groups of patients respectively. 98% patients of the 1st group had endocrine-metabolic disorders, in 85% the disease was complicated with cardio-vascular disorders, 80% had neuromuscular-skeletal-joint complications, in 42% the disease was complicated with the respiratory disorders, 25% having neoplasms. In the 2nd group endocrine-metabolic disorders were found in all patients, in 80% and 30% the disease was complicated with cardio-vascular and respiratory complications respectively, neuromuscular-skeletal-joint disorders and neoplasms being found in 85 and 20% respectively. Two months later the repeated examination of the 1st group patients showed that average daily GH level was $5.6\pm1.2$ mIU/l, the one remaining high in the 2nd group patients ($26.6\pm8.5$ mIU/l). In the 1st group the decrease in the incidence of endocrine-metabolic (78%), cardio-vascular (45%) and respiratory (15%) disorders was observed. In the 2nd groups patients the incidence of endocrine-metabolic disorders preserved (100%), cardio-vascular (90%) and respiratory (40%) disorders tended to increase.
Conclusions
Somatostatin and its analogues are the first line drugs in treatment of acromegaly. Post-radiotherapy use of sandostatin as the part of the combined therapy of acromegaly reducing both the process activity and complications is the evidence for its efficacy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1095

**Clinical manifestations of depression in patients with primary hyperparathyroidism**

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Aim
The work was initiated to study depression manifestations in patients with primary hyperparathyroidism.
Materials and methods
Symptoms of anxiety and depression were assessed in 67 patients with primary hyperthyroidism aged from 16 to 30 years examined by means of Spielberger–Khanin's test and Beck's Depression Inventory.
Problem of marginal psychoneurotic disturbances in patients with various somatic pathologies has become urgent for the last decade. Psychoneurotic disturbances can be leading for quite a long time masking other manifestations of an underlying disease. Prevalence of depressive conditions was found to grow for the last years; by 2020 depression is estimated to occupy a leading place in general structure of disease incidences.
Results
Parameters of both personal ($n=62$, 92, 3%) and situational ($n=60$, 88.3%) anxiety were found high. (Spielberger-Khanin's test).
Fatigability ($n=60$, 88.3%), sleep disturbance ($n=58$, 89.4%), irritability ($n=52$, 79.1%), work inhibition ($n=46$, 68.6%), sadness ($n=44$, 66.7), self accusations ($n=38$, 56.7%), pessimism and dissatisfaction ($n=33$, 49.2%), self dislike ($n=28$, 41.7%), were among clinical manifestations of depression

registered among the examinees. (Beck's Depression Inventory).
Conclusions
Registered in most examinees with primary hyperthyroidism depressive reactions were found to aggravate severity of general illness. The study demonstrates necessity to timely diagnose affective impairments in patients with primary hyperparathyroidism to be corrected by means of combined therapy with antidepressants aiming at improvement of the patients' life quality.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1096

**Discovery of a novel peptide, neuroendocrine regulatory peptide-2 regulating feeding behavior and glucose metabolism**

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Discovery of novel peptides leads to the elucidation of unknown regulatory systems in the body and to the clinical development of diagnosis and therapeutics for diseases. We have used peptidomics approach to profile a complete set of secretory peptides from cultured human endocrine cells and identified two novel carboxy-terminally amidated peptides, 25-amino acid neuroendocrine regulatory peptide (NERP)-1 and 38-amino acid NERP-2, derived from a neurosecretory protein VGF (JBC 2007). NERPs modulate vasopressin release in both *ex vivo* and *in vivo* experiments. *Vgf* unll mice exhibited dwarfism and hypermetabolic rates, suggesting that VGF or VGF-derived peptides play important roles in energy metabolism. NERP-2 was abundant in the lateral hypothalamus, one of orexigenic centers, and colocalizes with orexigenic neuropeptides, orexins. NERP-2 stimulated orexin release from isolated hypothalamic explants. Central administration of NERP-2 activated orexin neurons to enhance feeding, body temperature, oxygen consumption and locomotor activity (AJP 2010). NERP-2's activities did not appear in orexin-deficient mice. C-terminal amidation of NERP-2 was essential for its biological activities. NERP-2 was also abundant in the pancreatic islets of humans and rodents and colocalizes extensively with insulin in double immunohistochemistry. NERP-2 enhanced glucose (16.7 mM)-stimulated insulin secretion from MIN6 β cells and isolated islets in a dose-dependent manner. Peripheral administration of NERP-2 significantly elevated plasma insulin in both normal rats and mice and melanocortin 4 receptor knockout mice, an obese diabetic mouse model. Fura-2 calcium imaging showed that NERP-2 elevated cytosolic calcium level in MIN6 β cells. NERP-2 is a novel regulator of food intake and glucose metabolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1097

**Effects of low sodium concentrations on neuronal cell models**

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Hyponatraemia is the most frequent electrolyte disorder encountered in hospitalized patients. Hyponatraemia causes a wide variety of neurological signs and symptoms, depending on the rate and degree of serum sodium drop. The negative effects of low extracellular sodium concentrations ((Na+)) on neuronal cells are known to be due to the entry of fluids into the cells, as a consequence of reduced plasma osmolality. To date, it has not been investigated whether low (Na+) have direct effects on neuronal cells. To this purpose, by generating media with physiological or low (131, 127, 121, 115, 100 and 90 mM; Na+), we studied whether low (Na+) are able *per se* to cause negative biological effects in two different neuronal cell lines (SK-N-AS and SH-SY5Y). We evaluated cell viability and adhesion after adapting to and maintaining cells in culture for 7 days at target (Na+) media. Results showed a significant decrease in cell viability starting from 115 mM (Na+) for SK-N-AS (49.6±1% vs control) and from 90 mM (Na+) for SH-SY5Y (22 ±0.4% vs control). Cell adhesion was also decreased vs control cells (SK-N-AS 58±0.7%; SH-SY5Y 48.7±2%). Similar results were obtained also when the reduced osmolality due to low (Na+) were corrected by adding mannitol. Moreover, we observed a downregulation of the expression of antiapoptotic genes (*bcl2, mdm2*) and of the neuroprotective gene *DHCR24* at the same reduced (Na+). Finally, by using electrophysiological techniques, we observed that 115 mM (Na+) for SK-N-AS and 90 mM (Na+) for SH-SY5Y significantly shifted the reversal potential towards more negative voltages, also when osmolality was corrected. This result could be mainly ascribed to a decrease of intracellular ($Ca^{2+}$) possibly involving $Ca^{2+}$ and Na/K pumps and could lead to a reduced cell response in hyponatraemic conditions. In summary, these findings show for the first time that low (Na+) directly cause detrimental effects on neuronal cells, independent of extracellular osmolality.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1098

**SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development**

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Background
Kallmann syndrome (KS) is a genetic disorder associating pubertal failure with anosmia/hyposmia. KS is related to defective neuronal development affecting both the migration of olfactory nerve endings and GnRH neurons. The discovery of several genetic mutations responsible for KS led to the identification of signaling pathways involved in these processes, but the mutations so far identified account for only 30% of cases of KS. Here we attempted to identify new KS responsible genes by using a pangenomic approach.
Methods
From a cohort of 120 KS patients, we selected 48 propositi with no mutations in known KS genes and normal caryotype. They were analyzed by CGHarray, using Agilent 105K oligonucleotide chips with a mean resolution of 50 kb. Copy-number variations were considered significant if they were defined by three or more oligonucleotides spanning at least 50 kb, contained at least one gene, and were not listed in the Database of Genomic Variants.
Results
One propositus was found to have a heterozygous deletion of 213 kb at locus 7 q21.11, confirmed by real-time qPCR, deleting 11 of the 17 SEMA3A exons. This deletion was absent in 520 subjects without KS analyzed with the same CHGarray, was also absent in the BACH genomic database and co-segregated in this family with the KS phenotype, that was transmitted in autosomal dominant fashion. The KS was not associated with other neurological or non neurological clinical disorders in all affected kindred.
SEMA3A codes for semaphorin 3A, a protein that interacts with neuropilins and KO mice lacking semaphorin 3A expression have been very recently showed to have a Kallmann-like phenotype.
Conclusion
SEMA3A is therefore a new gene whose loss of function is involved in KS. These findings validate the specific role of semaphorin 3A in the development of the olfactory system and in neuronal control of puberty in humans.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1099

### Stimulatory role of neurokinin B in the control of the gonadotropic axis in the rat: developmental changes, sexual dimorphism and regulation by gonadal steroids

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Recent studies in various species have pointed out that Neurokinin B (NKB), encoded by Tac2 in rodents, and its receptor, NK3R, are important regulators of reproduction. NKB is co-expressed in Kiss1 neurons of the arcuate nucleus (ARC) and stimulates, via auto-regulatory loops, kisspeptin output onto GnRH neurons, therefore stimulating gonadotropin secretion. However, important aspects of the roles of NKB as regulators of the gonadotropic axis remain unknown. We report here that in the rat, LH secretory responses to the agonist of NKB, senktide, display sexual dimorphism, with greater responses in females regardless of the stage of postnatal maturation, and null NKB-induced LH secretion in males from puberty onwards. Such a sexual dimorphism manifested also in a higher number of NKB-positive neurons in the ARC in adult females. This may stem from differences in the sex steroid milieu during early critical periods of brain differentiation, as neonatal exposures to high doses of estrogen markedly decreased the number of NKB neurons and the circulating levels of LH, which were normalized by exogenous administration of senktide. Of note, estrogen also inhibited Tac2 expression in adult female rats in the ARC, while enhancing it in the lateral hypothalamus. Finally, despite null gonadotropic responses to senktide in adult male rats, adult females supplemented with testosterone retained LH responses to the NKB agonist. In sum, our study provides a comprehensive developmental analysis of the role of NKB in the control of the gonadotropic axis in the rat. Our observations disclose key aspects of this system, such as its maturational changes, sexual dimorphism and differential regulation by sex steroids, which may help to explain the spectrum of reproductive effects of the NKB system and the pathophysiological manifestations of its inactivation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1100

### Intracerebroventricular administration of metformin inhibits ghrelin-induced hypothalamic AMP-kinase signalling and food intake

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The antihyperglycaemic drug metformin reduces food consumption through mechanisms that are not fully elucidated. The present study investigated the effects of intracerebroventricular administration of metformin on food intake and hypothalamic appetite-regulating signalling pathways induced by the orexigenic peptide ghrelin. Rats were injected intracerebroventricularly with ghrelin (5 μg), metformin (50, 100 or 200 μg), AICAR (25 μg) and L-leucine (1 μg) in different combinations. Food intake was monitored during the next 4 h Hypothalamic activation of AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), Raptor, mammalian target of rapamycin (mTOR) and *P*70 S6 kinase 1 (S6K) after 1 h of treatment was analyzed by immunoblotting. Obtained results showed that metformin in a dose-dependent manner suppressed the increase in food consumption induced by ICV ghrelin. Ghrelin increased phosphorylation of hypothalamic AMPK and its targets ACC and Raptor, which was associated with the reduced phosphorylation of mTOR. The mTOR substrate S6K was activated by ICV ghrelin despite the inhibition of mTOR. Metformin treatment blocked ghrelin-induced activation of hypothalamic AMPK/ACC/Raptor and restored mTOR activity without affecting S6K phosphorylation. Metformin also reduced food consumption induced by the AMPK activator AICAR, while ghrelin-triggered food intake was inhibited by the mTOR activator L-leucine. Thus, metformin could reduce food intake by preventing ghrelin-induced AMPK signalling and mTOR inhibition in the hypotalamus.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1101

### Genetic variation of the hypothalamo-pituitary axis may increase susceptibility of postnatal depression

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Objective

To investigate whether genetic variants in the glucocorticoid receptor (GR) and corticotropin releasing hormone receptor 1 (CRHR1) genes are associated with increased susceptibility for postnatal depression (PND).

Methods

A prospective longitudinal cohort study at University Hospital, Coventry, England. Two hundred pregnant women were recruited and assessed for PND using the Edinburgh postnatal depression score (EPDS) upon recruitment and again 2–8 weeks post-natally. Five SNPs with established association to depression were genotyped. Association analysis was carried out in 140 patients that completed the study protocol.

Results

The bcl1 SNP of the GR and rs242939 SNP of the CRHR1 genes were over-represented in women with PND with significant allele association. Risk ratios for development of PND associated with presence of each minor allele were 2.9 (95% confidence interval: 1.2–6.9) for bcl1 and 4.9 (2 to 12) for rs242939. In contrast, no significant association was found between PND and the R23K, rs1876828 or rs242941 SNPs.

**Table 1** Patient demographics and single nucleotide polymorphism distribution in the study population

| Variable | Cases without postnatal depression number (%) | Cases with postnatal depression number (%) | *P*value |
|---|---|---|---|
| Total number | 106 (75.7) | 34 (24.3) | |
| SNPs Bcl1 HET/HOM WT | 51 (48.6) 54 (51.4) | 25 (73.5) 9 (26.5) | 0.011* |
| R23K HET/HOM WT | 9 (8.6) 96 (91.4) | 2 (5.9) 32 (94.1) | 0.61 |
| rs1876828 HET/HOM WT | 34 (32.4) 71 (67.6) | 14 (41.2) 20 (58.8) | 0.35 |
| rs242939 HET/HOM WT | 13 (12.4) 92 (87.6) | 14 (41.2) 20 (58.8) | 0.003** |
| rs242941 HET/HOM WT | 62 (59.6) 42 (40.4) | 26 (76.5) 8 (23.5) | 0.076 |

Het/hom, Heterozygous/homozygous allele, WT: wild type allele.

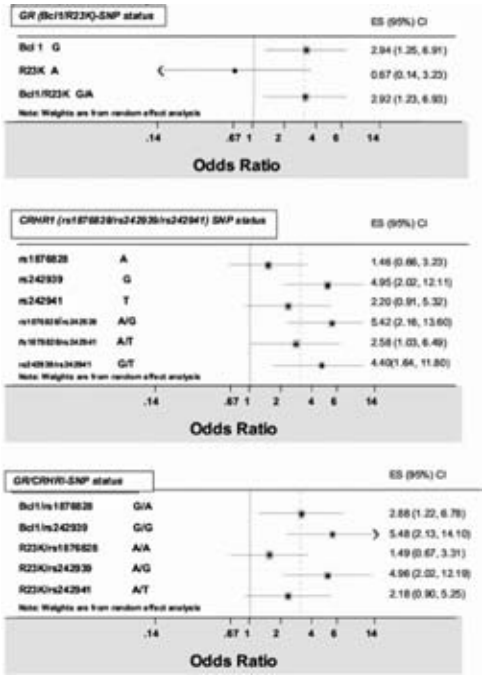


Figure 1. Forest plot for glucocorticoid and cortico tropin releasing hormone single nucleotide polymorphisms.

Conclusions

This is the first demonstration that specific SNPs of the GR and CRHR1 genes are associated with PND and might represent promising genetic biomarkers to help early identification of women at risk of PND.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1102

**Differential diagnosis of acromegaly: a new gene for pachydermoperiostosis**

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Case report
24-year. old man presented with change in facial appearance and joint pain. His symptoms began at puberty with the thickening and folding of the skin on the forehead and scalp and thickening of his fingers. These changes progressed over the next 5 years with marked seborrhoea, hyperhidrosis and linear palmar–plantar keratosis. The lower legs and forearms are cylindrically thickened, hands and feet increased in size, the terminal phalanges of the fingers show pronounced clubbing. Change of appearance was accompanied by swelling and pain in the joints, particularly in the knees. The patient complained of diarrhoea and abdominal pain, a colonoscopy revealed ulcerative colitis. This state was maintained for 4–5 years. At the age 22 he noticed slight improvement in the joint pains. His younger brother presented with very similar symptoms and signs at the age of 17. The parents were not related to each other. Acromegaly, Sotos syndrome, McCune–Albright syndrome and Carney complex were ruled out with clinical tests.
We diagnosed hypertrophic osteoarthropathy (pachyder moperiostosis). Due to the familial nature of the disease and no other underlying disease we considered primary hypertrophic osteoarthropathy, which can be due to deficiency of the enzyme 15-hydroxyprostaglandin dehydrogenase (HPGD), but no mutations were detected in this gene. As the phenotype of these patients is slightly different (teenager rather than childhood-onset, severe cutis gyrate on the forehead, lack of acroosteolysis), another causative gene was sought. A novel gene SLCO2A1 (solute carrier organic anion-transporter family member-2A1), also part of the PDE-pathway, was found to have compound heterozygote mutations c.(838C>T)+(1693T>G), P.(R280X)+(W565G) in both subjects. SLCO2A1 is a transporter, which mediates active uptake of prostaglandins, prior to their intracellular degradation. Hypertrophic osteoarthropathy can develop due cancer-induced reactive osteoarthropathy or in response to therapy with PDE1. These findings implicate local prostaglandin excess as the stimulus to hypertrophic osteoarthropathy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1103

**Magnetic risonance imaging (mri) of olfactory bulbs and other brain structures in kallmann syndrome**

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Kallmann syndrome (KS) is characterized by hypogonadotropic hypogonadism and other non-reproductive disorders including smell deficiency due to olfactory bulb developmental abnormalities. The aim of our study was to evaluate retrospectively the role of MRI in patients with clinical diagnosis of KS, searching for any abnormalities of the brain structures involved.
Methods
We evaluated 25 KS pts (14–32 years, 4F, 21 M). KS cases were classified as hyposmic (KSHO, *n* 9) or anosmic (KSAO, *n* 16) by using a modified smell test. Brain MRIs (SE T1 sagittal and coronal planes, TSE $T^2$ axial and coronal planes 2 mm thick, volumetric MPRAGE) of olfactory bulbs and sulci, corpus callosum and pituitary were blind assessed by two radiologists.
Results
In KSAO group eight showed olfactory bulbs and sulci agenesis, four bulbs agenesis (3 normal, one hypoplastic sulci), 4 bulbs and sulci hypoplasia. In KSHO two showed bulbs and sulci agenesis, two bulbs agenesis, two bulb and sulci hypoplasia, 1 hypoplastic bulbs, one normal bulbs and sulci but turbinates abnormalities. three cases were not evaluated for technical reasons. The pituitary was abnormal in 8% of cases: three pituitary hypoplasia, 1 a mucinous cysts of the pars intermediate, three a sellar arachnoid diverticulum. Hypoplastic corpus callosum was done in 2 out 25 pts.
Conclusions
Clinical and MRI data are sometimes not correlated. 50% of KSAO presented agenesis of both sulci and bulbs, 25% only bulbs agenesis and 12% bulbs and sulci hypoplasia. KSHO showed a highly variable phenotypic framework. Associated abnormalities of sellar and suprasellar regions are less frequent. MRI is important to detect abnormalities of olfactory structures, especially in prepubertal patients, where clinical diagnosis is not always easy. Moreover, imaging study may reveal other possible causes of smell dysfunction, allowing to minimize any diagnostic bias.
Declaration of interest
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## P1104

**Kisspeptin is the peptide linking metabolism and reproduction**

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There is a strong correlation between metabolic status (e.g. obesity, diabetes, alcohol use and abuse) and reproductive functions. Disruptions of metabolic status affecting the reproductive system are often manifested as decrease in secretion of hormone (GnRH) and (LH), disruptions of secretion of sex steroids and estrus cycles (in animals) or menstrual cycles (in women), and may even lead to infertility. Diabetes and obesity are also correlated with hypogondism, which is reported in adult and older men.
Kisspeptin is a hypothalamic peptide that has a key function in the regulation of reproduction. Recently it was shown that kisspeptin plays a role in the integration of metabolic and reproductive systems. However, the neuronal mechanisms responsible for integration between these systems and its disruptions by metabolic stressors are not well known yet.
Two rats models
i) prenatal ethanol/alcohol exposure (PAE); ii) diet induced obesity, diabetes typ her 1 and 2, are discussed in the context of the role of metabolic stressors on the reproductive and kisspeptin systems.
Data from the first model suggest that: i) PAE delays onset of puberty in females (delays vaginal opening) in females; ii) PAE causes lack of increase of estradiol and progesterone with age in females but has no effect on changes in testosterone levels with age in males; iii) In adult PAE females, in sex steroids replacement paradigm, kisspeptin neurons in the arcuate nucleus of the hypothalamus show increased sensitivity to estradiol and decreased sensitivity to progesterone.
Currently experiments are preformed to study the changes in the kisspeptin system in the second model.
Obtained data may allow us to better understand the role of kisspeptin in linking metabolic and reproductive systems, its disruptions under metabolic stress, as well as search for new treatment strategies for patients with fetal alcohol spectrum disorder (FASD), obesity and diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1105

**Topiramate treatment improves hypothalamic insulin and leptin signaling and action and reduces obesity in mice**

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Topiramate (TPM) is an anticonvulsant drug used for the treatment of epilepsy and prophylaxis of migraine. Weight loss is a side effect frequently reported in patients and animal models treated with TPM. Some studies showed that TPM may decrease energy storage, and thus increase energy expenditure and thermogenesis. However, the mechanisms by which TPM reduces body weight are not completely understood. Anorexigenic hormones such as insulin and leptin regulate the activity of distinct neuron populations that control energy balance via IR/PI3K/Akt/Foxo1 pathway or OBR/JAK2/STAT3 pathway respectively. However, whether TPM alters hypothalamic insulin or leptin sensitivity is not known. Thus, in the present study, we investigated whether TPM treatment alters energy balance by altering insulin and leptin action/signaling in the hypothalamus from control and diet-induced obesity (DIO) mice. Swiss mice fed with standard chow or high-fat diet were orally treated with TPM (100 mg/kg/day) during 7 days. TPM treatment led to decreased body weight gain in DIO mice, but not in control mice, with increased oxygen consumption, fat oxidation and UCP-1 expression in brown adipose tissue. Moreover, TPM treatment followed by intracerebroventricular insulin and leptin infusion increased hypothalamic insulin and leptin signaling and action in obese mice, respectively, which led to decreased food intake and increased energy expenditure, with higher levels of CRH, TRH and CART, and reduced levels of NPY, MCH and orexin mRNA expression. However, when mice were pair-fed, no significant differences were observed concerning body weight gain and hypothalamic insulin and leptin signaling in TPM-treated mice compared with non-treated animals. In conclusion, the reduced food intake and increased energy expenditure induced by TPM are associated with hypothalamic insulin and leptin regulation and may reduce obesity in mice on high-fat feeding, presenting an alternative therapy for the treatment of obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1106

**Insulin improves memory and cognition via protein kinase c delta (δ)**

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Older population and people with type II diabetes have a significantly higher rate of decline in cognitive function. However, the mechanisms are poorly understood. There are strong links between insulin and cognitive function supported by epidemiological data from humans and animal studies and *in vitro* research. Protein kinase C (PKC) signaling cascades and insulin pathways are closely integrated. The consequences of PKC activation by insulin in the CNS influence memory, cognition, synaptogenesis, and neuronal repair. In addition, PKCδ, a novel PKC has been implicated in memory, neuronal survival and proliferation. Insulin regulates the alternative splicing of mouse PKCδ splice variants: PKCδI promotes apoptosis while PKCδII functions as pro-survival protein. In vitro and *in vivo* studies demonstrated that apoptosis accounted for the neuronal loss and cognitive decline in the aging and diabetic mouse models. Our *in vivo* data demonstrates that intranasal insulin improves memory and cognition in aging and diabetic mice. Our *in vivo* data in mouse neural cells derived from the hippocampus, which is the seat of learning, memory and cognition, demonstrates that insulin increases the expression of PKCδII. PKCδII expression is not influenced by high glucose or thiazolidinediones thereby establishing that insulin increases PKCδII expression via its signaling pathways. To determine which pathways are involved in insulin-mediated PKCδ alternative splicing in neuronal cells, we used inhibitors of several pathways targeted by insulin in other tissues and show that insulin increases PKCδII expression via PI3K-Akt pathway. Constitutively active Akt2 kinase mimicked insulin effects in increasing PKCδII levels while WT-Akt2 kinase had no effect without insulin. Further, we show that PKCδII increases expression of pro-survival proteins Bcl-2 and Bcl-xL thereby increasing neurogenesis. In conclusion, we demonstrate that insulin improves cognitive functions via PKCδ.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1107

**The Tuebingen Cushing's disease quality of life inventory: development and normative data from 1784 healthy people**

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Objective

In part I of the study a health related quality of life (HRQoL) inventory for Cushing's disease (CD), the Tuebingen Cushing-25 quality of life inventory (Tuebingen CD-25) was developed. In the second part, we assessed normative data from healthy controls (HC) with which the individual patients' scores can be compared.

Methods

Sources for item generation consisted of technical literature, interviews with patients and the rating of neurosurgeons, endocrinologists and a neuropsychologist. A preliminary inventory with 64 items was handed out to 63 CD patients. Item reduction and scale generation followed the principles of Classical Test Theory. Validation was performed with the WHOQoL-BREF. For assessing normative data, the inventory was filled out by 1784 HC omitting the introductory sentence 'Because of my Cushing's disease' that was included in the CD group.

Results

The final version of the Tuebingen CD-25 contained 25 items, showed high reliability (Cronbach's $\alpha = 0.93$) and validity ($r = -0.65$) and includes the subdomains depression, sexual activity, environment, eating behavior, bodily restrictions and cognition. We found a non-linear correlation between the Tuebingen CD-25 scores and patients' age, younger and middle-aged patients having inferior HRQoL than patients between 31 and 50 years and older than 61 years. Preoperative 24 h UFC levels correlated significantly with the subscale cognition and only marginally failed significance level for the subscale Eating Behavior, while preoperative cortisol and ACTH levels did not correlate with any scale. In 28.6% of our CD patients we found slight and in 41.3% severe impairment in the Total Score of the Tuebingen CD-25 compared to HC. Less than one-third of our patient sample presented with unimpaired HRQoL.

Conclusion

The Tuebingen CD-25 is a feasible instrument to assess HRQoL in CD in a clinical and investigative setting and provides normative data for all age groups and genders.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1108

**Oligogenicity in the idiopathic central hypogonadism**

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Introduction

ICH is a rare and heterogeneous condition due to defects of GnRH secretion or action. Recent data indicate that ICH, though characterized by a strong genetic component, is a disease of multifactorial origin. Indeed, digenic defects have been described as a possible pathogenic explanation for ICH.

Subjects

We present two familial cases with particular clinical and genetic profiles, out of a cohort of 300 ICH patients.

Results

Proband 1 is a male diagnosed with normosmic ICH when he was 18 and then treated with testosterone. After 6 years of therapy he showed a complete recovery of a spontaneous GnRH function. Genetic analyses showed a heterozygous new nonsense mutation of the PROKR2 gene (15fsX43) and a known heterozygous loss-of-function mutation of the GnRHR (Q106R) respectively inherited from the unaffected father and mother. His sister has wild-type sequences but his brother,

despite a normal puberty and eugonadism, shows the same digenic genotype. Proband 2, a male diagnosed at 24 years with Kallmann syndrome (KS), was found to carry a new heterozygous missense substitution in the FGFR1 gene (L713P) and a disrupting duplication of the exons 4 to 14 of KAL1 gene. His father, younger brother and sister are unaffected with normal sequences. His mother and a elder brother were carriers of the KAL1 duplication but not of the FGFR1 variation. This brother had a normal puberty but is affected by daltonism and arched palate (two minor stigmata of KS), while the mother only by daltonism.
Conclusions
we describe two ICH families displaying a highly variable penetrance of the underlying multiple genetic defects. The first case shows that heterozygous digenic defects may not be sufficient to cause ICH, thus giving rise to the idea of a multifactorial origin of this heterogeneous condition.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1109

### Investigating glucose responsive neuropeptide release using a static hypothalamic incubation system

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The activity of certain hypothalamic neuronal populations is altered by changes in glucose. *in-vitro* studies have identified several glucose-sensitive neuronal populations in hypothalamic regions controlling energy homeostasis. Subsets of orexigenic arcuate nucleus Neuropeptide Y (NPY)-releasing neurones are known to be glucose responsive. These neurones may allow glucose to influence appetite. Cocaine and amphetamine regulated transcript (CART) is a neurotransmitter abundantly expressed in regions of the hypothalamus involved in energy homeostasis. At present, it is not clear if CART releasing neurones are glucose-sensitive. To assess hypothalamic neuropeptide release in response to changes in glucose, we investigated NPY and CART release by the hypothalamus at different concentrations of glucose, using a static hypothalamic incubation system.
Hypothalami from 20 male Wistar rats were incubated in artificial CSF (aCSF) for a 2-h equilibration period. The hypothalamic explants were then incubated for three 45 min periods in 600 µl aCSF containing 3, 8 (baseline) and 15 mM glucose in random order. Finally, the viability of the tissue was verified by 45-min incubation of aCSF containing 56 mM KCl. At the end of each incubation period, supernatants were removed and assayed for NPY and CART release by radioimmunoassay. Only explants that showed a greater secretion of NPY or CART with 56 mM KCl as compared to baseline were considered viable. Release of neuropeptide was expressed as percent of basal release.
There was a significant increase in NPY release with aCSF containing 3 mM glucose versus baseline glucose (3 mM: $285.2 \pm 92.8$ vs 8 mM: $100 \pm 36.1$ vs 15 mM $130.7 \pm 25.8$). There was a decrease in CART release with aCSF containing 15 mM glucose versus baseline glucose (3 mM: $95.1 \pm 10.6$ vs 8 mM: $100 \pm 6.8$ and 15 mM: $78.8 \pm 3.9$). These results suggest an important role for glucose-sensitive NPY-releasing hypothalamic neurones in mediating changes in energy homeostasis in response to glucose. They also suggest that subsets of CART-releasing hypothalamic neurones may be glucose responsive.
Declaration of interest
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## P1110

### Expanding the clinical and genetic spectrum of the reversal of congenital hypogonadotropic hypogonadism

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Introduction
Up to 10% of patients with congenital hypogonadotropic hypogonadism (HH) may undergo reversal of hypogonadotropism and some of them even attain normal sperm count in adulthood. However, clinical and molecular genetic features of these patients and the triggers leading to reversal of HH are not well understood. We studied whether Finnish reversal variants displayed a common phenotypic or genotypic feature that would predict the clinical course of HH.
Patients and methods
Thirty-two male HH patients with anosmia/hyposmia (Kallmann Syndrome, KS; $n=26$) or normal sense of smell (nHH; $n=6$), were enrolled (age range, 18–61 years). The patients were clinically examined, and reversal of HH was assessed after treatment withdrawal. KAL1, FGFR1, FGF8, PROK2, PROKR2, CHD7, WDR11, GNRHR, GNRH1, KISS1R, KISS1, TAC3, TACR3, and LHβ were screened for mutations.
Results
Six HH patients (2 KS, 4 nHH) were verified to have reversal of HH (median age, 23 years; range, 21–39 years). All of them had spontaneous testicular growth while on testosterone replacement therapy (TRT). One nHH subject was restarted on TRT due to a decline in serum T. Two KS patients had a mutation in CHD7 (p.Q51X) or in FGFR1 (c.91+2T>A), two nHH patients were compound heterozygotes for mutations in GNRHR (R139H/R262Q and R262Q/del309F), while only two remained without a molecular genetic diagnosis.
Conclusions
Considerable proportion of patients with HH recovered in early adulthood. Spontaneous testicular enlargement during TRT was highly suggestive for reversal of HH, but we did not find phenotypic features that would have predicted reversal. However, those with the GNRHR mutation R262Q accompanied by another GNRHR mutation may be prone to reversal. Conversely, even patients with a truncating mutation in CHD7 or a splice-site mutation in FGFR1 can recover. We recommend that all adolescents and young adults with congenital HH should be informed on the possibility of reversal.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1111

### Retinoic acid regulates growth-related gene expression in the rat hypothalamus

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The vitamin A-derived hormone retinoic acid (RA) is best known for its essential role in development, but components of the RA signalling pathway are also expressed in regions of the adult brain, including the hypothalamus, which regulates feeding behaviour, metabolism and body weight. RA is synthesised by the tanycytes lining the third ventricle and has recently been found to regulate cell proliferation in the hypothalamus. Some animals, such as the hamster and F344 rat, alter their feeding and body weight in response to changes in day length and photoperiod manipulation is a useful system for investigating growth- and weight-related changes in the hypothalamus. Expression of the RA synthetic enzyme retinaldehyde dehydrogenase 1 (RALDH1) is photoperiodically regulated in the hypothalamus of the photoresponsive F344 rat, with higher RALDH1 expression in rats kept on a long-day photoperiod (16 h light:8 h darkness; LD), relative to short-day (8 h light:16 h darkness; SD.). This suggests that RA levels are higher, and RA signalling more active, under LD conditions. To further investigate the role of RA signalling, hypothalamic slices from P10 male Sprague-Dawley rats were cultured in the absence of vitamin A for 4 days, then treated for 48 h with either DMSO or $10^{-6}$ M RA. qPCR analysis of treated tissue showed that RA treatment increased hypothalamic expression of GHRH, AGRP and POMC, genes known to be upregulated in LD-exposed rats. PCSK2, which encodes a prohormone convertase necessary for posttranslational processing of POMC, was also upregulated by RA. NPY and CART, genes associated with SD photoperiod, were unaffected by RA treatment. These results demonstrate that increasing hypothalamic RA levels is sufficient to upregulate genes associated with LD photoperiod and suggest that RA signalling contributes to the control of body weight by regulating the expression of growth-promoting genes in the hypothalamus.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1112

**Crf type 2 receptor mediates estradiol-induced increase in hypothalamic leptin sensitivity**

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Energy homeostasis is controlled by neural, endocrine and adipocyte factors and CNS plays a key role in this control. We have previously observed that ovariectomized (OVX) rats treated with estradiol have lower body weight gain associated with an increased hypothalamic CRF mRNA expression. We investigated the role of CRF on activation of hypothalamic CART neurons by leptin in estradiol-treated OVX rats. Female Wistar rats (200–250 g) were subjected to both OVX and cannula implantation into the lateral ventricle and treated with estradiol cypionate (OVX + E, 10 μg/kg Bw, s.c.) or vehicle (OVX, corn oil: 0.2 ml/rat, s.c.) for 8 days. I C V injection of recombinant leptin (10 μg/5 μμ) was performed with i c v pre-treatment with CRF-R2 antagonist (antisauvagin − 30. 5 μg/5 μl) or vehicle. Anesthetized rats were perfused 90 min after leptin or vehicle injection. Fos/CART expressions were analyzed in the arcuate nucleus (ARC) of the hypothalamus by immunohistochemistry. Leptin induced body weight loss and reduced food intake both in OVX and OVX + E and increased neuronal activation in the ARC in OVX animals with this effect being amplified by estradiol treatment (OVX: 5.0 ± 0.6 vs 17.8 ± 2.7; OVX + E: 3.3 ± 1.3 vs 28.3 ± 6.1; $n = 3$-5; $P < 0.05$; vehicle vs leptin, respectively). These effects of leptin were abolished by CRF-R2 antagonist pre-treatment only in OVX + E rats (5.2 ± 1.9; $n = 5$; $P < 0.001$). Leptin treatment increased Fos/CART double-labeling in the ARC in OVX and OVX + E animals (OVX: 1.7 + 0.9 vs 9.5 + 1.6; OVX + E: 0.3 + 0.3 vs 9.5 + 2.2; $n = 3$-5; $P < 0.001$), with no differences between these groups. CRF-R2 antagonist abolished leptin effect on Fos/CART double labeled neurons only in OVX + E rats (10.5 + 0.3; $n = 5$; $P < 0.001$). In conclusion, our data suggest that estradiol increases hypothalamic sensitivity to leptin and this central effect is mediated, at least in part, by CRF type 2 receptors.

Declaration of interest

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## P1113

**Retinal growth hormone: local regulation by GH-releasing hormone (GHRH)?**

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Introduction

The (GH) gene, identical to that in the pituitary, is expressed in retinal ganglion cells (RGCs) in chickens, rats, mice and humans. Retinal GH is a neuroprotective factor, as it promotes RGC survival and, in humans, the concentration of GH in vitreous fluid may be a marker for ocular disease. Increasing retinal GH expression could thus have therapeutic potential. The factors regulating retinal GH expression are, however, unknown. The possibility that GH-releasing hormone (GHRH) might be involved in the expression of retinal GH was therefore investigated.

Methodology

The possible presence of GHRH was determined by immunohistochemistry, using retinal sections from embryonic chicks and fixed QNR/D cells (a - commercial quail neuroretinal cell line). The possible GH-releasing activity of GHRH was determined by the *in vitro* culture of QNR/D cells with exogenous GHRH and by the measurement of mRNA, using of quantitative PCR.

Results

GHRH immunoreactivity was abundantly present in the cytoplasm of chick retinal RGCs and in QNR/D cells and in both cases was co-localized with GH immunoreactivity. After a 24 h incubation in exogenous GHRH (at 10–6 M) GH mRNA in the QNR/D cells was significantly increased.

Conclusion

These results demonstrate, for the first time, that GHRH is present in retinal RGCs and that exogenous GHRH promotes retinal GH expression. The co-localization of GH and GHRH also suggests that retinal GH regulation occurs locally by autocrine or paracrine mechanisms. Increased GHRH signaling might therefore be utilized therapeutically to increase retinal GH secretion.

Declaration of interest

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## P1114

**Cu–gnrh and pacap-induced cAMP/PKA and cGMP/PKG pathways activity in anterior pituitary cells *in vitro*.**

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Hypothalamic neuropeptide PACAP is a potent endogenous stimulator of adenylate cyclase synthesis in gonadotrope cells. Previously we found that, in contrast to GnRH, also Cu–GnRH complex is able to induce cAMP/PKA pathway activity.

The aim of the study was to determine whether Cu–GnRH can act via GnRH-R and/or PAC-1 receptors to stimulate cAMP/PKA intracellular signaling. Moreover, we compared PACAP and Cu–GnRH ability to activate cGMP/PKG system in anterior pituitary cells.

$5 \times 105$/ ml cells obtained from cycling female rats were preincubated for 72 h and then stimulated for 30 min, 1 and 3 h by $10^{-7}$ M of Cu–GnRH or PACAP. Peptides were also incubated with cells pretreated with $5 \times 10^{-7}$ M of specific receptor antagonists: PACAP 6–38 (for PAC-1 receptor) or antide (for GnRH receptor). Intracellular and extracellular cAMP, cGMP and LH medium concentration were measured by specific RIAs.

PACAP as well as Cu–GnRH activated cAMP synthesis although pattern of activation was different for both peptides. PACAP-induced increase in cAMP concentration was detected after 30 min, whereas Cu–GnRH -induced elevation required 1 h of incubation. In contrast to Cu–GnRH, PACAP-stimulated cAMP/PKA pathway activity remained time-dependent since an increase of cAMP concentration was found up to 3 h of incubation. cAMP synthesis was reduced when Cu–GnRH complex was incubated in the presence of PAC-1 receptor antagonist but was not changed in the presence of GnRH receptor antagonist. Obtained data also revealed that Cu–GnRH potently stimulated cGMP synthesis and inhibition of endogenous PKA activity resulted in an inhibition of cGMP production in anterior pituitary cells.

In conclusion, results indicate that Cu–GnRH-induced cAMP/PKA pathway activation might occur, at least partially, through an involvement of specific PACAP receptor. Moreover, Cu–GnRH complex may induce a cross-talk between cAMP/PKA and cGMP/PKG pathways in anterior pituitary cells.

Declaration of interest

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## P1115

**Corticosterone interferes with the stimulatory effect of cAMP on proTRH transcription by promoting a GR–PKAc interaction and not through chromatin remodelling**

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The activity of hypothalamus–pituitary–thyroid (HPT) axis is essential for energy homeostasis. TRH expression in the PVN is rapidly stimulated by energy demanding situations as cold exposure or locomotion, but blunted by stress-induced increased corticosterone serum levels. In primary cultures of rat hypothalamic cells (RHC), PKA activators as forskolin (Fsk) increase TRH mRNA levels, effect reverted by coincubation with dexamethasone. Fsk-dex coincubation also impedes binding of GR and pCREB to GRE and CRE in TRH-promoter (Díaz Gallardo, *J.Neuroendocrinol* 2010). In an attempt to elucidate the mechanism involved, we determined whether 1 h coincubation with dex or Fsk-dex induced chromatin condensation. ChIP analyses of RHC-isolated chromatin from Fsk treated cells revealed increased PCR signal of TRH promoter (−246/+69) in immunoprecipitates of pCREB, RNA-Pol II, H3ACK10 or H3ACK14 antibodies (Ab), and of GR from dex-treatment; these signals were diminished in Fsk-dex coincubates. No recruitment was obtained with Ab against deacetylases HDAC3 H99 in Fsk-dex compared to that detected in cells treated $T^3$. These results demonstrate that simultaneous activation of GR and PKA does not affect chromatin remodeling but impede GR and pCREB binding to TRH promoter. Antibodies against the catalytic subunit of PKA (PKAc), or against pCREB, incubated with a GR-immunoprecipitate from subcellular fractions of RHC

treated with dex-Fsk allowed to demonstrate the interaction of GR:PKAc. In living Fsk treated RHC, cytoimmunochemical analysis demonstrated increased pCREB in nuclei that was diminished with Fsk-Dex treatment. Furthermore, co-transfecting GR and TRH-Luc on SH-SY5Y cells reproduced the interfering effect dex-Fsk on Fsk stimulation; dex interference on Fsk stimulation was lost transfecting GR mutated on DBD site (L501P), and with GR-T171A or GR-S224A mutants that affect nuclear translocation and interaction with coregulators, supporting GR intrinsic characteristics for protein-protein interactions. These results demonstrate that GR interaction with PKAc provides a fast mechanism for avoiding pCREB activation of TRH transcription.

Declaration of interest

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## P1116

**Effects of mineralocorticoid agonists and antagonists on survival, proliferation and differentiation of adult rat hippocampal progenitor cells**

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Introduction

Hippocampus is a key area in the brain and influences the neuroendocrine functions, especially the hypothalamo–pituitary–adrenal (HPA) axis that is mainly regulated by corticotrophin-releasing hormone (CRH), vasopressin (ADH) and glucocorticoid (GC). This feed-back action is mediated by both glucocorticoid (GRs) and mineralocorticoid (MRs) receptors. GRs are distributed throughout the brain, but mostly in hypothalamic neurons, while the MRs highest expression has been detected in the hippocampus. Aim of the study was to clarify the role of fludrocortisone (MRs agonist) and spironolactone (MRs antagonist) on cell proliferation, differentiation and survival in adult rat hippocampal progenitor (AHP) cells, a totipotent cell line that can differentiate in neurons and glial cells. Furthermore we investigated the effect of fludrocortisone on AHP cell survival after treatment with amyloid β-protein (fragment 1–42).

Methods

AHP cells were cultured with neural stemline medium enriched with FGF-basic. The presence of GRs and MRs was evaluated by RT-PCR. Cell survival was measured by MTT assay and cell proliferation by BrdU incorporation. Apoptosis was studied by Caspase-3 activity analysis. Activation of survival signalling pathways was determined by Western blotting, i.e. phosphorylation of phosphatidylinositol 3-kinase/Akt (PI3K/Akt), glycogen synthase kinase-3 (GSK-3β) and of cAMP response element binding (CREB).

Results

Fludrocortisone, but not spironolactone, stimulates proliferation, and protects against growth factor deprivation-induced apoptosis. Fludrocortisone activates the GSK-3β, the PI3K/Akt and the CREB pathways. Moreover it counteracts the effect of the amyloid β-protein (1–42) on cell death and inhibition of cell proliferation.

Conclusion

The survival and proliferative effect of fludrocortisone in neuronal precursors candidate MRs agonists as potential molecules in the treatment of neurodegenerative conditions, such as Alzheimer′s disease.

Declaration of interest

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## P1117

**Sleep deprivation-induced changes in rat hypothalamic arginine–vasopressin content**

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Neurohypophysial hormone, arginine–vasopressin (AVP) concentrations in human plasma and in the plasma, and hypothalamus of the rat display diurnal variations with rising levels over the hours of sleep. Previous reports indicate that AVP infusions reduce rapid-eye-movement sleep in humans and increase the amount of time spent in waking in rats. Our aim was to determine whether AVP content of the rat hypothalamus is altered by sleep deprivation (S.D.). Adult male Sprague-Dawley rats were acclimated to a 12:12-h light–darkness cycle and were sleep deprived by gentle handling starting at light onset. Hypothalamic samples ($n = 8$–10 per group) were obtained after 4 and 8 h of S.D.and after 1 and 2 h of recovery following 8 h of S.D. Control samples were collected from undisturbed rats at corresponding time points. After extraction, AVP was determined by means of radioimmunoassay. The changes in AVP during and after S.D. were evaluated with respect to the time-matched control samples by means of two-way ANOVA. Hypothalamic AVP contents differed significantly between the control groups and the sleep deprived animals. AVP was normal after 4 h of S.D..and dropped to a low level by the end of h 8 of S.D.. AVP was significantly suppressed after 1 and 2 h of recovery. Similarly to our data S.D.. induced a significant fall in nighttime plasma AVP in healthy children. Current results confirm that diurnal variations in AVP secretion are in fact associated with sleep thus AVP could play a role in the physiological regulation of sleep.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1118

Abstract withdrawn.

## P1119

**Ghrelin, leptin and adiponectin plasma levels in narcolepsy with cataplexy**

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Introduction

Narcolepsy is a rare sleep disorder characterized by excessive daytime sleepiness and REM-sleep abnormalities. Lateral hypothalamic hypocretin/orexin neurones are critical for normal wakefulness and energy expenditure, and the reduction of their activity has been linked with narcolepsy. Their activity is inhibited by extracellular glucose and the 'satiety' hormone leptin but stimulated by the 'hunger' hormone ghrelin. Patients with narcolepsy and cataplexy are often overweight and display an increased prevalence of type 2 diabetes mellitus. Because of possible ghrelin/adypocytokines disbalance, we assessed plasma ghrelin, leptin and adiponectin periferial levels.

Description of methods

Plasma ghrelin, leptin and adiponectin concentrations were measured in 25 narcoleptic patients with cataplexy (mean age 40.76 ± 13.81 years, mean BMI 26.44 ± 5.21) and 20 healthy age and BMI matched controls (mean age 41.25 ± 14.05, mean BMI 26.04 ± 3.94). Body composition was measured using bioimpedance method. Levels of parameters were measured by RIA (kits LINCO Research).

Results

There were no stastically siugnificant differencies in plasma ghrelin, leptin, and adiponectin concentrations between narcoleptic and healthy individuals. As expected there was a positive correlation between leptin levels and fat mass in patients with narcolepsy and cataplexy.

Conclusion

Our study suggests that possible metabolic changes and tendency to owerweight previously reported in narcolepsy with cataplexy may be caused by other mechanisms but not ghrelin/adypocitokines disbalance.

Declaration of interest

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## P1120

### High serum cortisol levels in patients with Alzheimer's Disease

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Introduction
Alzheimer's Disease (AD) is a multifactorial disease. The hypothalamo-pituitary–adrenal (HPA)-axis shows extensive changes in AD. It is observed a hyperactivation of corticotropin-releasing hormone (CRH) neurons with age, which is significantly present in men. The aim of this study is to compare serum levels of cortisol in patients with Alzheimer's disease and normal controls.
Material and Methods
Ninety patients with AD and 95 normal controls matched for age and gender were included. The diagnosis of Alzheimer dementia was based on standard criteria provided by the DSM-IV TR system and the NINDS-ADRDA. Blood samples were frozen at $-80^{\circ}$C until analysis. Cortisol levels were measured using a commercial routine immunoassay (electrochemiluminescent assay, ECLIA). Differences in cortisol levels were assessed between the two groups using the Mann–Whitney method. Linear regression analysis was also used to adjust for characteristics shown to be associated with cortisol and cognitive decline. These characteristics were sex, age, weight and smoking. The significant level was set at $P<0.05$.
Results
From the patients with AD, 74 were women and 16 were men (mean age $80.53\pm6.03$, mean body weight $71.49\pm8.33$) and from the control group 78 were women and 17 men (mean age $79.27\pm6.86$, mean body weight $70.23\pm6.73$). Serum cortisol levels were significantly higher in patients with AD compared to normal controls ($16.95\pm9.53$ in AD patients vs $9.90\pm5.49$ in normal controls, $P<0.001$, Mann–Whitney $U$).
Conclusions
The study showed that patients with AD have high serum cortisol levels. Because of the inhibitory control of the hippocampus on the HPA-axis, damage to this structure was expected to disinhibit the HPA-axis, and to cause a positive feedforward cascade of increasing glucocorticoid levels over time. This 'glucocorticoid cascade hypothesis' of stress and hippocampal damage was proposed to be causally involved in age-related accumulation of hippocampal damage in disorders like Alzheimer's disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1121

### Melatonin levels, sleep and psychosocial impairment in patients after radiotherapy for brain tumors or leukemia

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Objective
Melatonin is a hormone secreted by the pineal gland which modulates sleep and wakefulness rhythms. Decreased melatonin secretion has been shown to be associated with increased daytime sleepiness and disturbed circadian rhythm in (irradiated) survivors of childhood craniopharyngeoma. The present study was performed to explore possible relationships between disturbed sleep, psychosocial impairment and melatonin secretion in patients treated with cranial radiotherapy as a part of brain tumour or leukaemia treatment.
Patients and methods
Thirty four (16 m, 18 f) patients who had received radiotherapy as part of brain tumour treatment or leukaemia were investigated at least 3 year after completion of radiotherapy. In all patients overnight urine was collected and melatonin concentration and melatonin secretion/h was measured. Sleep quality was assessed using the pittsburgh sleep quality index (PQ3I) and daytime sleepiness using the epworth sleepiness scale (ESS). Quality of life was assessed with the short form- 36 (SF–36) and depression with the beck depression inventory-II (BDI-II). Tumors were divided into midline or near-midline tumours ($n=15$; i.e. germinoma medulloblastoma), non-midline ($n=12$; i.e.hemispheric) tumours and patients with leukaemia ($n=7$).
Results
Self-assessed sleep quality in the PQ3I or daytime sleepiness (ESS) were not linked to melatonin secretion and neither were BDI-II nor SF-36 scores. Melatonin concentrations were not dependent on radiation dose, elapsed time since radiotherapy, age at radiotherapy, or childhood versus adult-onset of brain tumour disease (n.s.). Patients irradiated for midline tumours had a significantly lower overall melatonin secretion and hourly melatonin secretion than patients irradiated for non-midline tumours ($P=008$).
Conclusion
Melatonin secretion was linked to brain tumour site and, thus, perhaps also radiation dose to the pineal gland. Since melatonin secretion was not associated with sleep quality or psychosocial impairment, the medical use of exogenous melatonin in such patients must be questioned.
Declaration of interest
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## P1122

### Ten years of gastroenteropancreatic neuroendocrine tumours: evolution of the classification and correlation with follow-up in 50 patients

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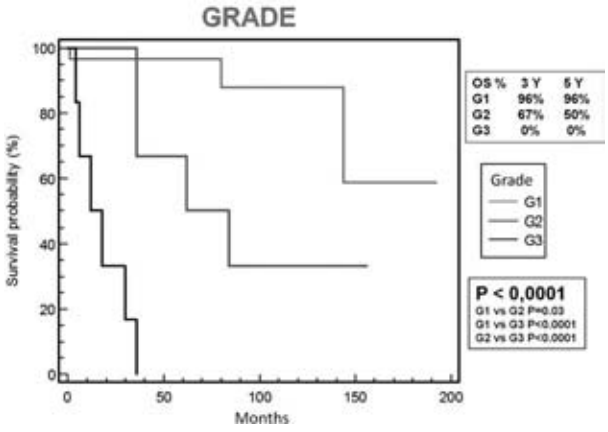
Gastroenteropancreatic (GEP) neuroendocrine tumours (NETs) are rare neoplasms with heterogeneous clinical behavior and potential long-term survival. In the last decade GEP–NET nomenclature has been twice reviewed. However, the 2000 WHO classification had poor prognostic power in well differentiated (WD) neoplasms. This led to the introduction of two new important parameters by the European Neuroendocrine Tumors Society (ENETS), grade and stage; the former became part of the new 2010 WHO classification. Considering the recent introduction of grade and stage, their external validation has been strongly requested.
Since these are important tools in the management of patients with NETs, our aim was to reclassify retrospectively, with grade and stage, our series and correlate with follow-up results.
From the histopathology archive at our centre, 170 GEP–NETs (1993–2011) were identified. Only 50 patients have been reclassified so far and the work is still in progress. Mean age at diagnosis was 56 years, F:M ratio was 1:1.2 and mean follow-up was 56 months (2–196).
Correlating differentiation with overall survival (OS), WD NETs had strikingly better outcome with respect to poorly differentiated ones. However only grade identified a subgroup of patients with WD lesions with less favorable clinical behavior (OS at 5 years: G1–96% vs G2–50%). Stage was also useful, though not statistically significant in our series so far.
Our data confirm the importance of grade and stage in prognostic stratification. Indeed, the new 2010 WHO classification has a better prognostic power and better predict the clinical course of WD disease. Therefore, in view of the long survival of patients with NETs, the retrospective classification of patients who may not



have been previously graded or staged, may influence the choice of therapeutic options, especially in the future.
Overall survival of GEP NETs stratified according to the grading.
Declaration of interest
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## P1123

**Orexigenic neuropeptide 26RFa: new evidence for an adaptive profile of appetite regulation in anorexia nervosa**

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Background
Restrictive anorexia nervosa (AN) presents an adaptive appetite regulating profile including mainly high levels of ghrelin. Because this adaptive mechanism is not effective on food intake, other appetite regulating peptides need to be explored. 26RFa is a hypothalamic neuropeptide that stimulates appetite, gonadotropin release and bone metabolism. The aim of the current study was to evaluate circadian levels of 26RFa in AN patients compared with healthy subjects, other eating disorders and constitutional thinness (CT).
Subjects and methods
Twelve-point circadian profiles of plasma 26RFa levels were measured in five groups of age-matched young women were included in the study: 19 restrictive AN (AN-R), 10 AN with bingeing/purging episodes (AN-BP), 14 with CT, 10 bulimic (BN) and 10 normal weight controls.
Results
Significant circadian variations of 26 RFA were noticed in controls with higher values in the morning and abrupt decrease at noon. 24-h mean 26RFa levels were significantly increased in AN-R and AN-BP ($P<0.001$), predominantly in the afternoon and evening when compared to controls. Pre-prandial rises of 26 RFA were noticed in AN patients. Mean 26RFa trend to be higher in CT than in controls ($P=0.06$) and significantly lower than in AN. BN patients presented a circadian profile of 26RFa similar to that of controls.
Conclusion
High levels of circulating 26RFa observed in AN patients reflects an adaptive mechanism of the organism to promote energy intake and to increase fat stores in response to a deficit in energy balance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1124

**Autoimmune thyroid disease and neuroendocrine gastric tumors: early detection and efficacy of somatostatin analogue treatment**

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Autoimmune chronic thyroid disease (AITD) is associated with atrophic gastritis (CAG) in 29–42% of the cases. CAG and the consequent hypergastrinemia, listed as precancerous lesion, can predispose to neuroendocrine cells hyperplasia and the development of neuroendocrine gastric tumors (NET). Aim of this study was to evaluate the association of AITD with risk of gastric NET and the efficacy of somatostatin analogues treatment.
Patients
From 2009 to 2011, we selected 92 AITD patients referred to the author's endocrinology practice (83 female, 9 male, aged 16–70 years). All cases underwent hormonal profile, including serum gastrin, CgA, parietal cell antibodies (PCA). Patients with gastrin and CgA significantly higher than normal level (cut off were $>$ 250 pg/ml and $>$80 ng/ml respectively) underwent to gastro esophageal endoscopy (EGDS). The patients positive for neuroendocrine cells hyperplasia at EGD, underwent somatostatin receptor scintigraphy and treatment with Sandostatina LAR 30 mg 1 fl i.m. each 28 days. Gastrin and CgA assays were repeated every three months and after one year they performed EGDF.
Results
Out of 92, only 58 patients exhibited gastrin and CgA. In 11 patients (18%) gastrin and CG were higher than normal (552,6 $\pm$ 250 pg/ml; 288 $\pm$ 152.3 ng/ml respectively), and only eight were PCA positive. Eleven patients with hypergastrinemia underwent EGDS, that showed in 2 patients diffused hyperplasia of the neuroendocrine cells of the body and in one a carcinoid. These patients, all negative to Octreoscan, were treated with Sandostatina LAR 30 mg fl every 28 days. After 3 months of treatment gastrin and CG levels were significantly reduced ($P<0.001$) and patients referred a decrease of clinical symptoms. After one year they showed normal gastrin and CgA values and EGDS detected absence of neuroendocrine hyperplasia.
Conclusions
We show that in 18% of AITD with hypergastrinemia was found neuroendocrine hyperplasia, a precancerous condition predisposing to NET risk. The remission, shown by EGDS, after one year of treatment, gives us evidence of the efficacy of somatostatin analogue in the prevention of gastric NET risk.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1125

**Contemporary microsurgical treatment of Cushing's disease**

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Introduction
Transsphenoidal surgery (TSS) is the treatment option of first choice for Cushing's disease (CD). The traditional microsurgical technique has recently been challenged by endoscopic surgical methods. We present endocrine outcome of TSS in CD with a modified contemporary microsurgical concept.
Methods
Eighty three patients underwent TSS for newly-diagnosed CD (72 micro-adenomas and 11 macroadenomas). An enlarged resection was performed in 36 patients. A modified exploration technique with radial incisions was performed in 19 patients in whom an adenoma was not readily detectable. Inferior petrosal sinus sampling (IPSS) was performed in only nine cases. Normal (or decreased) urinary cortisol and suppression below 2 μg/dl during a low-dose dexamethasone suppression test were required for endocrinological remission.
Results
An initial remission rate of 87.5% (63/72) was achieved in microadenomas. Six patients with microadenomas were re-operated for persistence and hypercortisolism was corrected in five of them. With re-operation included, the overall remission rate for microadenomas was 94.4%. In macroadenomas, a remission rate of 63.6% was achieved. No procedure-related complications occurred in primary surgery.
Of the patients in remission, 72.5% had early postoperative random cortisol levels below 2 μg/dl, 17.4% had cortisol levels between 2 and 5 μg/dl, and 10.1% had cortisol levels $>$5 μg/dl.
15.2% of the patients with microadenomas developed postoperative partial hypopituitarism and 3% diabetes insipidus. No increased rate of hypopituitarism was found with enlarged adenomectomy compared to selective adenomectomy. Only a slightly higher rate of partial hypopituitarism (23.1%) was found if extensive exploration was required.
Conclusion
Transsphenoidal microsurgery is highly effective as initial therapy of CD. Early re-operation is a successful option if CD persists. If pituitary dependence has been established on the basis of endocrine functional testing, IPSS is not obligatory even if MRI is negative. Enlarged resection for poorly-demarcated micro-adenomas yields a high remission rate without compromising pituitary function.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1126

**Effect of ghrelin on aromatase gene expression and estradiol 17-β concentrations in ventromedial and lateral hypothalamic area**

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The goal of this study was to determine whether ghrelin increase aromatase gene expressions and estradiol 17-β concentrations in ventromedial (VMH) and lateral hypothalamic area (LH). Forty rats were randomly divided into four groups. Animals in different groups were received either saline, 1, 2 or 4 ng ghrelin into their VMH and LH. After infusion of ghrelin, VMH and LH tissue were separated and homogenized. Homogenized sample were assayed for aromatase gene expression by PCR technique and estradiol 17-β ($E_2$) concentrations by RIA techniques. Infusion of 2 and 4 ng ghrelin into VMH and LH area significantly increased the mean aromatase gene expressions and $E_2$ concentrations in the VMH and LH of the rats. Infusion of 1 ng ghrelin into VMH and LH area did not change the mean aromatase gene expressions and $E_2$ concentrations in the VMH and LH of the rats.The result of this study indicated that ghrelin may depolarize neurosteroids of VMH and LH and consequently increase aromataze gene expressions and $E_2$ concentrations in the brain of the rats.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1127

**Regional distributions of kisspeptin and gonadotropin-inhibiting hormone peptides in ovine and rat brains**

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The reproductive system is controlled by (GnRH) secretion from the brain, which is finely modulated by a number of factors, including gonadal sex steroids and inhibins. It was recently claimed that kisspeptin (KP) and gonadotropin inhibitory hormone (GnIH) neurons are regulators of GnRH secretion. Therefore, we have developed highly specific and sensitive fluoro-immunoassays (FIAs) using Eu-labeled antigens for KP 10 and GnIH. In this study, the distributions of KP 10 and GnIH in rat and ovine brains were determined by FIA, and they were compared those of GnRH.

Using Eu-labeled KP-10 and GnIH, highly sensitive immunoassays for KP-10 (IC50, 87 pg/ml) and GnIH (IC50, 15 pg/ml). After decapitation, rat and ovine brain tissues were boiled for 10 min in 1-N acetic acid, after which they were homogenized in 10 volumes (v/w) in 1-N acetic acid. Aliquots of supernatant were evaporated and dissolved well in assay buffer.

When the distribution of KP was examined in rat brain, KP was detected only in the hypothalamus (35 pg/100 mg wt), with negligible KP in other tissues. However, the amount of GnIH was high in rat brain; the highest concentration was found in the thalamus (410 pg), followed, in order, by the hypothalamus, medulla oblongata, telencephalon, and cerebellum. In ovine brain tissues, the highest concentration of KP (116 pg) was observed in the diencephalon, and the amount of KP in other tissues ranged between 20 and 40 pg. On the other hand, the concentration of GnIH in ovine brain was highest in the diencephalon (155 pg), followed, in order, by the midbrain, pons, and medulla oblongata, with GnIH concentration in other brain tissues being $<60$ pg.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1128

**Right hemicolectomy in the treatment of patients with appendiceal neuroendocrine tumors: does size matter?**

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Background

A recent study of a small series of patients with appendiceal neuroendocrine tumors (ANETs) fulfilling various criteria for right hemicolectomy (RHC) revealed that $\approx$ 25% may harbor identifiable extra-appendiceal disease. The residual disease might not have been detected using the latest European Neuroendocrine Tumors Society (ENETs) revised pathological criteria.

Aims

To evaluate the latest pathological criteria for completion right hemicolectomy in an extended series of patients with ANETs.

Methods

The medical files of 27 consecutive patients who underwent RHC for ANETs in three tertiary hospital NETs clinics were retrospectively assessed. Demographic, clinical, laboratory data were collected; surgical specimens were reviewed for the indications for completion RHC: tumor diameter of $\geq 2$ cm, tumor location at the appendiceal base, extensive mesoappendiceal invasion (EMI) of $>3$ mm, vascular invasion (VI) or a KI67 proliferation index of $\geq 2\%$.

Results

5/27 patients were found to have residual disease. In 8/27 patients (30%) the tumor diameter was $<1$ cm; the indications for RHC included: tumor presence in surgical margins (3), EMI (5), VI (2), KI67 $\geq 2\%$ (3); residual disease was present in one patient (13%). In 12/27 patients (44%) the tumor diameter was 1–2 cm; the indications for RHC were as follows: tumor presence in surgical margins (1), EMI (10), VI (2), KI67 $\geq 2\%$ (2); residual disease was present in two patients (17%). In 7/27 patients (26%) tumor diameter was $\geq 2$ cm. In this subgroup, RHC is accepted practice; the tumor was present in surgical margins (3), EMI (7), VI (5), KI67 $\geq 2\%$ (5). Residual disease was present in two patients (28%).

Conclusions

In patients with ANETs it is well established that tumor size and the EMI are the most significant factors in taking the decision about the extension of the surgery. Our present data suggests that in the subgroup of patients with a primary tumor size between 1–2 cm (the 'gray zone') the risk of residual disease high. Using the latest criteria for RHC, residual disease may be missed in 7% of ANETs patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1129

**Delay in the onset of puberty of intrauterine growth retarded female rats cannot be rescued by hypernutrition after birth**

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Perinatal undernutrition is known to disturb reproductive development, in particular by delaying the onset of puberty in certain species. Using a rat model, we studied whether hypernutrition after birth can rescue the delayed onset of puberty in intrauterine undernourished female rats. Pregnant rats were divided into two groups the maternal normal nutrition (mNN, $n=8$) and maternal undernutrition (mUN, $n=9$) groups. In the mUN group, dams received 50% of the daily food intake of the mNN group from day 15 of pregnancy until delivery. Pups from both the mNN and mUN dams were then separated into two groups, based on their postnatal feeding

Conditions

control-normal nutrition (control-NN), control-hypernutrition (control-HN), IUGR-normal nutrition (IUGR-NN), and IUGR-hypernutrition (IUGR-HN). Litter sizes of the hypernutrition groups were controlled to five pups per dam, and normal nutrition groups to 12–13 pups per dam. From postnatal day 30, pups were inspected daily for vaginal opening (VO). The age of VO in the IUGR-NN group was $35.7 \pm 2.4$ days (mean $\pm$ S.D.), which was significantly delayed compared to that of the control-NN group ($33.8 \pm 0.8$ days). The age of VO in the IUGR-HN group was $35.5 \pm 2.3$ days, which was significantly delayed compared to that of the control-HN group ($33.5 \pm 0.8$ days). Interestingly, the age of VO did not differ between the IUGR-NN and IUGR-HN groups. In conclusion, maternal undernutrition delays puberty in female offspring, and this delay in puberty cannot be rescued with hypernutrition after birth.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1130

### Fasting induces a delayed increase of thyrotropin-releasing hormone degrading ectoenzyme activity in the tanycytes of the median eminence of the rat hypothalamus

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Fasting produces a rapid reduction of the activity of the central arm of the hypothalamic–pituitary–thyroid (HPT) axis, with a drop in biosynthesis of thyrotropin releasing hormone (TRH) in periventricular and medial parvocellular neurons of the paraventricular nucleus (PVN), and decreased TRH release into the pituitary portal capillaries. These events contribute to fasting-induced suppression of serum thyroid hormone levels, an adaptive mechanism to conserve energy. Pyroglutamyl peptidase II (PPII), the TRH degrading ectoenzyme, is expressed by the β2 tanycytes, in close apposition with TRH nerve endings in the external layer of the median eminence, and controls TRH levels in the extracellular space and serum TSH levels. To test the hypothesis that tanycyte PPII activity is regulated during fasting, we measured PPII expression and activity in the median eminence of 250 g BW male Wistar rats. Rats were transferred into individual cages and submitted to fasting for 24–72 h, with water ad libitum. Control animals were submitted to isolation for the same time than food-deprived animals. Animals were sacrificed at various time points after fasting initiation, but always 3–5 h after lights were on. Compared to isolation, fasting reduced animal weight, PVN TRH mRNA levels, and concentration of total $T_4$ (but not $T_3$) in serum; a delayed drop in serum TSH concentration was observed between 48–60 h. Semi-quantitative *in situ* hybridization indicated that PPII mRNA levels increased in β2 tanycytes 48 h after fasting initiation. This increase was transitory (not detected at 72 h), and followed by an increase of PPII activity in the median eminence, with a peak response between 60 and 72 h. We conclude that during fasting a delayed increase in median eminence PPII activity may facilitate the maintenance of the profound reduction of HPT axis activity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1131

### Estradiol effects on hypothalamic stat3 phosphorylation induced by leptin

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Besides its critical functions as a reproductive hormone, 17β-estradiol ($E_2$), plays an important role in the control of energy homeostasis. In experimental animals, reduction of circulating estrogen levels by bilateral ovariectomy leads to the development of obesity, which can be reversed or prevented by $E_2$ treatment. $E_2$ effects on energy balance resemble many similarities to the actions of leptin, key molecule involved in the control of energy homeostasis. In this study, to assess the effects of $E_2$ in the activation of STAT3 by leptin in the hypothalamus, we used ovariectomized Wistar rats treated with $E_2$ (OVX +E) or corn oil (OVX), during eight consecutive days. At the eighth day of treatment, they received i.c.v injection (ICV) of leptin or vehicle. We evaluated the phosphorylation of STAT3 protein in the mediobasal hypothalamus (MBH) by Western Blotting or in the hypothalamic nucleus retrochiasmatic (RCA), arcuate (ARC), paraventricular (PVN) and ventromedial (VMN) by the quantification of p- STAT3 positive neurons by immunochemistry. Leptin induced a significant increase in p-STAT3 expression in the MBH, with no difference between OVX e OVX +E groups. Similarly, compared with respective saline group, leptin increased the number of p-STAT3 positive neurons in the RCA, ARC, PVN and VMN of the groups OVX and OVX +E, with no difference between both groups. Interestingly, we observed a higher number of p-STAT3 positive neurons in the VMN of OVX +E rats that received vehicle ICV, compared to OVX rats. These findings suggest that $E_2$ could act directly in the VMN, activating the STAT3-mediated signaling and this $E_2$ effect can facilitate leptin actions to reduce body weight and food intake.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1132

### Evaluation of cushings disease remission and serum cortisol dynamic early after transsphenoidal surgery

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Aim

serum cortisol dynamic after transsphenoidal pituitary surgery (TSS) in predicting remission and recurrence of CD.

Methods

A cohort of 103 CD patients from a referral center was prospectively analyzed at 111 TSS in 6.0±4.8 years of follow-up. Twenty patients received glucocorticoids in transoperative and had serum cortisol measured at 10–12 days after TSS (Protocol I). Eighty six patients had serum cortisol measured at each 6 h in the first 24 h, at 48 h and 10–12 days after TSS and glucocorticoid administration only if adrenal insufficiency (Protocol II).Remission (defined as clinical hypercortisolism absence plus serum cortisol $< 3$ μg/dl in overnight dexamethasone test and/or normal free urinary cortisol) during follow-up and recurrence (loss of remission criteria at least a year after TSS.

Results

Eighty percentage of remission after first TSS, and 8% of the patients had recurrence Serum cortisol nadir $\leq 3.5$ μg/dl at 48 h after TSS had specificity of 100% and sensitivity of 73%, and serum cortisol nadir $\leq 5.7$ μg/dl at 10–12 days after surgery had specificity of 100% and sensitivity of 92% in predicting surgery remission.

Conclusion

Recurrence could not be predicted. These results state the importance of serum cortisol values after TSS in predicting CD remission.

Relative risk for postoperative cortisol as Cushing's disease predictor, in different adjusted models.

**Table 1**

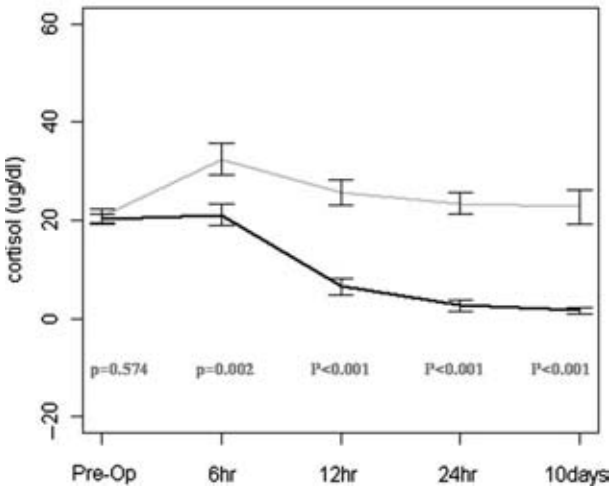
| | RR | CI 95% | P |
|---|---|---|---|
| Model 1 Nadir 48 h PO | 0.90 | 0.84–0.98 | 0.0016 |
| Model 2 Nadir 48 h PO | 0.89 | 0.83–0.96 | 0.003 |
| Model 3 Nadir 48 h PO | 0.90 | 0.83–0.97 | 0.009 |
| Model 4 Nadir 48 h PO | 0.86 | 0.76–0.96 | 0.011 |
| Model 5 Nadir 48 h PO | 0.90 | 0.83–0.96 | 0.004 |
| Model 1 Nadir 10–12 days PO | 0.91 | 0.83–1.0 | 0.063 |
| Model 2 Nadir 10–12 days PO | 0.88 | 0.80–0.96 | 0.005 |
| Model 3 Nadir 10–12 days PO | 0.89 | 0.81–0.97 | 0.008 |
| Model 4 Nadir 10-12 days PO | 0.77 | 0.58–1.0 | 0.069 |
| Model 5 Nadir 10–12 days PO | 0.88 | 0.81–0.96 | 0.004 |

Model 1, adjustment for microadenoma or macroadenoma in pituitary image; model 2, adjustment for normal pituitary image; model 3, adjustment for presence of adenoma at histopathology; model 4, adjustment for presence of adrenocortotrophic hormone at immunohistochemistry; model 5, adjustment for presence of hypopituitarism after transsphenoidal surgery.

The curve of the postoperative serum cortisol for the remission and failure group after transsphenoidal pituitary surgery.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.



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## P1133

### Hyponatremia in hospitalised patients: are we investigating and managing them properly?

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Introduction
Hyponatremia is common in hospitalised patients and can be due to a number of aetiologies. Investigations and management are often quite varied.
Aims
To find out the prevalence, aetiology, investigations and management of hyponatremia in our hospitalised patients over a 2 week period (1 May and 15 May 2011).
Methods
Case notes and electronic data assessment of all patients admitted with sodium level $<$130 mmol/l.
Results
One hundred and seventy patients had hyponatremia (hospital range 136–143 mmol/l). Complete data was available in 27/46 patients (15 males, 12 females, average age 65 years) with sodium $<$one hundred and thirty mmol/l. Mean sodium was 127 mmol/l. 6 (4%) had sodium $<$ 125 mmol/l. Hyponatremia was mentioned as a diagnosis in 7(26%) and SIADH specifically in only 2 (7%). Hydration status recorded were as follows: euvolemic–seven (26%), hypovolemic–five (18%), hypervolemic–six (22%), not mentioned–nine (33%). Only 2 patients had proper investigations for SIADH. Speciality ward allocation were Acute Medicine - 8, Cardiology - 3, Gastroenterology - 2, Endocrinology - 2, Care of Elderly - 1, Respiratory - 1, Orthopaedics - 3, Surgery (various) - 3, Obstetrics - 2, Gynaecology oncology - 1. Working diagnosis on admission were reported as: Hypovolemia - 6 (22%), Hypervolemia - 1, SIADH (cancer lung and ovary) - 2, Sepsis - 7 (26%). 6 (22%) patients received fluid restriction, IV fluid - 1, Demeclocycline - 1, diuretics withheld - 1, IV antibiotics - 3, ITU admission - 3. 5 (18%) patients died and 8 (30%) were discharged with Sodium level $<$ 130 mmol/l. Average Length of stay was 15.6 days (range 1–51 days).
Conclusions
Hyponatremia is poorly understood & recorded, frequently overlooked and inadequately investigated and managed in hospitalised patients. A variety of specialities deal with hyponatremia. Endocrinologists are rarely consulted to investigate and rule out SIADH - which can often be challenging. Plan is to increase awareness among clinical staff and develop pathways to enable correct investigations and management and re-audit the whole process.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1134

### Epidemiology, clinical behaviour and prognostic factors of gastro-enteropancreatic neuroendocrine tumours in Castilla-La Mancha (Spain) from 1995 to 2010

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Neuroendocrine gastroenteropancreatic tumours (GEP–NETs) are considered rare malignancies that are characterized by their ability to produce a great variety of peptides that may cause characteristic hormonal syndromes. In this study, we examined the epidemiology, clinical behaviour and prognostic factors of GEP–NETs identified in Castilla La Mancha (Spain) from 1995–2010.

Patients and methods
Two hundred and sixteen patients with GEP–NETs were analyzed in retrospect. Data regarding demographic characteristics, functional syndrome, diagnostic procedures,localization of the primary tumour and stage at diagnosis, therapeutic interventions and survival were collected.
Results
The estimated prevalence was 11.9 cases/1,00,000. 47.7% men, mean age 56.9 $\pm$ 18 years, 13% hypersecretion symptoms, 4.6% associated with MEN. No significant differences according to gender were observed. Incidental diagnostic in 47.6% of cases. Computed tomography (69.9%), ultrasound (47.7%) and octreotide scintigraphy (39.8%) were the most commonly imaging studies carried out. Chromogranin A and urinary 5-hydroxyindole acetic acid were done in 41% and 14.3% and were increased in 79.8% and 28.6% of tested patients respectively. Tumour characteristics are expressed in Table 1. Two-thirds of the patients underwent surgery. Local-regional therapies (chemoembolization, radiofrequency or other ablative techniques) were uncommon ($<$3%). 35.7% received systemic therapy (18.5% somatostatin analogues, 2.3% interferon, 13% chemotherapy).The median overall survival was 12 years, with 72.9% alive at 5 years (95% CI 65.5%–80.32%) and was significantly greater in younger patients, in patients with hormonal syndrome, early stage and lower grade. Prognosis differed according to tumour type and primary tumour site. Multivariate analysis confirmed Ki-67 index as the only independent prognostic factor for survival.
Conclusions
This study provided comprehensive information on the prevalence, management and outcome of this type of tumours from a region of Spain.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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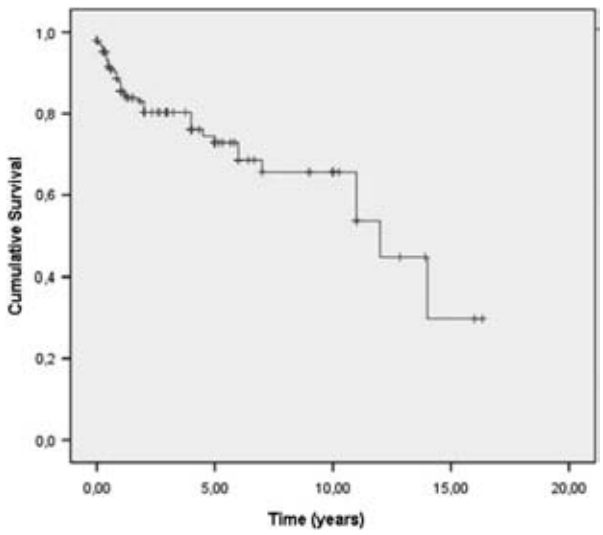
**Table 1** Tumour characteristics

| | N=216 | % |
|---|---|---|
| Tumour types: Carcinoid Pancreatic non functional NETs Metastasis of unknown primary Insulinoma Gastrinoma Glucagonoma Vipoma | 150 34 8 12 9 1 2 | 69,4 15,7 3,7 5,6 4,2 0,5 0,9 |
| Primary Tumour site: Gastrointestinal tract Pancreas Unknown | 155 52 8 | 72 24 4 |
| Stage at diagnosis: Local Regional Distant Unknown | 129 9 50 28 | 59,6 4,2 23,1 13 |
| Localization of metastasis: Liver Lymph nodes Peritoneum Lung Bone CNS Multiple | 35 19 4 2 1 1 5 | 63,6 15,8 7 3,5 1,8 1,8 8,8 |

CNS: Central nervous system



## P1135

**Effect of galanin on aromataze gene expression and estradiol 17-β concentrations in ventromedial and lateral hypothalamic area**

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The goal of this study was to determine whether galanin increase aromataze gene expressions and estradiol 17-β concentrations in ventromedial (VMH) and lateral hypothalamic area (LH). Forty rats were randomly divided into four groups. Animals in different groups were received either saline, 1, 2 or 4 ng galanin into their VMH and LH. After infusion of galanin, VMH and LH tissue were separated and homogenized. Homogenized sample were assayed for aromataze gene expression by PCR technique and estradiol 17-β ($E_2$) concentrations by radio immunoassay techniques. Infusion of 4 ng galanin into VMH and LH area significantly increased the mean aromataze gene expressions and $E_2$ concentrations in the VMH and LH of the rats. Infusion of 1 and 2 ng galanin into VMH and LH area did not change the mean aromataze gene expressions and $E_2$ concentrations in the VMH and LH of the rats.The result of this study indicated that galanin may depolarize neurosteroids of VMH and LH and consequently increase aromataze gene expressions and $E_2$ concentrations in the brain of the rats.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1136

**Leptin resistance and desensitization of hypophagia during prolonged inflammatory challenge are associated with high ptp1b expression in the hypothalamus**

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Repeated exposure to lipopolysaccharide (LPS) leads to endotoxin tolerance. Desensitization of hypophagia in response to repeated exposure to endotoxin is related to an inability of leptin to phosphorylate STAT-3 protein. Protein tyrosine phosphatase 1B (PTP1B) activity is known to contribute to leptin resistance by inhibiting leptin signaling. In this study, we induced endotoxin tolerance, injecting repeated LPS doses (6LPS) (100 μg/kg, i.p.) in comparison with single (1LPS) treatment and control group treated with saline. Food intake, body weight and plasma leptin levels were determined after lps or saline injection. Brain tissue was collected from another set of rats for determination of PTP1B expression in the mediobasal hypothalamus by western blotting. A third set of rats was pre-treated with PTP1B inhibitor (3 nM/rat in 5rl, i.c.v.v) for determination of food intake and body weight after LPS or saline injection. 1LPS group, but not 6LPS group, showed decreased food intake and body weight, associated with an increased plasma leptin concentration. 6LPS, but not single LPS rats, showed higher expression of PTP1B in the mediobasal hypothalamus. Hypophagia and body weight loss induced by 1LPS were potentiated by PTP1B inhibitor, when compared with vehicle treatment. Interestingly, pre-treatment with PTP1B inhibitor induced hypophagia in animals treated with 6LPS. The present data suggest that increased PTP1B activity contributes to the development of leptin resistance during tolerance to endotoxin. The present model of prolonged inflammatory challenge may contribute to further investigations on mechanisms of leptin resistance.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1137

**Obestatin does not modify weight and nutritional behavior but is associated to metabolic syndrome in old women**

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Introduction
Ghrelin gene products ghrelin and obestatin have apparent opposite orexigenic and anorexigenic effects, although this latter has not been firmly demonstrated for obestatin in humans. So far, no information has been reported in relation to its potential association to metabolic syndrome (MS).
Objective
To study obestatin concentrations in relation to nutritional parameters and eating behavior in old women.
Methods
One hundred and ten women (age 76.93 ± 6.32) from the Mataró Ageing Study were included. Individuals were phenotypically characterized by anthropometric variables, lipids, glucose, blood pressure, MS components (ATP-III criteria), anorexia assessed by a questionnaire and nutritional status by MNA-SF. A further reevaluation was performed at 2-years follow-up. Obestatin was measured by an IRMA.
Results
58.2% of the subjects had metabolic syndrome; at 2-y follow-up 24.1% had a weight loss > 5% and 7.2% > 10%, and 26.4% changed their MNA score to risk of malnutrition category. Anorexia was present in 38.4%. Obestatin levels were not related to either change of weight, MNA and anorexia, but a positive correlation was found with waist circumference (WC) ($P = 0.039$, rs = 0.200), to the absolute Δ between basal vs 2-y WC ($Ps < 0.001$; rs = 0.459), relative Δ between basal vs 2-y WC ($Ps < 0.001$; rs = 0.447); both absolute and relative WC Δ remained significant after adjusting for age and BMI. When obestatin was divided in quartiles, a significant lineal trend was observed in relation to WC ($P = 0.049$), absolute and relative Δ between basal vs 2-y WC (both $P < 0.01$). Glucose impairment was associated to obestatin (69.0% in 4th quartile had glucose impairment vs 44.4% in 1st; $P = 0.021$) and to MS by ATP-III (77.8% had MS in 4th quartile, 36% in 3rd, 60% in 2nd vs 57.7% in 1st; $P = 0.025$).
Conclusions
Obestatin is elevated in aged women bearing MS but is otherwise not associated to other nutritional parameters, weight loss or anorexia.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1138

**Hypopituitarism 3 and 12 months after TBI and SAH**

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Introduction
Recent studies have found hypopituitarism (HP) to be a common complication of traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH). In Iceland most patients with moderate TBI and all patients with severe TBI and SAH are transferred to Landspitali University Hospital (LSH). The aim of this study was to assess the prevalence of HP following TBI/SAH in Iceland, 3 and 12 months after the incidence.
Methods
All adult patients with moderate and severe TBI and SAH transmitted to LSH through one year were included, a total of 40 patients. Four patients died shortly after admission. Twenty-four patients accepted participation 3 months after TBI/SAH, 12 and 12 patients respectively. Twenty-five patients were screened 12 months after TBI/SAH, 14 and 11 patients respectively. Baseline hormone levels were measured and an insulin tolerance test (ITT) was performed. If ITT was contraindicated a Synachten test and a GHRH-arginine test were performed.
Results
Three months after TBI/SAH, 7 of the 24 patients (29.2%) had HP, 4 TBI patients (33.3%) and 3 SAH patients (25%). Two had (GHD), confirmed with GHRH-arginine test. One had elevated prolactin level. Two premenopausal women had oligomenorrhea. Three men had low testosterone and inappropiately low gonadotropin levels, one of them also had GHD. Twelve months after TBI/SAH, 6 of the 25 patients (24%) had HP, 5 TBI patients (35.7%) and 1 SAH patient. Three patients had GHD confirmed with GHRH-argine test (1 SAH patient). Two men had low testosterone and inappropiately low gonadotropin levels. One patient had mildly raised prolactin levels.
Conclusion
HP was confirmed in 29.2% and 24% of patients after 3 and 12 months respectively. Interestingly none of the GHD patients were diagnosed using the ITT. These results do emphasize the need for screening of pituitary function after TBI and SAH.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1139

### Pituitary incidentaloma: clinical presentation and endocrine evaluation in a Spanish population

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Introduction
The incidence of previously unrecognized lesions within the pituitary has been studied by MRI. The data suggest that up to 10% of people have clinically unsuspected pituitary tumors, the majority being $<10$ mm. Currently, little information is available about the prevalence of incidentally-discovered sellar masses.
Objective
To perform a clinical audit on pituitary incidentalomas in order to establish an appropriate clinical approach to them.
Patients and Methods
Twenty three patients were referred to our endocrine department between January 1990 and July 2011 for evaluation of incidentally-discovered pituitary lesions. All patients underwent a complete history, physical examination, laboratory evaluation for hormone hypersecretion and hypopituitarism and a formal visual field examination. Clinical presentation, type of imaging used and endocrine status are evaluated retrospectively.
Results
Twenty three patients (16 female) with a mean age of $41.3 \pm 19.2$ years were included. 91.3% of them were diagnosed through brain magnetic resonance imaging (MRI) and 8.7% through Computed Tomography (CT). The reasons for head CT and/or MRI are shown in Table 1. 16 patients were diagnosed with non-functioning pituitary adenomas (69.5%), two with microprolactinomas (8.6%), two with hypophysitis (8.6%), one with Cushing's Disease (4.3%) and one with craneopharingyoma (4.3%). Of 16 patients diagnosed with non-functioning pituitary adenoma (mean size $7.9 \pm 6.3$ mm, range 2–23 mm), 13 patients had a microadenoma without pituitary dysfunction. Of the three macroadenomas, 2 had hypogonadotrophic hypogonadism.
Conclusions
Despite the high prevalence of pituitary incidentalomas, the literature reporting on the natural history of this entity is scarce. In our study we found an important prevalence of endocrine abnormalities whose adequate diagnosis is essential.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Reasons which led to MRI and/or CT.

| | Total ($n=23$) |
|---|---|
| Headache | 6 (26.1%) |
| Stroke | 3 (13.0%) |
| Obesity | 2 (8.7%) |
| Amenorrhoea | 2 (8.7%) |
| Infertility | 2 (8.7%) |
| Head injury | 1 (4.35%) |
| Basal cell cancer | 1 (4.35%) |
| Other brain diseases | 3 (13.0%) |
| Unknown | 3 (13.0%) |

## P1140

### Comparison of the effect of chronic restraint and crowding on hypothalamic-pituitary-adrenocortical response to acute restraint stress

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Stressful events affect numerous physiological processes including central nervous system and endocrine responses. Repeated stress alters basic mechanisms for the maintenance of hypothalamic-pituitary-adrenocortical (HPA) homeostasis. The purpose of the present study was to determine whether, and to what extent prior repeated restraint or social crowding stress during 3 days affects the HPA response to an acute short-lasting restraint stress. Male Wistar rats were restrained 2 times a day for 3 days or subjected to crowding stress for 3 days before exposure to acute restraint stress in metal tubes for 10 min. Immediately after the restraint or 1 h, 2 and 3 h later the rats were decapitated and their trunk blood was collected for the measurement of plasma ACTH and corticosterone levels. Chronic stress alone (restraint 2 x 10 min/day for 3 days and crowding for 3 days) induced a moderate increase in plasma ACTH level compared to the corresponding level in control rats without prior stress exposure. Acute restraint stress for 10 min considerably increased plasma corticosterone and ACTH levels immediately after restraint and no substantial increase was observed 1, 2 or 3 h after stressor termination. Restraint and crowding for 3 days significantly and to a similar extent inhibited the restraint stress-induced increase in ACTH and corticosterone secretion. These results indicate that repeated restraint or crowding stress potently attenuates the acute restraint stress-induced stimulatory action of the HPA axis what suggests adaptive action of moderate stress on the HPA axis response to acute stress. The results also suggest that a short-lasting hypersecretion of corticosterone during psychological stress may induce a prolonged feedback inhibition of the HPA axis activity.
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## P1141

### Circulating interleukin 6 and soluble forms of interleukin 6 receptor and glycoprotein 130 in women with anorexia nervosa

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Introduction
Anorexia nervosa (AN) is an eating disorder, resulting in sustained low weight and marked decrease in fat mass. Interleukin 6 (IL-6) may play a role in appetite, energy expenditure and body weight control in central nervous system. IL-6 acts through binding with membrane receptor (IL-6R) and activates glycoprotein 130 (gp130) signaling. Both IL-6R and gp130 are present in the blood in the soluble forms (sIL-6R and sgp130, respectively). sIL-6R sensitizes cells towards IL-6, whereas sgp130 inhibits gp130 signaling. The aim of the present study was to estimate circulating IL-6/sIL-6R/sgp130 system and its relationships with body weight and resting energy expenditure (REE) in AN women.
Methods
We examined 19 women with AN and 27 healthy normal weight female controls. Euglycemic hyperinsulinemic clamp, indirect calorimetry and the measurement of serum IL-6, sIL-6R and sgp130 concentrations were performed in all the subjects.
Results
We did not observe differences in insulin sensitivity between AN and control subjects. REE was decreased in AN women ($P<0.001$). Serum IL-6 was higher in AN women in comparison with control group ($P=0.04$). Serum sIL-6R was lower ($P=0.009$) and serum sgp130 was higher ($P=0.004$) in AN women in comparison with controls. In the entire study population, BMI and REE were inversely related to IL-6 ($r=-0.45$, $P=0.02$ and $r=-0.62$, $P<0.001$, respectively) and positively to sIL-6R ($r=0.36$, $P=0.015$ and $r=0.42$, $P=0.013$, respectively). Correlation between IL-6 and REE was also present in AN group ($r=-0.55$, $P=0.013$).
Conclusions
Increased IL-6 in AN seems to be compensated by the changes in sIL-6R and sgp130 which are directed towards inhibition of IL-6 action. The balance between these factors might play a role in the regulation of energy expenditure in AN.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1142

### Ghrelin and growth hormone levels are decreased in Saudi autistic children

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Introduction
Autism is a neurodevelopmental disorder with a pathogenesis that is not completely understood. Although a genetic origin has been recognized, a potential role for environmental factors, immune dysfunctions, and variations of hormonal levels was suggested. Ghrelin is a peptide hormone which can stimulate growth hormone (GH) release by activating GH secretagogue receptor. In addition, ghrelin exerts neuroprotective effects, inhibits apoptosis in hypothalamic neurons and has positive effects on learning and memory.
Design
A case-control study was employed to investigate acylated ghrelin (AG), des-acyl ghrelin (DAG), leptin and growth hormone (GH) levels in Saudi autistic children.
Methods
We investigated AG, DAG, leptin and GH in 31 Saudi autistic male patients in comparison to those levels in 28 healthy age-matched children. Hormones were measured in blood samples using commercially available ELISA kits. Independent t-test was used to investigate statistical significance in study groups with a (p) value of less than 0.05 was considered significant.
Results
In autistic group AG, DAG and GH levels were significantly lower compared to the control group by 31.6, 28.4, and 47.9%, respectively. Leptin concentration was significantly higher by 106.2% in autistic children. While leptin levels showed positive correlation ($r = +0.57$) with body mass index (BMI) in autistic group, DAG levels had no significant correlation with BMI in the same group.
Conclusion
Our results demonstrate a novel decrease of AG, DAG and GH levels in autistic children in comparison with age-matched control group. Considering ghrelin's capacity to affect neuroinflammatory and apoptotic processes that were shown to be linked to autism, this study indicates a potential role for ghrelin in the pathogenesis of autism and further studies are needed to elucidate the role of ghrelin as a diagnostic biomarker and a potential therapeutic agent in autism.
Declaration of interest
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## P1143

### Basal and stimulated GH secretion in active cushing's syndrome and 2 years after remission

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Impaired GH secretion occurs in Cushing's syndrome (CS), reflecting hypothalamic and pituitary alterations, without IGF-I impairment. Obesity is known to blunt GH release, leading to BMI-related tests for GHD. Evaluation of GH in CS after remission is difficult, due to chronic previous hypercortisolism and persistent overweight. Aim of this study was to evaluate in 22 patients with Cushing's syndrome (16 Cushing disease, CD, 6 adrenal adenomas, CS) GH response to GHRH + ARG during active disease and 2 years after surgical-induced remission, according to BMI-related cut-off levels. Basal IGF-I and pituitary function were also assayed. Active disease: 10 patients were obese (45%), 8 overweight (36%), 4 normal (18%). A severe GHD was shown in 12 patients (54%) (8 CD and 4 CS, 3 obese, 6 overweight and 3 normal). IGF-I were in the normal range. 8 CD showed 1 or multiple pituitary deficiencies.
Remission
5 patients persisted obese (22,7%), 7 overweight (31%) and 10 became normal (45%). A severe GHD was shown in 8 patients (36%, CD only, 2 obese, 3 overweight, and 3 normal). 13 CD showed 1 or more pituitary deficiencies. No CS patient showed pituitary deficiencies. GHRH + ARG-induced GH levels were independent of either age/BMI. No correlation between GH peaks and HPA parameters were found, while IGF-I levels were positively correlated ($P < 0.01$) with UFC during active disease and after surgery.
In conclusion
(1) CS shows an impaired GH release even according to BMI-related cut-off levels, together with a dissociation between GH and IGF-I secretion, due to a stimulatory cortisol action on the liver; (2) chronic hypercortisolism seems to override the influence of age and BMI on GH/IGF-I activity; (3) glucocorticoid-induced GHD is no longer evident 2 years after remission, thus suggesting that this could be a good time for the evaluation of GHD in patients suspected for surgery-induced hypopituitarism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1144

### Overly rapid correction of severe hyponatremia with vasopressin receptor antagonists poses a risk of inducing osmotic demyelination syndrome

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Rapid correction of chronic hyponatremia can cause osmotic demyelination syndrome(ODS). Recent days, vasopressin receptor antagonists(VAs) are clinically used for the treatment of congestive heart failure or hyponatremia including SIADH. To date, few cases of ODS caused by VAs have reported; however, it is presumed that the treatment with VAs for severe hyponatremia may be associated with an increased risk of ODS. Therefore, in this study, we investigated whether rapid correction of hyponatremia with tolvaptan has a risk of inducing ODS in SIADH model rats. Hyponatremia was induced by liquid diet feeding and subcutaneous desmopressin infusion using osmotic pumps. Seven days later, hyponatremia(approximately 105 mEq/l) was corrected by the following ways; i) bolus administration of hypertonic saline (HS group), ii) oral administration of tolvaptan 1 mg/kg (T1 group), iii)5 mg/kg (T5 group), iv)10 mg/kg (T10 group), or v)removal of osmotic pumps (PO group). The serum sodium levels were increased approximately by 30 mEq/l in 24 h in all groups and there was no significant difference in the degree of sodium elevation. Accordingly, most rats in all groups showed serious neurological impairment. Five days after the correction, the incidence of neurological findings including gait abnormality, paralysis, seizure, or death was respectively 75%, 50%, 66%, 66%, and 88% in HS, T1, T5, T10, and PO group, respectively. Immunohistochemical analysis confirmed the demyelination and the accumulation of microglia in the cerebral cortex and the neighborhood of the red nucleus in the midbrain in all groups. These results suggest that overly rapid correction of severe hyponatremia with a relatively high dose of VAs associated with the blockage of vasopressin action as well as hypertonic saline poses a risk of inducing ODS and that clinicians using VAs to treat hyponatremia need to monitor serum sodium carefully to avoid overcorrection.
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## P1145

### Relationship between leptin, markers of bone turnover, bone mineral density, and body weight in patients with schizophrenia treated with long acting risperidone (LAIR)

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Introduction
Leptin, adipose-tissue-derived hormone has a prominent role in bone remodeling by activating, through CNS relay, the sympathetic tone which inhibits bone formation. Schizophrenia is a CNS disease associated with low bone mass and changes in bone remodeling. Risperidone, atypical antipsychotic, may be related to increase in body weight (BW). Increased BW is thought to be protective to the bone.
Aim
To determine relations between leptin, bone remodeling parameters (osteocalcin and C-terminal telopeptide of type I collagen-CTx), bone mineral density (BMD-

lumbar spine et femoral neck) and BW in patients with schizophrenia treated with LAIR for at least 6 months up to 3 years.
Patients
Cross-sectional study, we investigated 26 out-patients(12 males, age 31.3±1.3 years, BMI 28.2±1.0 kg/m2) with stable schizophrenia in real-life conditions. They were treated with LAIR for mean 18.0±1.6 months with mean dose 38±2 mg/two weeks. Thirty-five matched subjects (11 males) served as healthy controls.
Results
The increase in BW was confirmed in 14(54%) patients treated with LAIR on average 8.7±1.6 kg. Leptin levels adjusted for BMI in females were significantly higher in patients than in healthy female subjects ($P=0.018$). Significant positive correlations between leptin and BW was found in patients and healthy controls. BMD tended to be lower but did not reach significance in patients with schizophrenia ($p=0.094$). CTx, was slightly increased in patients on LAIR ($P=0.023$). Significant negative correlation between leptin and osteocalcin ($r=-0.376$, $P=0.050$) was found only in patients with schizophrenia. Leptin did not correlate with BMD in schizophrenic patients while it did in healthy subjects (femoral neck $r=0.366$, $P=0.031$).
Conclusion
Higher serum leptin levels in female patients after adjusting for BMI may suggest leptin resistance. Leptin has been shown to inhibit bone formation so it is tempting to speculate that weight gain together with leptin resistance during long term therapy with LAIR may be protective to the bone in patients with schizophrenia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1146

**Estrogen receptor alpha PvuII polymorphism in female acute stroke patients: no associations with disease severity and short-term prognosis**

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Introduction
Several studies have examined the association of the common PvuII polymorphism of the estrogen receptor alpha (ERα) gene with the risk of stroke. Data linking the polymorphism with the severity and outcome of cerebrovascular disease is lacking. We looked for possible associations of the PvuII polymorphism with stroke severity and short-term prognosis in a cohort of female stroke patients.
Patients-Methods
We studied 302 postmenopausal Caucasian female patients suffering an acute stroke, hospitalized in two tertiary hospitals over a period of two years. In all patients, a detailed medical history and recording of classical stroke risk factors was performed. The neurological severity on admission was assessed using the National Institutes of Health Stroke Scale and the one month functional outcome using the modified Rankin Scale. ERα polymorphism at position c.454–397 T>C (PvuII) was genotyped. Circulating androgen and estradiol levels were measured in all patients, the latter using a high sensitivity assay.
Results
The prevalence of CC genotype was 21%, CT 50% and TT 29%. There was no difference in the neurological severity on admission or in the short-term functional outcome and mortality between the three genotype groups. No association was found between the PvuII polymorphism and classical stroke risk factors. Estradiol levels were higher with increasing frequencies of the C allele ($P$ 0.04). An association of the CC genotype with venous thromboembolism history was recorded ($P$ 0.05).
Conclusions
There was no association between the PvuII polymorphism and stroke severity and short-term outcome in the studied female stroke population. One cannot exclude the possibility that the long-term estrogenic action reflected by the genetic polymorphism may be attenuated in older age.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1147

**The incidence of acromegaly in iceland from 1955 through 2010**

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Objective
Acromegaly is a rare disease with multiple serious comorbidities. The aim of this study was to gather information on patients diagnosed with Acromegaly from 1955 through 2010.
Materials and methods
Information on patients with pituitary adenomas diagnosed from 1950 has aldready been gathered and and imported to patient journals at the National University Hospital in Iceland. In this retrospective study information on patients diagnosed with Acromegaly in the years 1955–2010 was gathered from these journals as well as from files at private clinics where some patients recieved follow-up care. All practising endocrinologists in Iceland were contacted to make sure all known cases were included.
Results
Forty-six ($n=46$, 19 women, 27 men) were diagnosed with Acromegaly during the study period. During the years 1955–1964: 2 patients were diagnosed; 1965 – 1974: 3 patients; 1975–1984: 7 patients, 1985–1994: 11 patients, 1995–2004: 7 patients and in the last 7 years, from 2005–2010: 16 patients. The average age at diagnosis was 43.11 years. Seven patients have died. Symptoms had been present for more than three years in most cases but in three cases for at least 15 years. The most common symptoms at presentation were enlargement of hands and feet as well as changes in facial features. At diagnosis, 21 patients had hypertension.
Conclusion
We found the incidence for Acromegaly to be much higher than earlier reported. During the last 6 years of the study 8.6 patients were diagnosed per million per year which is more than two times higher than what should be expected according to earlier reports (3–4 cases per million per year). Interestingly almost 46% of the patient population had hypertension at diagnosis indicating the importance of endocrine disorders in the aetiology of hypertension.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1148

**The incidence of hypoglycaemia in non-diabetic hospital patients outside critical care estimated by a capture-recapture technique: retrospective analysis**

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Introduction
Unexplained or a cluster of non diabetic hypoglycaemia has at times been the only clue to malicious use of Insulin in hospitals. We wished to establish the incidence of hypoglycaemia in non-diabetic adult patients outside the intensive care unit (ICU) in a 1200-bed university hospital.
Methods
We retrospectively analysed data for 2010 from three distinct sources to identify patients: bedside and laboratory blood glucose measurements; medication records for those treatments (high-strength glucose solution and glucagon) commonly given to reverse hypoglycaemia; and diagnostic codes for hypoglycaemia. We excluded from the denominator admissions of patients with a diagnosis of diabetes or prescribed diabetic medication. The denominator included patients admitted to ICU, as they almost always have a period of stay outside ICU (susceptible population).Case notes of all patients identified were reviewed to ascertain hypoglycaemia and where it occurred. We used capture-recapture methods based on log linear models to establish the likely true rate of hypoglycaemia in non-diabetic in-patients outside ICU. Analysis was carried out at different cut-off points for hypoglycaemia. We also recorded co-morbidities that might have given rise to hypoglycaemia.
Results
Among the 37 898 admissions we identified a total of 71 admissions of non-diabetic patients with hypoglycaemia at or below 3.3 mmol/l and 37 admissions at or below 2.7 mmol/l. Using capture-recapture methods the estimated incidence was 50 (95% CI 33–93) and 13 (95% CI 11–19) per 10 000 admissions at

3.3 mmol/l and 2.7 mmol/l cut-off values. Admissions of patients aged above 65 years were 50% more likely to have an episode of hypoglycaemia. Commonest relevant co-morbidities linked to hypoglycaemia were sepsis, renal disease, alcohol dependence, pneumonia, liver disease, cancer and self harm with hypoglycaemic agents.
Conclusion
Hypoglycaemia is rare in non-diabetic patients, and usually associated with important co-morbidity. Our study suggests a way to monitor an unusual increase in hypoglycaemia in hospital.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1149

### An initial dose of 7.5 mg Tolvaptan is safe and effective in the treatment of hyponatremia caused by SIADH

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Tolvaptan (TV), an ADH V2-receptor blocker, is useful in treating SIADH-induced hyponatremia. Maximum aquaresis following 15 mg TV occurs on day 1, when excess body water is greatest. This can cause a sharp rise in natremia, and poses a risk of overcorrection. Our aim was to evaluate an initial dose of 7.5 mg.
Methods
We studied 7 hospitalized patients with SIADH (4 females), median age 80 (57–95), with nadir natremias ($Na_p$) ranging from 111 to 126 mmol/l, $Na_u$ 39–92 mmol/l, $Osmolality_p$ 224–257 mOsmol/kg, $Osmolality_u$ 152–462 mOsmol/kg. Patients were euvolemic, with normal adrenal, thyroid, and renal function. 5 patients with initial $Na_p < 120$ mmol/l were first treated with 3% hypertonic saline and/or furosemide and oral salt. All 7 had presented an inadequate response to water restriction, or a Furst formula predicting non-response. TV dose was 7.5 mg (day 1), and was increased to 15 mg on day 2 (patients 2-7) or on day 3 (patient 1).
Results
$Na_p$ increments are all calculated from baseline. Day 1: Median baseline $Na_p$: 126 mmol/l (121–133). After 6 hours median $Na_p$ variation was 2 mmol/l (0–4), with median $Na_p$ 128 mmol/l (122–135). Day 2: median $Na_p$ increase was 5 mmol/l (1–10), median $Na_p$ 134 mmol/l (125–136). Day 3: patients 2–7: Median $Na_p$ rise was 7 mmol/l (5–12), Median $Na_p$ 134 mmol/l (128–138). Patient 1's $Na_p$ had risen 6 mmol/l on day 3 and 13 mmol/l on day 4. 12 h $Na_p$ was determined in the first 3 patients, descending 1 mmol in 2/3 and rising 2 mmol in a 3rd vs 6-h natremia. Maximum 24-h and 48-h $Na_p$ increments were 10 and 12 mmol/l respectively. Side Effects: one woman experienced intense thirst.
Conclusion
In our patients, starting tolvaptan at a dose of 7.5 mg was both safe and effective.
Evolution of natremia following initiation of tolvaptan therapy

**Table 1**

| Patient | Nadir $Na_p$ - mmol/l | Baseline (Day 1) $Na_p$ - mmol/l | 6-hour $Na_p$ (increase from baseline) mmol/l | Day 2 $Na_p$ (increase from baseline) mmol/l | Day 3 $Na_p$ (increase from baseline) mmol/l |
|---|---|---|---|---|---|
| 1 age 84 female | 118 | 122 | 122 (0) | 127 (5) | 128 (6) |
| 2 age 66 female | 126 | 126 | 130 (4) | 130 (4) | 133 (7) |
| 3 age 94 male | 119 | 124 | 125 (1) | 134 (10) | 136 (12) |
| 4 age 75 male | 122 | 128 | 128 (0) | 134 (6) | 135 (7) |
| 5 age 80 female | 118 | 127 | 130 (3) | 136 (9) | 132 (5) |
| 6 age 52 female | 111 | 133 | 135 (2) | 134 (1) | 138 (5) |
| 7 age 85 male | 116 | 121 | 123 (2) | 125 (4) | 128 (7) |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1150

### Role of endocrine nurse in simultaneous bilateral inferior petrosal sinus sampling (BIPSS): single center experience

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Background
Simultaneous bilateral petrosal sinus sampling is valuable in differentiating the etiology of ACTH dependent Cushing's syndrome. It is used to locate the source of ACTH production when pituitary magnetic resonance imaging is negative.
Nurses' role
Specially trained endocrine nurses are part of the team for inferior petrosal sinus sampling. They prepare necessary material and documentation, mark all test tubes, handle the samples taken from both catheters record the time and localization of each sample, take samples from the peripheral vein, give iv bolus of CRH and keep track of time for sampling after CRH administration. Nurses are also responsible for adequate and timely transportation of samples for ACTH (on ice) and cortisol measurement to laboratory and proper material submission to the lab. They prepare patient for the procedure and monitor him/her through and after the procedure.
Aim
To analyze results of all bilateral inferior petrosal venous sampling studies performed in our center during period 2009/2010.
Patients and methods
In six patients with ACTH dependent Cushing's syndrome BIPSS was performed. Patients' records, catheter and laboratory protocols were analyzed retrospectively for the period 2009/2010.
Results
During 2009/2010 period 7 studies were performed in our (tertiary) center. In all 7 cases simultaneous bilateral petrosal venous sampling with CRH administration was performed. In two patients whole body venous sampling was performed disclosing the ectopic source of ACTH secretion in one patient. In two patients unilateral venous malformations were encountered and samples were taken from the inferior jugular vein. In one patient results were inconclusive and study was repeated. In 5 cases results of the catheter study confirmed the diagnosis of Cushing's disease.
Conclusion
Role of endocrine nurse in coordination with other members of the team is important for the success of catheter studies in patients with Cushing's syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1151

### PTHrP-induced hypercalcemia: a rare and challenging presentation in metastatic pancreatic neuroendocrine tumour

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Introduction
Hypercalcemia due to parathyroid-hormone-related-peptide (PTHrP) is rare in neuroendocrine tumours.
A 63-year-old lady initially presented to ENT surgeons with symptoms of reflux, dysphagia, and weight loss. Past medical history included hysterectomy eight years previously for uterine prolapse. There was no family history of note. Investigations revealed corrected calcium was elevated at 2.94 (2.1–2.5) mmol/l. ALP: 184 (30–135) U/l. CA19-9 was normal. PTH was suppressed <3 (14–72)

ng/l. PTHrP was elevated 4.1 (0–1.8) pmol/l.
CT scan demonstrated an 11×9 cm pancreatic mass arising from the tail of pancreas involving spleen, left kidney, left colon and para-aortic lymph nodes. There were multiple lesions in the liver. EUS guided FNA was performed revealing well differentiated neuroendocrine carcinoma of the pancreas. Subsequent biochemistry revealed chromogranin-A 154 (0–60) pmol/l, Chromogranin B 152 (0–150) pmol/l. Urinary 5-HIAA was normal.
The tumour was locally advanced and surgical resection was not possible. Patient was treated with somatostatin analogues for tumour stasis of NET. Hypercalcemia was treated with intravenous pamidronate but recurred rapidly and was >3 mmol/l 1 week after. Prednisolone 10–15 mg po od was added with no improvement in calcium concentration. Zolendronic acid was administered with a rapid and sustained normalisation of calcium to 2.25 mmol/l.
Conclusions
Hypercalcemia due to parathyroid hormone related-peptide (PTHrP) is rare in neuroendocrine tumours. Managing hypercalcemia was initially challenging however responded to zolendronic acid. Sunitinib is being considered as systemic therapy and it will be interesting to see if calcium concentration falls with use of this agent.
Declaration of interest
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## P1152

**Profile of neurosarcoidosis observed in internal medecine practice**

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Introduction
Neurological achievements of sarcoidosis or neurosarcoidosis 'NS' is rare (5%), but revealing of the disease in 50%. Neurological and endocrinous signs vary according to the topography of the damage. Cerebral forms results from the infiltration of the meningeal spaces, the granulomatous process spreads secondarily in the cerebral and spinal nerves, in the vessels and in the cerebral parenchyma. NS are individualized better with the contribution of the neuro-imaging. Precocity of the diagnosis improves the prognosis.
Objectives
To review through some case reports clinical polymorphism of the NS and the diagnostic and therapeutic difficulties observed.
Patients and methods
Retrospective Study over five consecutive years on January 2005-December 2010.
Results
In total, 11 inclusive patients, 9 Women and 2 Men, age means 37 2 years (24–56). Achievement is encephalic (5), medullar (2) and orbital (3). Its joins to a hypothalamic-pituitary localization (4), thoracic (5), cutaneous (4, liver (2), eyes (3) and is inaugural at three patient's. Revealing clinical pictures are intracranial hypertension 'ICH' (4), inflammatory tumour of the orbit (2), myelitis (2) and endocrine's signs as insipid diabetes or hypopituitarism (4). Treatment imposes hormonal substitution, immunosuppressive drugs (4) and peritoneal derivation (2). The course is fatal (1), conducts to cognitive disorders (2), to definitive endocrine insufficiency (3), sever osteoporosis (3), optic atrophy (1) and to meningeal infections (2).
Discussion
NS is polymorphous with hypothalamic-pituitary achievements, ICHT, pseudo-tumour of the orbit, etc. witch constitute circumstances of diagnostic wandering, and sometimes compromise the prognosis by their resistance of corticoadrenal therapy and the secondary infections caused by a lymphocytopenia and an adverse effects of therapy.
Conclusion
NS diagnosis is based (after exclusion any infectious or tumour process), on the identification of a neurological localization accessible to identify the sarcoidal granuloma. MRI is at present a decisive element for the diagnosis and the monitoring of the NS. Considering the functional and vital risks of this a corticotherapy must be prematurely prescribed.
Declaration of interest
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# Nuclear receptors and Signal transduction

## P1153

**Effects of estradiol on tight junction in the human breast cancer cell line MCF-7**

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Tight junctions (TJ) exist as macromolecular complexes comprised of several types of membrane proteins, cytoskeletal proteins and signaling molecules. Recently, it has been observed that some components are regulated during epithelial–mesenchymal transition in cancer cells; however, the effects of estradiol ($E_2$) have been poorly studied. This study sought to determine the role of $E_2$ on the expression and cell localization of the TJ-associated proteins Zonula Occluden-1 (ZO-1), occludin and the ZO-1-associated transcription factor (ZONAB), and in the activation of the epidermal growth factor receptor-2 (HER-2) and Rous sarcoma virus (SRC), in the human breast cancer cell line MCF-7. We demonstrated that $E_2$ increases ZO-1 and ZONAB mRNA and protein expression after 6 h of incubation, and HER-2 after 24 h. Since it is known that ZO-1 and ZONAB act as co-activator and transcription factor respectively, in the promoter of the HER-2 gene, immunolocalization assays (Western blot and confocal microscopy) were performed. After 30 min of incubation with $E_2$, maximal translocation of ZO-1 and ZONAB proteins to cell nucleus was observed. This effect was not precluded when an estrogen receptor (ER) antagonist was used; therefore, ZO-1 and ZONAB protein translocation can be associated to SRC protein, since active SRC increased significantly after $E_2$ incubation. We also demonstrated that $E_2$ decreased dramatically occluding levels in MCF-7 cells, effect that can be associated with an increase in paracellular permeability. We conclude that $E_2$ can induce genomic and non-genomic changes in TJ proteins that induce epithelial-mesenchymal transition and cell proliferation in breast cancer cells.
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## P1154

**Insufficiency of the chaperones GRP78 and GRP94 links TLR4 signaling to endoplasmic reticulum stress**

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TLR4 activation and the induction of endoplasmic reticulum stress (ERS) are two of the most important mechanisms connecting excessive dietary fat with insulin resistance. TLR4 activation is a primary event in the induction of cellular stress that contributes to increased inflammatory gene expression in metabolic diseases. However, the mechanisms linking these molecular events are unknown. The chaperones GRP78 and GRP94 play an important role during the assembly of newly translated TLR4 molecules. In addition, GRP94 escorts TLR4 to the cell membrane. Under prolonged activation the demand for newly synthesized TLR4 molecules increase, and thus, the demand for new chaperones. Therefore, we hypothesized that under increased activation of TLR4 the synthesis of the protein would not be matched by the expression of chaperones, thus, triggering ERS. To test this hypothesis, the monocyte cell line THP-1 was incubated with LPS and the expression/activation of proteins involved in ERS was determined. Time-course experiments revealed that LPS led to a 2.5-fold increase of TLR4 expression starting as early as 8 h, peaking after 24 h and remaining significantly increased after 48 h. The expression of GRP78 underwent a three-fold increase with a sharp rise at 24 h, while GRP94 increased by only 1.5-fold with a peak at 2 h and an early return to base-line levels. None of the chaperones were increased after 48 h. LPS-induced ERS was detected as early as 4 h after stimulus as detected by the evaluation of PERK/eIF2α, IRE1 and ATF6 pathways. Strong signals of ERS were still present after 48 h. The pre-incubation of THP-1 in glucose-deprived medium produced 2.5- and 11-fold increases of GRP94 and GRP78, respectively. Upon glucose deprivation, LPS could no longer induce ERS. Inhibition of chaperone expression by siRNA completely abrogated the effect of glucose deprivation to protect cells from LPS-induced ERS. Thus, insufficient LPS-induced chaperone expression links TLR4 signaling to ERS.

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## P1155

**Central diabetes insipidus associated with impaired renal aquaporin-1 expression in mice lacking the oxysterol receptor liver X receptor β**

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We have previously shown that in pancreatic ductal epithelial cells, the expression of the water channel aquaporin-1 is under the control of the oxysterol receptor liver X receptor β. The aim of the present study was to investigate water balance in mice lacking each LXR isoform.
Given free access to water, LXRβ−/− mice demonstrated an abnormal daily excretion of highly diluted urine, polyuria, with increased water intake compared to WT animals, polydipsia. After 24-h dehydration, LXRβ−/− mice were able to decrease the 24-h urine volume and to concentrate urine but the urine output was higher than that in dehydrated WT mice. Interestingly, in LXRα−/− there were no detectable defects in urine volume and concentration.
Polyuria and polydipsia are the clinical features of a condition called diabetes insipidus that according to the etiology may be classified in central and nephrogenic. The first one results from a defective production/secretion of the antidiuretic hormone arginine vasopressin while the nephrogenic one is due to a defective vasopressin action in the kidney.
To pinpoint the genesis of the LXRβ-diabetes insipidus, WT and LXRβ−/− mice were injected intraperitoneally with arginine vasopressin. There was no change in the urine volume after 3 and 6 h, but the urine osmolality was significantly increased at 6 h in LXRβ−/− mice indicating a partially conserved kidney capability to respond to vasopressin. Therefore the central vasopressin production was investigated. Surprisingly, in LXRβ−/− mice, there was an important loss of vasopressin-producing neurons both in the supraoptic and in paraventricular nuclei of the hypothaluamus. In addition, the vasopressin content of the 24-h urine was lower than that in WT mice, indicating a central genesis of their diabetes insipidus. Moreover, in addition to the central effects, there was a nephrogenic involvement since aquaporin-1 expression in the kidney was reduced in LXRβ−/− mice.
In conclusion this paper shows that LXRβ plays a crucial role in regulating water balance both centrally and peripherally and it can be suggested both as genetic predisposition in human central diabetes insipidus and as possible therapeutic target in disorders of water balance.
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## P1156

**PAK1-Nck interaction regulates prolactin-dependent cyclin D1 promoter activity**

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Introduction
Prolactin (PRL) is a hormone utilized at both endocrine and autocrine levels. PRL regulates cyclin D1 expression through JAK2-mediated transcriptional activation of cyclin D1 promoter. Serine–threonine kinase PAK1 is also implicated in regulation of cyclin D1 gene expression. We previously showed that PAK1 binds to and is Tyr phosphorylated by JAK2. Herein we linked PRL and PAK1 as JAK2 substrate to stimulation of cyclin D1 promoter activity.
Methods
Two-dimensional peptide mapping and LC–MS/MS mass spectrometry identified three Tyr(s) of PAK1 (153, 201 and 285) which are phosphorylated by JAK2. We measured induction of cyclin D1 promoter activity in T47D cells expressing cyclin D1 promoter-luciferase and either PAK1 WT or PAK1 Y3F (three tyrosines are mutated). We used confocal immunofluorescence and cell fractionation to study a role of PAK1 nuclear localization for cyclin D1 promoter regulation.
Results
PAK1 activates cyclin D1 promoter in response to PRL. Mutation of JAK2 phosphorylation sites on PAK1 decreases both PRL-induced PAK1 nuclear translocation and cyclin D1 promoter activity by 55%. Mutation of PAK1 nuclear-localization signals (NLS) decreases PRL-induced cyclin D1 promoter activity by 46%. A PAK1 Y3F mutant lacking NLSs decreases cyclin D1 activity by 68% suggesting that there is another PAK1-dependent mechanism to activate cyclin D1 promoter. We found that adapter protein Nck sequesters PAK1 in cytoplasm and co-expression of PAK1 and Nck inhibits amplifying effect of PRL-induced PAK1 on cyclin D1 promoter activity (95% inhibition). This inhibition is partially abolished by disruption of PAK1-Nck binding.
Conclusion
We propose two PAK1-dependent mechanisms to activate cyclin D1 promoter activity in response to PRL: via nuclear translocation of Tyr-phosphorylated PAK1 and via formation of Nck-PAK1 complex that sequesters PAK1 in cytoplasm.
Declaration of interest
I fully declare a conflict of interest. Details below.
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## P1157

**Estrogen priming is critical for effective progesterone receptor engagement with DNA in breast cancer cells: ChIP sequencing and microarray studies**

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One in 11 women develop breast cancer before the age of 75, and mortality is significant. Estrogens and progesterone (PROG) are mediators of breast cancer aetiology and progression, and are targets for both diagnostics and therapy. The actions of estrogens via estrogen receptor alpha (ERα) have been extensively studied, but the genomic actions of PROG via the progesterone receptor (PGR) are still not fully understood. This study investigates the genomic actions of PGR in breast cancer cells treated with PROG with or without 17-β estradiol ($E_2$).
Microarray expression analysis in breast cancer ZR-75-1 cells identified virtually no effect of PROG-$E_2$ co-treatment on global transcription. However, priming with $E_2$ markedly increased transcriptional response to subsequent PROG treatment. Analysis of PGR binding sites by ChIP-seq supports these findings, with PROG treatment alone generating $<10\%$ of the number of PGR binding sites following priming with $E_2$ (444 vs 4566 at equivalent peak threshold). Compared to PROG alone, the primed PGR cistrome was highly conserved amongst species, and enriched for 3-keto steroid receptor response elements and binding sites for the pioneer factor, FOXA1. Candidate ChIP confirmed these findings in 100% (12/12) of sites. Importantly, $E_2$ enhancement of PGR binding could be virtually eliminated by tamoxifen co-priming, but not by short-term tamoxifen co-treatment. In T-47D cells that have naturally high PGR levels, $E_2$ priming was not required for efficient PGR/DNA interaction.
Our data suggests that the effective engagement of PGR in genomic activity is dependent on cellular PGR levels, which is modulated by $E_2$ activity, and steroid receptor collaborations such as FOXA1. Further, $E_2$ and PROG collaboratively shape a distinctive transcriptional response in breast cancer cells. These findings suggest a need to reevaluate the meaning of ERα and PGR levels in breast cancer, and better define the context of PGR contribution to breast cancer.
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## P1158

**Hypoxia-induced estrogen receptor alpha activation is mediated by both the MAPK and PI3K phosphorylation pathways**

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The estrogen receptor (ER) plays an important role in breast cancer development and progression. Hypoxia was shown to modulate the level of ERα expression and induce ligand-independent transcriptional activation of ERα, which may be intimately associated with the biology of breast carcinomas. Given that phosphorylation affects the transcriptional activity and stabilization of ERα, we examined the changes in phosphorylation of ERα under hypoxic conditions. Hypoxia induced phosphorylation of ERα at serine residues 118 and 167 in the absence of estrogen. Phosphorylation-defective ERα mutants with serine-to-alanine replacements at residues 118 and 167 had impaired hypoxia-induced ERα activation, showing that serine residues 118 and 167 are involved in hypoxia-induced ERα activation. The hypoxia-induced ERα-mediated transcriptional response was dependent on both the ERK1/2 MAPK and PI3K pathways, but not the p38 pathway, as assessed using chemical inhibitors. Both the A/B and DEF domains of ERα were activated by hypoxia, indicating that both the N- and C-termini are involved in activation. These data show that ERα phosphorylation via both the MAPK and PI3K pathways is one mechanism leading to ERα activation under hypoxia.

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## P1159

**GH-STAT5 signaling pathway is down-regulated by Liver X Receptor agonists in liver**

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The Liver X Receptor (LXR) agonists have been shown to influence the development of hyperlipidemia and atherosclerosis in mouse models. It has also been demonstrated that some LXR agonists can cause hepatic steatosis in experimental animals. GH is also known to regulate hepatic metabolism and absence of the hepatic GH receptors leads to hepatic steatosis. In this study we analyzed whether actions of LXR agonists could involve interference with GH signaling. We showed that LXR agonist impairs GH signaling in hepatocytes. LXR agonist treatment attenuated GH induction of SOCS2, SOCS3, and CIS mRNA levels in BRL-4 cells. Likewise, the activity of the GH sensitive reporter vector was inhibited by a simultaneous treatment with LXR agonist. The inhibitory effect of LXR agonist on GH signals can be mimicked by overexpression of the LXR regulated factors SREBP1a and SREBP2 in hepatic cells. In both cases, total and phosphorylated STAT5b protein levels were significantly reduced. DNA binding assays demonstrated that SREBP1 binds to an E-box within a previously defined GH responsive element in the SOCS2 gene promoter but does not compete with STAT5b binding to a nearby site in the same response element. Taken together, our findings indicate that the inhibitory effects of LXR agonists on GH signaling are mediated by SREBP1, through the downregulation of STAT5b gene transcription and stimulation of STAT5b protein turnover. The described findings provide new insight in understanding the molecular actions of LXR agonists, which may be of relevance for their pharmacological actions.

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## P1160

**Post-translational modification of the thyroid hormone receptor interferes with its transcriptional activity**

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The thyroid hormone receptor (TR) belongs to a family of transcription factors which activity is modulated by the presence or absence of their particular ligand. Additionally, the transcriptional activity of TR is modulated by post-translational modifications such as phosphorylation, acetylation or ubiquitination. Here, I investigate whether TR might also be regulated by sumoylation. Sumoylation of transcription factors at defined lysine residues often leads to an alteration of their transcriptional activity.

Various fusion proteins of the Gal4 DNA binding domain (Gal4) fused to different fragments of TRα were investigated in reporter gene assays. These fusion constructs show a strong activation of reporter gene activity in the mammalian one-hybrid assay upon stimulation with $T_3$ (up to 100-fold induction rates). Several point mutations at suggested sumoylation sites were introduced into the Gal4-TR construct. These mutated constructs show (i) an increased basal reporter activity in the absence of $T_3$ and (ii) reduced fold induction rates upon stimulation with $T_3$. These data indicate that sumoylation defective point mutants of TR have defective repressor function. TR wild type and sumoylation deficient mutants were investigated on natural promoter sequences of both positively and negatively regulated $T_3$ target genes. These investigations confirmed an alteration of ligand-depending activation by some of the mutants. Chromatin immunoprecipitation assays revealed an increased concentration of the transcriptional corepressor NCoR in the presence of TR wild type compared to its sumoylation deficient mutant in the absence of the ligand $T_3$.

All these data suggest that post-translational modifications of TR are essential for appropriate transcriptional silencing by unliganded TR.

Declaration of interest

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## P1161

**Impact of receptor tyrosine kinase ErbB2/Her-2 and chemokine receptor CXCR4 crosstalk on estrogen receptor β activity**

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Objective

Estrogen receptors α and β are members of the nuclear receptor family which regulate target gene expression in response to estrogens and to growth factor receptors-mediated pathways. CXCR4 is a chemokine receptor whose physiological ligand is the stromal-derived-factor-1 (SDF-1), a chemokine with proliferative and chemotactic functions. Recently, we reported that ERβ activity can be modulated by the tyrosine kinase ErbB2/ErbB3 receptors and also by the CXCR4 receptor. We sought to determine the crosstalk between ErbB2, CXCR4 and the transcriptional regulation of ERβ.

Methods

The interplay between CXCR4 and ErbB2 receptors was analyzed by co-immunoprecipitation and Western analyses, as well as the signaling pathways involved. The effect on ERβ transcriptional activity was determined by luciferase reporter assay.

Results

Our results indicate that ERβ transcriptional activity was repressed by ErbB2 activation, an effect which was relieved by CXCR4. However, addition of CXCR4 ligand SDF-1 did restore the reduction in ERβ activity, suggesting a ligand-dependent effect of CXCR4. Under these conditions, Akt kinase activity was diminished in presence of CXCR4 and increased with SDF-1 treatment. We also found that CXCR4 altered PI3-K recruitment to ErbB2/ErbB3 receptor dimer, resulting in a decreased interaction between ERβ and Akt in response to ErbB2 when CXCR4 was expressed. Interestingly, these interactions were restored back in presence of SDF-1.

Conclusion

These results indicate a regulatory crosstalk that impacts ERβ transcriptional activity through the ErbB2 signaling pathway modulated by the presence of CXCR4 and its ligand SDF-1, and suggest that it is a PI3-K/Akt dependent process.

Declaration of interest

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## P1162

**Characterisation of the N321K mutation in the type-2 vasopressin receptor causing nephrogenic diabetes insipidus**

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Background
Loss of function mutations of the type-2 vasopressin receptor (V2R) in kidney lead to nephrogenic diabetes insipidus (NDI). The function of mutant receptors can be rescued using ligands resulting sufficient amount of plasma membrane localized V2Rs in a conformation which are able to generate cAMP signal. We examined a previously described, not characterized mutation. In order to determine personalized therapy for the individual the properties of the mutant receptor were characterized. Based on the findings we could frame a therapeutical strategy.
Materials and methods
We constructed a highly sensitive Epac-based BRET (bioluminescence resonance energy transfer) biosensor to perform real-time cAMP measurments. The β-arrestin binding of the receptors was examined with luciferase-tagged β-arrestin and mVenus-tagged V2Rs using BRET technique. The BRET measurements were performed on transiently transfected HEK293 cells using 96-well plates and Berthold Mithras LB 940 multilabel reader. Cell surface expression and localization examinations were implemented with fluorescent tagged receptors visualised with with Zeiss LSM510 confocal laser-scanning microscope.
Results
The previously described N321K mutation is in the 7th transmembrane helix of the V2R. Determination of the ligand induced cAMP generation of the mutant receptor showed increased EC50 in arginine-vasopressin (AVP) stimulation and lack of signal in desmopressin stimulation. BRET experiments revealed decreased β-arrestin binding of N321K-V2R. The mutant receptor also showed different sensitivity for V2R antagonists compared to wild-type receptor.
Conclusions
N321K mutant V2R showed cell surface expression, the physiologically essential cAMP generation of the receptor can be rescued with elevated dose of AVP, while with clinically used desmopressin was not efficient. Different internalization of the receptor may occur through altered β-arrestin binding. Administration of vasopressin receptor antagonists may be useful to avoid the side effects of high dose AVP in an NDI patient.
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## P1163

**Protein metabolism in human skeletal muscle after 72 h fasting; increased muscle protein breakdown and impaired insulin signaling**

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Background
Intermittent fasting increase maximum lifespan in several species and this effect is associated with reduced protein synthesis signaling. During fasting, a progressive loss of protein in human skeletal muscle is evident. Furthermore, fasting is associated with increased lipid oxidation and insulin resistance.
Aim
To define which protein metabolic mechanisms are activated in skeletal muscle during fasting.
Methods
We investigated the response to 72 h of fasting in eight healthy men. Skeletal muscle protein metabolism was assessed using labelled phenylalanine tracer combined with arteriovenous catheterization technique. Insulin sensitivity was assessed by hyperinsulinemic–euglycemic clamp. In addition, substrate oxidation and intramyocellular signaling to protein synthesis and breakdown was assessed.
Results
Peripheral insulin sensitivity was reduced and substrate oxidation shifted toward lipid oxidation during fasting. Net muscle protein breakdown was increased, mTOR signaling to protein synthesis was reduced, and FOXO3a signaling to local protein breakdown was unaltered. Furthermore, insulin signaling to protein synthesis was impaired downstream of Akt. However, fasting did not affect the upstream kinases Akt and AMPK or amino acid sensitive phosphorylation of eIF2a.
Conclusion
This work defines the physiological adaptations to fasting. The findings suggest that the increased net protein breakdown is due to reduced mTOR mediated protein synthesis. Furthermore, impaired insulin signaling to protein synthesis is not due to amino acid deprivation or AMPK activation. Our findings suggest that inhibition of mTOR signaling is a central mechanism triggering reduction of protein synthesis in muscle during fasting – and perhaps increasing longevity in humans and other species.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1164

**Chronic and acute effect of IL1β on sex hormone-binding globulin by decreasing HNF-4α via JNK/c-Jun pathway**

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Patients suffering from low-grade chronic inflammatory diseases such as rheumatoid arthritis, osteoarthritis, diabetes and obesity have low plasma SHBG levels. These diseases are characterized among other features by high plasma IL1β levels. The aim of the present study is to explore whether IL1β could regulate hepatic SHBG production to account for low SHBG levels in these diseases. We provide evidence that daily IL1β treatment reduces SHBG production in HepG2 cells. The IL1β effect on human SHBG expression was mediated by the downregulation of HNF-4A via the JNK/c-Jun signalling pathway. Daily TNFα treatment of human SHBG transgenic mice reduced plasma SHBG and hepatic HNF-4α mRNA and protein levels. Importantly, SHBG levels were reduced by IL1β within 24 h *in vitro* in HepG2 cells and *in vivo* in human SHBG transgenic mice. The human SHBG promoter did not respond directly to c-Jun and its transcription was reduced by the rapid decrease in HNF-4α levels that occurs within 2–4 h of IL1β treatment. Our results show the molecular mechanisms by which IL1β reduces hepatic SHBG production suggesting that IL1β plasma levels are an important factor in accounting for the low SHBG levels in chronic low-grade inflammatory diseases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1165

**LPS administration modulates hypothalamic AMPK and glucose homeostasis in mice**

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Introduction
Hypothalamic AMP-activated protein kinase (AMPK) has emerged as a key molecular player in energy homeostasis. It participates of glucose homeostasis modulating liver glucose production. It is known that endotoxin shock presents various complicated metabolic aspects. Among them, in later phase circulatory collapse and hypoglycemia are induced with a deterioration of energy metabolism. Here we evaluated the participation of hypothalamic AMPK in the modulation of hepatic gluconeogenesis by lipopolysaccharide (LPS).
Methods
Swiss mice were used to this study. When necessary the mice were submitted to stereotaxic surgery to implant of guide cannula into the lateral ventricle (I.C.V.).

Lypopolissacaride (1 mg/kg) was administered via I.P. in fasted mice and after 4 h it were killed by decapitation and hypothalamus and liver were quickly removed and stored at −80°C. When necessary the food intake was measured during dark period. The protein analyses (AMPK, ACC, PEPCK and STAT3) were performed by western blot and the level of mRNA of PEPCK and G6Pase were evaluated by real time PCR. Blood glucose was determined by glucose oxidase method.
Results
The administration of LPS IP reduced food intake (60%) and glycemia (56%) as well as the AMPK (45%) and ACC (70%) phosphorylation when compared to control mice. Besides, total NFkB expression in the hypothalamus was higher in LPS-mice than control mice. These effects were accompanied by reduced level of mRNA and protein of PEPCK (25 and 40%, respectively) and increase in tyrosine phosphorylation of STAT3 (40%) in the liver. However, the ICV injection of AICAR prevented the dephosphorylation of hypothalamic AMPK and hypoglycemia associated to LPS-IP administration. In TNFR1−/− mice the glycemia was not significantly modified by LPS injection.
Conclusion
These results suggest that hypoglycemia associated to endotoxin shock also can be triggered by TNFα signaling and inactivation of hypothalamic AMPK leading to metabolic complications.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1166

**Evaluation of K+ flux-dependent regulation of mitochondrial potential (ΔΨm) by α-adrenergic agonist phenylephrine in single hepatocytes**

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It is generally accepted that hepatic responses to α-adrenergic agonists are dependent on the redistribution of intracellular and also extracellular $Ca^{2+}$. Besides, phenylephrine induces significant $^{K+}$ flux changes and plasma membrane hyperpolarisation. Earlier it was shown that $^{K+}$ channel blockers as well as chelating of extracellular $Ca^{2+}$ with EGTA could inhibit phenylephrine-induced metabolic responses and activation of mitochondrial processes. Taking into account the significance and interplay of $Ca^{2+}$ and $^{K+}$ ions in α-adrenergic regulation of liver metabolism and mitochondrial responses we have investigated phenylephrine-induced ΔΨm changes in single hepatocytes at different conditions of $Ca^{2+}$ or $^{K+}$ fluxes.
Quantitative microfluorimetry with using mitochondrial probe JC-1 allowed evaluating the ΔΨm changes in single hepatocytes. The fluorescence of J-aggregates at 590 nm gives an index of ΔΨm.
It was shown that ΔΨm increased within 15 min of the phenylephrine action on hepatocytes (140±7% relative to control) and fell gradually to 104±6% during 30 min experiment. $^{K+}$ channel blockers – $Ba^{2+}$(2 mM) and 4-aminopyridine (5 mM) decreased the mitochondrial response. ΔΨm was 115±8% and 102±9% in the presence of $Ba^{2+}$ and 4-AP, respectively (15 min). ΔΨm progressively fell down below the control values (up to 75% in both cases at 30 min). But 0.1 mM $Ba^{2+}$ did not change the agonist-simulated ΔΨm response (147±14% and 112±10% 15 and 30 min, respectively). Plasma membrane depolarization with elevated extracellular $^{K+}$(140 mM) turned effect of phenylephrine to opposite and decreased ΔΨm below control values (49±8% within 15 min). 1 mM EGTA influenced on phenylephrine-induced ΔΨm changes in the same way as extracellular high $^{K+}$(49±9%).
The data obtained suggest that complete or partial plasma membrane depolarization due to alterations of $^{K+}$ fluxes causes changes in transmission of α-adrenergic agonists signal to mitochondrial compartments.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1167

**Altered muscle mitochondrial amount and physical performance in 5/6 nephrectomy CKD model mice**

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Background
Chronic kidney disease (CKD) in human has been believed to decrease muscle weight and physical performance. High protein diet would increase muscle mass and strength especially in athletes, but is not recommended for CKD patients because of the risk for exacerbation of uremia. Therefore, we investigated the muscle character especially focused on the mitochondrial activity and physical performance in 5/6 nephrectomy CKD model mice, which fed on different protein contents in diets.
Methods
C57bl/6 mice which were 5/6 nephrectomized at 6–7 weeks old were fed on diets containing 3–50%Kcal protein. Muscle strength and exercise endurance were examined at 16 weeks old. Muscle weight, the mitochondrial activity, and the phospholization levels of S6K1 and AMPK were examined at 20 weeks old. C2C12 myotubular cells were treated with amino acids cocktail and investigated for mitochondrial energy metabolism.
Results
Exercise endurance was decreased in 5/6 nephrectomized mice at 16 weeks old with decreasing phosphlization level of AMPK and mitochondrial amount in skeletal muscle, although muscle weight and strength were preserved. Exercise endurance and mitochondrial function were further decreased in 30% Kcal protein diet group compared with 10% Kcal protein group. Serum lactate level after exercise was remarkably elevated in the high-protein diet group, associated with a decrease in muscle pyruvate dehydrogenase (PDH) activity. Amino acids cocktail treatment in the C2C12 cells decreased oxygen utilization and ATP content and increased lactate level in the culture medium, associated with a decrease in the PDH activity.
Conclusion
We found that muscle mitochondrial amount and exercise endurance were decreased in 5/6 nephrectomy CKD model mice at 16 weeks old, associated with a decrease in muscle AMPK phospholization and mitochondrial amount. A protein-restricted diet containing 10% Kcal protein contributed to maintain exercise endurance in the CKD model mice.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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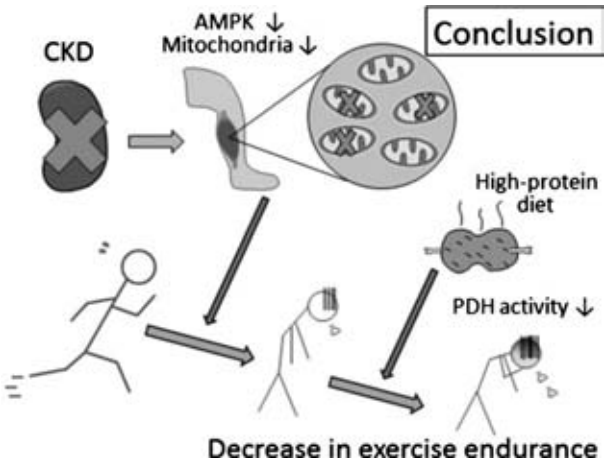

## P1168

**PPARγ activator may improve sepsis symptom by inhibiting inflammation in adipose tissue**

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Introduction
Postoperative sepsis exhibits high mortality. Prevention of postoperative infection and palliation of sepsis symptom are important issues in the patient's care after surgical operation. Adipose tissue as an endocrine organ that secretes various cytokines. Circulating adiponectin, an anti-inflammatory cytokine, and inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α are derived partially from adipose tissues. Recent studies have suggested that adipose tissue may play an important role in systemic inflammatory response. Our previous report also demonstrated that preoperative circulating adiponectin levels may be a risk factor of postoperative infection. We examined here whether a PPARγ activator, pioglitazone (PGZ) which increases serum adiponectin level may suppress excessive inflammatory reaction accompanying with sepsis in the mouse sepsis model.
Method
Seven-week old mice were treated with PGZ (10 mg/kg, i.p.) for 7 days and sepsis was induced by cecal ligation and puncture (CLP). The survival rate was monitored, and inflammatory factors in peritoneal fluid (PF) and abdominal adipose tissue were analyzed by real-time PCR and ELISA 24 h after CLP. In addition, abdominal adipocyte was cultured in the presence or absence of CLP mice-derived PF with PGZ to determine direct effects of PF on inflammatory cytokine expressions in adipocytes.
Result and conclusion
Serum adiponectin levels before CLP were elevated after PGZ treatment and the survival rate after CLP was improved. CLP increased IL-6 and TNF-α mRNA expressions in abdominal adipose tissues, and also TNF-α and endotoxin levels in PF. PGZ pretreatment inhibited TNF-α expression without the suppression of endotoxin. PF collected from CLP mice stimulated IL-6 and TNF-α expression in cultured abdominal adipocytes. However, these cytokines levels were significantly lower when PF derived from PGZ-treated CLP mice was applied in adipocytes. These results suggest that pretreatment of PPARγ activator possibly improves the survival rate by down-regulating excessive inflammation in adipose tissues.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1169

**Effects of benzbromarone on serum CRP in patients with gout**

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Introduction
C-reactive protein (CRP), an acute reactant protein and member of the pentraxins family has a primitive defense function against exogenous organisms. Although its production is predominantly under the control of IL-6, IL-1 and tumor necrotic factor also contribute to hepatic synthesis and CRP secretion. CRP is mainly produced in the liver and possibly other tissues, such as atherosclerotic lesions. C-reactive protein (CRP) is associated with increased risk for cardiovascular diseases (CVD), while increased serum uric acid level is suggested to be independently associated with an increased risk of CVD. Accordingly, in the present study, we compared serum CRP levels between healthy controls and patients with gout who were matched in regard to parameters associated with serum CRP levels. In addition, since benzbromarone decreases the level of urate in serum, based on its uricosuric action and activates peroxisome proliferater-activated receptor alpha (PPARalpha), we also examined whether benzbromarone had effects on serum CRP levels in patients with gout and the expression of the messenger mRNA in hepatoma cell line, HuH7.
Methods
In the first experiment, blood samples were drawn from 40 healthy males and 40 male patients with gout after an overnight fast. In the second experiment, 42 male patients with gout were given uric acid-lowering therapy with benzbromarone. Blood samples were drawn after an overnight fast before and 1 year after beginning benzbromarone treatment. In the third experiment, the effects of benzbromarone on IL1beta-induced CRP expression were determined in HuH7 cells
Results
Serum CRP levels were not significantly different between the patients with gout and healthy subjects, while serum CRP levels were decreased by 11% after benzbromarone treatment, as compared to the values before treatment ($P<0.01$). In addition, our *in vitro* findings suggested that benzbromarone, as well as fenofibrate, down-regulated IL1beta-stimulated CRP gene expression via PPARalpha pathway.
Conclusions
These results suggest that hyperuricemia may not contribute to an increase in serum CRP level, while benzbromarone may have a favorable effect on CRP.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Obesity

## P1170

**Association of 3111t/c polymorphism of the clock gene with binge eating disorder in obese children and adolescents**

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Introduction
The etiopathogenesis of obesity is represented by the endogenous circadian clock system, which has been shown to have a part in the regulation of body weight and energy homeostasis. The gene Circadian locomotor output cycles kaput (CLOCK) is part of the positive regulatory branch of the system. Polymorphism in CLOCK genes in human disorders characterized by alterations of endogenous circadian rhythms. Moreover, since in up to 30% of obese subjects seeking treatment obesity seems to be associated with binge eating disorder (BED) we explored whether the 3111T/C CLOCK gene SNP was associated specifically to this phenotype.
Objective
To investigate the association of 3111T/C CLOCK gene SNP with BED in obese children and adolescents.
Methods
A total of 319 obese children and adolescents (35.7% boys; aged $10.6 \pm 1.4$ years, BMI $30.2 \pm 4.5$ kg/m$^2$, ZBMI $2.3 \pm 0.3$, 45.7% pubertal) were genotyped using Assay by Design kits Applied Biosystem. 121 cases completed the Binge Eating Scale questionnaire and were classified about the grade of eating compulsivity. SPSS v16 (SPSS Inc.) was used for data preparation and initial analysis of the data.
Results
Genotype frequencies did not significantly differ between sex ($X2 = 1.78$; d.f. $= 2$; $P = 0.41$). According to Binge Eating Scale questionnaire the sample consisted of 39.6% patients have BED, and allele CC was found in 75.0% of them, only 37.9% of the patients with allele TT presents BED ($P = 0.015$). BED score was strongly associated in girls with presence of allele CC ($19.8 \pm 2.36$ vs $14.6 \pm 0.90$; $P = 0.047$) as compared to the TT genotype.
Conclusions
Present findings show the 3111T/C SNP of the CLOCK gene is associated to BED in obese children and adolescents. This association provides a critical starting point for an understanding of the likely polygenic contributions towards eating disorders.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1171

### Nicotine induces negative energy balance through hypothalamic AMP-activated protein kinase

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Introduction
Smokers around the globe commonly report increased body weight after smoking cessation as a major factor that interferes with their attempts to quit. In addition, numerous controlled studies in both humans and rodents have reported that nicotine exerts a marked anorectic action. Nicotine's effects on energy homeostasis have been mostly pinpointed in the central nervous system, but the molecular mechanisms controlling its action are still not fully understood.
Objective
The aim of this study was to investigate the effect of nicotine on hypothalamic AMPK and its effect on feeding and brown adipose tissue function.
Methods
We used adult male Sprague–Dawley rats. Subcutaneous and intracerebroventricular treatments. Stereotaxic microinjection of adenoviral expression vectors. Analysis by *in situ* hybridization, enzymatic assays, western blotting and real-time quantitative PCR. Analysis of energy expenditure, locomotor activity, respiratory quotient and lipid utilization. Nuclear magnetic resonance analysis.
Results
Here, we demonstrate that nicotine-induced weight loss is associated with decreased orexigenic signaling in the hypothalamus, inactivation of hypothalamic AMP-activating protein kinase, alterations in fuel substrate utilization and upregulation of thermogenic markers in brown adipose tissue. Conversely, nicotine withdrawal or genetic activation of hypothalamic AMPK in the ventromedial nucleus of the hypothalamus reversed nicotine-induced hypophagia and weight loss.
Conclusions
Overall, these data demonstrate that nicotine's effects on energy balance involve specific modulation of the hypothalamic AMP-activating protein kinase-brown adipose tissue axis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1172

### Ac 2-26, an annexin A1-derived peptide, reduces inflammation in human SGBS adipocytes after hypoxia treatment

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Previous studies demonstrated that the N-terminal peptide of annexin A1 (AnxA1) (peptide Ac2-26) can mimic the anti-inflammatory actions of the full-length protein in various systems. In the current study, we report the effectiveness of the peptide Ac2-26 as an anti-inflammatory agent in a model of human SGBS adipocytes. We have previously demonstrated that plasma AnxA1 protein is significantly inversely correlated with BMI, body fat level and waist to hip ratio. We have also shown that ANXA1 gene is expressed in human SGBS adipocytes and hypoxia reduces the expression of ANXA1 gene showing that AnxA1 may act as a counter regulator of adipose tissue inflammation. Given that low-level systemic inflammation is seen in metabolic syndrome-associated chronic pathologies, here, we investigate if Ac2-26 peptide can have an effect on the inflammatory markers in human SGBS cells. To mimic the relative hypoxia found in the white adipose tissue of obese humans, mature adipocytes (14 days post-confluent) were treated with 4, 8 and 24 h hypoxia treatment (1% $O_2$). The cells were also treated with 10-5 M Ac2-26 peptide or vehicle (DMEM/F12 medium supplemented with insulin (20 nM). Cells were collected and SYBR green PCR analysis performed for CRP, IL-6, adiponectin and leptin genes which have been all shown previously to be altered by hypoxia (1% $O_2$) in SGBS cells. We found that CRP expression was significantly down-regulated following 4, 8 and 24 h of hypoxia treatment in the cells also treated with Ac2-26 peptide compared to vehicle alone ($P=0.027$, $P=0.042$ and $P=0.014$, respectively). IL-6 was also found to be significantly down-regulated after 24 h hypoxia treatment in the Ac2-26 treated cells compared to vehicle ($P=0.013$). There was no significant difference in the expression of adiponectin, or leptin at any of the time points measured with either Ac2-26 treated cells or vehicle controls.
Here, we demonstrate for the first time that an AnxA1 mimetic, Ac2-26 peptide, regulates pro-inflammatory markers in human SGBS adipocytes. These data show the effectiveness of the peptide Ac2-26 as an anti-inflammatory therapeutic agent in a human SGBS adipocyte model. Furthermore, AnxA1 may be an important modulator of inflammatory and pro-resolution pathways necessary to restore homeostasis in the inflamed adipose tissue of the obese.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1173

### Pro-inflammatory wnt5a and anti-inflammatory sFRP5 are differentially regulated by nutritional factors in obese human subjects

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Background
Obesity is associated with macrophage infiltration of adipose tissue. These inflammatory cells affect adipocytes not only by classical cytokines but also by the secreted glycopeptide wnt5a. Healthy adipocytes on the other site are able to release the specific wnt5a inhibitor sFRP5. This protective effect, however, was found to be diminished in obesity. The aim of the present study was to examine i) whether obese human subjects exhibit increased serum concentrations of wnt5a and ii) whether wnt5a and/or sFRP5 serum concentrations in obese subjects can be influenced by caloric restriction.
Methodology
In total, 23 obese human subject (BMI $44.1 \pm 1.1$ kg/m$^2$) and 12 age- and sex-matched lean controls (BMI $22.3 \pm 0.4$ kg/m$^2$) were included into the study. Obese subjects were treated with a very-low-calorie-diet ($\sim$ 800 kcal/d) for 12 weeks. Body composition was assessed by impedance analysis, insulin sensitivity was estimated by the leptin-to-adiponectin ratio and wnt5a and sFRP5 serum concentrations were measured by ELISA. sFRP5 expression in human adipose tissue biopsies was further determined on protein level by immunohistology.
Principal findings
Pro-inflammatory wnt5a was not measurable in any serum sample of lean control subjects. In patients with obesity, however, wnt5a became significantly detectable consistent with low-grade inflammation in such subjects. Caloric restriction resulted in a weight loss from $131.9 \pm 4.0$ to $112.3 \pm 3.2$ kg in the obese patients group. This was accompanied by a significant decrease of the leptin-to-adiponectin ratio, indicating improved insulin sensitivity. Interestingly, these metabolic improvements were associated with a significant increase in serum concentrations of the anti-inflammatory factor and wnt5a inhibitor sFRP5.
Conclusions/significans
Obesity is associated with elevated serum levels of pro-inflammatory wnt5a in humans. Furthermore, caloric restriction beneficially affects serum concentrations of anti-inflammatory sFRP5 in such subjects. These findings suggest a novel regulatory system in low grade inflammation in obesity which can be influenced by nutritional therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1174

**Zinc-α2-glycoprotein in adipose tissue is related with insulin resistance and lipolytic genes in morbidly obese patients**

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Objective
Zinc-α2 glycoprotein (ZAG) stimulates lipid loss by adipocytes and may be involved in the regulation of adipose tissue metabolism. We analyze ZAG expression levels in adipose tissue from a group of morbidly obese patients, and their relationship with lipogenic and lipolytic genes and with insulin resistance (IR).
Design
mRNA expression levels of PPARgamma, IRS-1, IRS-2, lipogenic and lipolytic genes and ZAG were quantified in visceral (VAT) and subcutaneous adipose tissue (SAT) of 25 nondiabetic morbidly obese patients, 11 with low IR and 14 with high IR. Plasma ZAG was determined by ELISA.
Results
The morbidly obese patients with low IR had a significantly higher ZAG gene expression in VAT as compared with the patients with high IR ($P=0.023$). In the patients with low IR, the VAT ZAG gene expression was significantly greater than that in SAT ($P=0.009$). ZAG expression correlated strongly between SAT and VAT ($r=0.709$, $P<0.001$). In VAT, ZAG expression correlated significantly and positively with the expression of PPARgamma, ACSS2, DGAT1, ATGL, IRS-1, IRS-2 and adiponectin. In SAT, ZAG expression correlated significantly and positively with PPARgamma, ACC1, DGAT1, ATGL, HSL and adiponectin. VAT ZAG expression was mainly predicted by insulin, HOMA-IR, circulating adiponectin levels and expression of adiponectin and ACSS2. SAT ZAG expression was only predicted by expression of ATGL.
Conclusions
ZAG is involved in modulating lipid metabolism in adipose tissue and is associated with insulin resistance.
Declaration of interest
I declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1175

**Longitudinal changes in body mass index and body composition among 417 adult survivors of childhood cancer**

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Introduction
Obesity, represented by high body mass index (BMI), is a major complication after treatment for childhood cancer. High amount of total and visceral fat and low lean body mass are described as more reliable determinants, predicting the development of cardiovascular disease. In this study longitudinal changes of BMI and body composition in adult childhood cancer survivors were evaluated.
Methods
Data of 417 adult childhood cancer survivors, who had visited the late effects clinic twice, were analyzed retrospectively. Median follow up time was 16 years (interquartile range 11–21) and time between visits was 3.2 years (2.9–3.6). At both time points BMI was measured and body composition was assessed by dual X-ray absorptiometry (Lunar Prodigy). BMI and body composition measures were compared with those of healthy Dutch references and calculated as standard deviation scores (SDS). Pituitary dysfunction and hormonal replacement were evaluated in all survivors. GH deficient subjects treated with GH replacement at time of follow up were excluded from further analyses.
Results
BMI SDS at first assessment was only significantly higher in female cranial radiotherapy (CRT) survivors as compared to healthy Dutch references (SDS = 0.40, $P=0.02$). Increase of BMI over time (expressed as units per year) was only significantly higher in male survivors (0.27 vs 0.02 in controls ($P<0.001$)). Percentage fat was significantly higher than controls in both men (SDS 1.37, $P<0.001$) and women (SDS 1.05, $P<0.001$) in all therapy groups, with the highest SDS after CRT (mean SDS 1.73 in men, 1.48 in women, $P<0.001$). Only in men, increase in total fat percentage was significantly higher as compared to controls (delta SDS = 0.22, $P<0.001$). Lean body mass did not significantly change over time.
Conclusion
Significantly greater increase of BMI and total fat percentage as compared to healthy references was found, especially in adult male survivors of childhood cancer.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1176

**Brown rice and its component, γ-oryzanol, attenuate the preference for dietary fat by decreasing hypothalamic endoplasmic reticulum stress in mice**

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It is known that brown rice (BR) prevents obesity and type 2 diabetes in humans. Previous studies have shown that exaggerated endoplasmic reticulum (ER) stress in the hypothalamus of obese mice is linked to hyperphagia. Here we tested the hypothesis that BR might decrease ER stress in hypothalamus, leading to the attenuation the preference for dietary fat.
Eight-week-old mice were randomly divided into three groups: the control, BR-containing diet, and the WR-containing diet groups. To explore the impact of BR on the preference for dietary fat, we performed the food choice tests between CD and HFD. The mice were allowed free access to both diets. The control group strongly preferred the HFD. On the contrary, BR group preferred CD (∼12.5-fold vs control, ∼3.7-fold vs WR), leading to the suppression of body weight gain. To assess the effect of BR on hypothalamic ER stress, the mRNA levels of ER stress-responsive genes were analyzed. BR significantly decreased the mRNA levels of these genes in the hypothalami of the mice fed HFD. In the food choice tests, the mice treated with 4-phenyl butyric acid (120 mg/kg per day i.p.), a well known ER stress eraser, significantly preferred CD. We investigated the effect of γ-oryzanol (Orz), a mixture of major bioactive components in BR, on the hypothalamic ER stress and the preference for dietary fat. Oral administration of Orz (20, 80, 320 mg/kg per day p.o.) decreased hypothalamic ER stress in the mice fed HFD. In the food choice tests, Orz-treated mice preferred CD (∼2.1-fold vs vehicle).
Our study is the first demonstration that BR and its component, Orz, decrease hypothalamic ER stress and consequently attenuate the preference for dietary fat in mice. BR and Orz may be unique tools to ameliorate metablic syndrome through modulating feeding behavior.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1177

**Weight-perception in male career US firefighters and its association with cardiovascular risk factors**

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Objective
To evaluate the associations among body mass index, weight perception and cardiovascular risk factors in male career US firefighters.
Design
The present cross-sectional study was nested within a federally-funded multicenter, prospective cohort investigation of cardio-respiratory fitness (CRF),

health and employment outcomes among career firefighters.
Subjects
Seven hundred and sixty-eight male career firefighters from two US Midwestern States. A physical examination was performed. Fasting blood samples were taken. CRF was determined from symptom- limited maximal treadmill exercise testing with ECG monitoring and estimation of oxygen consumption (METS) following the Bruce protocol. Self-reports of weight perception were extracted from responses to a health and lifestyle questionnaire with standardized written instructions to complete the multiple choice survey regarding eating, health, exercise, sleep, and work habits.
Results
We found that a high proportion of overweight and obese male career firefighters underestimate their weight categories (68%) and the risk of underestimating one's weight category increased by 24% with each additional unit of BMI after adjustment for age and CRF. When divided into six groups based on combination of measured BMI category and weight self-perception, there were significant differences among the groups for most cardiovascular risk factors. After adjustment for age and BMI, these differences remained statistically significant for CRF, amount of weekly exercise, prevalence of metabolic syndrome, body fat percentage and cholesterol measurements.
Conclusion
The majority of overweight and obese firefighters incorrectly perceive themselves as belonging to lower weight categories. As a result, they are unlikely to fully appreciate the negative health consequences of their excess weight, which were clearly demonstrated on objective testing. Efforts to improve the accuracy of weight status awareness are probably needed in order to reverse the trend of increasing obesity among public safety occupations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1178

### GH secretagogue receptor gene 171 C/T polymorphism association with metabolic features in obese children and adolescents

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Introduction
Ghrelin, enhances appetite and increases food intake in healthy humans. The endogenous ligand ghrelin is the GH secretagogue receptor (GHSR), and effects of ghrelin are mediated via GHSR. The GHSR system plays a significant role in regulating the process of obesity and type 2 diabetes that are linked to metabolic syndrome (MS). Therefore, the GHSR gene 171C/T (rs495225) polymorphism is a good candidate for susceptibility to obesity and excellent MS.
Objective
To assess the frequencies of 171C/T (rs495225) GHSR polymorphism in obese children and adolescents (OCA) comparing to normal weight healthy controls and to investigate associations with MS and metabolic features in OCA.
Methods
A total of 428 Brazilian children: 91 normal healthy weight (43.9% boys; aged $10.6 \pm 1.5$ years; BMI $17.0 \pm 2.4$ kg/m$^2$; ZBMI $-0.3 \pm 0.9$, 40.6% pubertal) and 337 obese (35.5% boys; aged $10.7 \pm 1.4$ years; BMI $30.6 \pm 4.7$ kg/m$^2$; ZBMI $2.3 \pm 0.3$; 46.3% pubertal; 43.0% with MS) participated into the study. The polymorphic region of GHSR gene was amplified by PCR and products were bidirectional sequenced. MS was diagnosed by adult treatment panel III (ATP III) adapted for children. SPSS v16 (SPSS, Inc.) was used for data preparation and initial analysis of the data.
Results
Genotype frequencies did not significantly differ between normal weight controls and obese children ($X^2 = 0.66$; df = 2; $P = 0.7$). However, obese patients carrying the TT genotype in 171C/T polymorphism had significantly higher values of HOMA ($4.21 \pm 0.19$ vs $3.66 \pm 0.26$; $P = 0.02$), higher frequency of MS (44.1 vs 15.2%; $P = 0.04$), hypertriglyceridemia (47.7 vs 14.1%; $P < 0.001$) and trend toward a hyperglycemia (56.5 vs 17.4%; $P = 0.08$) as compared to the CC genotype.
Conclusions
The results support the notion that the studied 171C/T (rs495225) polymorphism of GHSR might be a genetic risk factor for MS in OCA. Our findings also suggest a complex genotype-environmental interaction on obesity risk.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1179

### Using $^1$H-nuclear magnetic resonance spectroscopy to identify changes in the plasma metabolome of rats fed low-carbohydrate/high fat diets

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We previously reported the impact of low-carbohydrate/high-fat (LC–HF) diets on different endocrine systems in rats. To unravel metabolic pathways involved, we now studied if pair-feeding isoenergetic amounts of three different diets – all composed of macronutrient from the same sources but at different relative abundance, – leads to specific changes of the plasma metabolic profile.
Methods
Plasma was collected from male Wistar rats ($n \geq 7$/group) which were pair-fed for 4 weeks either a control chow diet (CH), or two LC–HF diets (LC–HF-1: high in fat and matched in protein content to CH; LC–HF-2: very high in fat, but low in protein). $^1$H-NMR spectra were recorded on a DRX-600 NMR spectrometer (Bruker BioSpin). OPLS-DA models were carried out between diet groups. Statistical analyses were performed with SIMPCA P + 12.0 (Umetrics AM, Sweden).
Results
The best separation was found between LC – HF-2 and CH. The OPLS-DA model had a predictive ability Q2Y = 94.9% and explained a total variance of R2X = 61.0%. Furthermore, the model showed that the variance related to the class separation was R2pX = 24.5%. As expected, NMR spectra revealed the ketone metabolite 3-hydroxybutyrate as one major metabolite allowing clear discrimination of the groups. After exclusion of 3-hydroxybutyrate corresponding ppm-regions the OPLS-DA model still showed a Q2Y of 91.9% and a R2X of 73.6% (R2pX was 20.3%). Also LC–HF-1 and CH groups were clearly distinguishable, although no differences in 3-hydroxybutyrate were detected. When comparing spectra from LC–HF-1 and LC–HF-2 groups and excluding the metabolite 3-hydroxybutyrate, the OPLS-DA model showed a Q2Y of 72.3% and a R2X of 81.0%.
In conclusion, NMR-spectroscopy and OPLS-DA allowed precise discrimination between dietary groups. As expected, 3-hydroxybutyrate is one major metabolite differing between LC-HF-2 and LC-HF-1 or CH fed rats. However, even exclusion of this metabolite allows good group separation paving the way to indentifying previously unexpected metabolic pathways.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1180

### Metabolic syndrome after liver transplantation: pre- and post-operative risk factors

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Background
Metabolic syndrome (MS) is a common condition among liver transplanted patients and it contributes to late morbidity and mortality by favoring the development of cardiovascular disease (CVD).
The aim of this study was to assess the prevalence of MS in the first 6 months after orthotopic liver transplantation (OLT) and the associated pre-operative and post-operative risk factors.

Methods
Seventy-one cirrhotic patients were evaluated at baseline and after OLT. The presence of MS was assessed accordingly to 2004 revised ATP III criteria. Nutritional habits were assessed using 3-day-food records.
Results
The prevalence of MS was 8/71 (11.3%) before OLT, and 23/71 (32.4%) and 21/71 (29.6%) 3 and 6 months after OLT respectively ($P=0.006$). The factors independently associated with MS were older age ($P=0.042$), family history of diabetes ($P=0.005$) and excess body weight at baseline ($P=0.025$). The analysis of daily food intake after transplantation showed an increase in caloric intake, with redistributions of macronutrients. As compared to subjects without MS, MS patients showed an higher intake of total energy (3 months: $2607\pm873$ vs $2155\pm595$ kcal/die, $P=0.03$; 6 months: $2132\pm500$ vs $1864\pm417$ kcal/die, $P=0.074$), saturated fatty acids (3 months: $11\pm3.6$ vs $8.0\pm2.6\%$, $P=0.007$; 6 months: $11.5\pm3.5$ vs $8.8\pm2.8\%$, $P=0.065$) and cholesterol (3 months: $367\pm170$ vs $275\pm174$ mg, $P=0.02$; 6 months: $276\pm122$ vs $208\pm83$ mg, $P=0.04$). MS patients showed higher prevalence of graft rejection (45.4 vs 31.7% respectively), infections (41 vs 26.8% respectively) and major cardiovascular events (11.6 vs 6.7% respectively), as compared with non-MS patients, although these differences did not reach statistical significance.
Conclusion
Transplanted patients are at high risk of developing MS. Both pre- and post-operative factors predispose patients to the onset of MS. Control of modifiable risk factors, such as body weight and dietary intake, may reduce the prevalence of MS after liver transplantation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1181

**Metformin in normoglycemic obesity: a systematic review**

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Objective
Metformin was considered as a weight lowering agent in diabetes patients, while it remained unclear in simple obesity patients. We aimed to investigate if metformin is effective and safe in the therapy of normoglycemic obesity.
Methods
We systematically searched MEDLINE (1950–2011), EMBase (1950–2011), and CCTR (4th Issue 2011), and collected and screened all randomized controlled trials compared metformin and placebo in obese participants excluding hyperglycemia, hypertension, and other chronic diseases.
Results
Twelve trials with follow-up duration 3–24 months were included in our study, eight of which included adolescence as subjects. Compared with placebo, metformin can effectively reduce BMI (overall MD $-1.14$ kg/m$^2$, 95% CI $-1.61$ to $-0.67$ kg/m$^2$, $P<0.00001$) and body weight (overall MD $-2.52$ kg, 95% CI $-3.84$ to $-1.20$ kg, $P=0.0002$), especially in adolescent patients (BMI, overall MD $-1.61$ kg/m$^2$, 95% CI $-2.61$ to $-0.61$ kg/m$^2$, $P=0.002$; body weight, overall MD $-3.11$ kg, 95% CI $-4.28$ to $-1.95$ kg, $P<0.00001$). The overall fat mass (overall MD $-1.77$ kg, 95% CI $-4.19$ to 0.66 kg, $P=0.15$) and waist circumferential (overall MD 0.57 cm, 95% CI $-1.33$ to 0.20 cm, $P=0.15$) reduction due to metformin did not meet statistical significance, while waist circumference could be reduced by metformin in adolescent subgroup (overall MD $-1.20$ cm, 95% CI $-2.18$ to $-0.22$ cm, $P=0.02$). Furthermore, fasting insulin (overall MD $-18.09$ pmol/l, 95% CI $-28.92$ to $-7.26$ pmol/l, $P=0.001$) and HOMA-IR (overall MD $-3.13$, 95% CI $-5.95$ to $-0.31$, $P=0.03$) but not fasting blood glucose (overall MD $-0.15$ mmol/l, 95% CI $-0.3$ to 0.01 mmol/l, $P=0.06$) decreased significantly after metformin therapy compared with placebo. Gastrointestinal adverse effects in metformin group were not significantly different from those in placebo.
Conclusion
Metformin could markedly reduce body weight and insulin resistance in the treatment of normoglycemic obesity, especially in adolescent patients.
Keywords: metformin, obesity, systematic review.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1182

**Growth differentiation factor-15 and transforming growth factor-β1 in healthy adults with and without obesity**

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Introduction
GDF-15 and TGF-β1 are growth factors involved in immune modulation and development of arteriosclerosis. Aim of this study was to assess whether GDF-15 and TGF-β1 concentrations are higher in adipose vs non-adipose individuals and whether this indicates early immune modulation with higher risk of arteriosclerosis.
Description of methods
We examined 84 healthy individuals: 38 non-obese (average age 24.9 years) with mean BMI of $20.5+0.2$ (S.E.M.) and 46 adipose (average age 34.8 years) with mean BMI of $37.7+0.9$ (S.E.M.). In both groups, we determined GDF-15 and TGF-β1 concentration in plasma with ELISA (Quantikine, R&D Systems, Inc., MN, USA).
Results
GDF-15 concentration did not differ significantly between non-obese individuals (mean value 433 pg/ml $+25$ S.E.M.) and adipose individuals (mean value 434 pg/ml $+38$ S.E.M.) (Mann–Whitney $U$ test, $P=0.3185$). TGF-β1 concentrations were significantly higher in adipose individuals (mean value 5784 pg/ml $+308$ S.E.M.) than in non-adipose individuals (mean value 3061 pg/ml $+133$ S.E.M.) (Mann–Whitney $U$ test, $P<0.0001$).
Conclusion
In the tested adipose individuals, TGF-β1, but not GDF-15 concentrations were significantly higher than in the non-adipose individuals. Hence, in young adipose individuals, changes in immune modulation may be assessed earlier with TGF-β1 than with GDF-15.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1183

Abstract withdrawn.

## P1184

**The effect of 'The Programme of weight loss' in women, Arkhangelsk, Russia**

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Background
Different programmes are offered to help people to reduce weight. 'The Programme of Weight Loss' is working in the outpatient clinic in the Arkhangelsk, Russia, but its effectiveness was not assessed before.
Aim of the study was to assess the effect of the complex course 'The Programme of Weight Loss'.
Methods
The panel study was conducted and included women participated in the two-week Programme from the 1st of January to the 31st of July 2010 with following observation during 6 months. We collected anthropometric and laboratory data, questioned them with the impact of weight on quality of life questionnaire (IWQOL-Lite) before the course and after six months. Statistical analysis was performed using Student's paired *t*-test, non-parametric tests, multiple linear regression and logistic regression.
Results
We analyzed the results of 55 women. After 6 months the reduction of body mass (−11.32 kg, 95% CI: −9.24; −13.4), body mass index (−2.74 kg/m$^2$; 95% CI: −0.69; −6.18), waist (−9.76 cm; 95% CI: −7.13; −12.39) and hip (−12.66 cm; 95% CI: −6.28; −19.04) circumferences, mass (−5.86 kg; 95% CI: −0.11; −11.84) and volume (−4.69%; 95% CI: −3.3; −6.08) of fat tissue, systolic (−12.97 mm Hg; 95% CI: −4.18; −21.76) and diastolic (−9.55 mm Hg; 95% CI: −5.68; −13.41) blood pressure, and total cholesterol (−1.1 mmol/l; 95% CI: −0.63; −1.57) were estimated. Lower basal waist circumference, shorter the length of the disease and lower basal level of total cholesterol were significantly associated with waist circumference reduction. Lower level of cholesterol also significantly predicted better decrease of body mass. Data from IWQOL-Lite indicated the significant improvement in self-assessment after six months. Initial body mass (OR = 1.16; 95% CI: 1.02; 1.33), smoking habits (OR for ex-smokers = 0.17; 95% CI: 0.04; 0.8); OR for current smokers = 0.05; 95% CI: 0.00; 0.7) significantly influenced on possibility to refuse from the follow-ups.
Conclusions
«The Programme of Weight Loss» was effective in reduction of body mass, waist and hip circumference, blood pressure and level of total cholesterol after 6 months.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1185

**Efficacy of orlistat vs placebo in the improvement of lipid profile among overweight and obese patients: a meta-analysis**

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Background
According to the 6th National Nutrition Health Survey, the Filipino is generally unfit, with increasing incidences of major risk factors for cardiovascular disease like hypertension, abnormal lipid profile, obesity and diabetes over the last 5 years. Orlistat, a lipase inhibitor is one of the medications used to facilitate weight loss in obese patients. Given that weight loss is known to be associated with an improvement in the serum lipid profile, concurrent improvement in concentrations of cholesterol and triacylglycerols should result from therapy with orlistat.
Objective
To determine the efficacy of orlistat compared with placebo in lowering lipid levels among overweight and obese patients with or without other comorbidities
Methodology
We performed a systematic literature search of Randomized Controlled Trials (RCTSs) using MEDLINE, Cochrane Library Cochrane Register of Controlled Trials, ScienceDirect, and Herdin from inception to 2008. Manual searching was also done through reviews of bibliographies of previous meta-analysis for orlistat. RCTs comparing orlistat and placebo reporting mean changes from baseline in serum lipid levels in both overweight and obese patients were included.
Results
A total of seven (7) RCTs met out inclusion criteria. All studies compared patients receiving orlistat 120 mg/tab TID with placebo. Statistically significant improvement in mean change from baseline levels of total cholesterol (mean difference was −0.33 (95% CI −0.45, −0.42)), low density lipoprotein (mean difference was −0.28 (95% CI −0.36, −0.19) and trigylceride (mean difference was −0.13 (95% CI −0.19, −0.06)) was seen in orlistat group than the placebo, but no statistically significant improvement in mean change from baseline level in high density lipoprotein (mean difference was −0.03 (95% CI −0.05, −0.02)).
Conclusion
Orlistat produces statistically significant improvement in total cholesterol, low density lipoprotein and triglyceride levels in overweight and obese patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1186

**Insulin resistance and acne: a new risk factor for men?**

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Introduction
Acne is a common skin disease that occasionally can be influenced by endocrine abnormalities. In females, a clear relationship has been highlight between acne onset and peripheral insulin resistance and hyperinsulinemia, whereas few data are available in male. The aim of this study was to investigate the relationship between acne and insulin resistance as well as other metabolic impairment in male subjects with acne.
Methods/design
Twenty-two subjects with inflammatory acne, resistant to common therapy guidelines of acne treatment, have been compared to 22 healthy subjects matched for age and gender. Acne was gradued using the global acne grading system score (GAGS). Clinical and biochemical parameters of glucose and lipid metabolism and serum levels of androgens were evaluated. Oral glucose tolerance test (OGTT) was performed and the homeostasis model assessment of insulin-resistance (HOMA-IR) was calculated.
Results
Males with acne had higher BMI ($P<0.01$), WHR ($P<0.05$), SBP ($P<0.01$), DBP ($P<0.01$), basal ($P<0.05$) and 120 min. OGTT serum insulin concentrations ($P<0.01$), basal glucose concentrations ($P<0.05$), HOMA-IR ($P<0.01$) and lower HDL cholesterol than controls ($P<0.01$). Among the subgroup of subjects with BMI <25, HDL cholesterol ($P<0.05$) and 120 min. OGTT serum insulin concentrations ($P<0.05$) resulted to be independent predictors of acne at the multivariate analysis.
Conclusions
These findings highlight that male subjects with acne are affected with a metabolic imbalance. Insulin resistance and low HDL-cholesterol seem to play the main role for the development of acne in males and could represent effective targets for therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1187

**Metabolic syndrome in benign and malignant nodular thyroid diseases**

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Introduction
Metabolic syndrome (MetS) is a group of metabolic abnormalities where insulin resistance (IR) plays a major role. Previous studies have shown that thyroid volume and nodule prevalence were increased in patients with IR. The aim of our study is to evaluate MetS and its components in patients with benign and malignant nodular thyroid disease (NTD).
Methods
A total of 800 patients (430 euthyroid benign and 370 euthyroid malignant NTD) followed in the outpatient clinic of Endocrinology department were analyzed. Fine needle aspiration cytology and histopathological examination, whichever eligible, was considered for the pathological classification of nodules. Serum insulin levels and the level of IR estimated by homeostasis model assessment, as well as other MetS parameters were evaluated. The results were also compared with a reference population study on MetS prevalance.
Results
MetS was detected in 59.8 percent ($n=478$) of 800 patients. The prevalance was 33.8% in the reference population study ($P<0.001$). There was no significant difference between benign and malignant NTD groups related to prevalence of the MetS (61.4 and 57.8% respectively, $P>0.05$). When MetS components were analysed, the most common component was abdominal obesity ($n=520$, 65%), followed by low HDL-C level ($n=518$, 64.8%), and high blood glucose level ($n=246$, 30.8%). There was no significant difference between benign and malignant NTD groups in terms of insulin levels ($9.06\pm5.3$ and $8.68\pm4.8$ respectively, $P>0.05$) and insulin resistance ($2.74\pm2.9$ and $2.4\pm2.2$ respectively, $P>0.05$). Conclusion: When the reference population data is considered, the results suggest that patients with NTD have significantly increased metabolic syndrome prevalence compared to patients without NTD (59.8%, 33.9% respectively). There was no significant difference between benign and malignant NTD in terms of prevalence and distribution of components of MetS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1188

Abstract withdrawn

## P1189

**A pilot study in obese patients who refer to plastic surgery: temperamental-personality traits**

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Literature evidenced correlation between psychiatric disorders and overweight/-obese (BMI>25) conditions, management and treatment. There are involved psychiatric diseases and temperamental traits. Our study, born from collaboration between Psychiatric and Plastic Surgery Clinic of Padua University, wants to detect psychiatric disorders, temperamental traits and body image perception in overweight-obese patients who seek surgical liposuction or abdominoplastic. We studied (March 2008–June 2011), 28 consecutive patients who refer for surgical enhancement (age 18–60 years) with BMI <34.9 at the recruitment. Exclusion criteria are: organic disorders or drugs that could interfere with obesity; cognitive deficit and psychosis. Thirty patients refused psychiatric evaluation. This clinical population has been compared to a control group ($n=25$) from general population. Psychiatric evaluation was based on clinical information, Mini-International Neuropsychiatric Interview, Beck Depression Inventory, Yale Brown Scale, Paykel Life Events Scale, NEO Five Factory Inventory, Tridimensional Personality Questionnaire, Body Shape Questionnaire. Case group evidenced higher scores in lifetime depression and BQ6 scores with a moderate/mild concern with body shapes; focus on personality traits, TPQ revealed higher score in subscale dependence/independence and significant higher presence at YBS for obsessive-compulsive characteristics; NEO-FFI Openness to experience is higher in control. It's unmistakable that affective sphere is relevant but also obsessive-compulsive and temperamental traits and negative body shape perception. These aspects are implicated in treatments adhesion and clinical outcome. Possibility to individuate patients who present those characteristics is an instrument to avoid early post-treatment relapse and give the possibility to refer to psychiatric care before and after surgery.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1190

**Evaluation of success of an obesity consultation (2004–2010)**

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Introduction
Moderate weight loss (5–10%) translates into health benefits, including improvement in cardiovascular risk. It is essential to assess the results and indicators of success in treating obesity, which is the objective of this work.
Methods
A retrospective study of patients with overweight and obesity, followed in Nutrition consultation between 2004 and 2010. We analyzed biodemographic and anthropometric variables, consultation reason and weight history and treatments. We used the *t*-test for independent samples, $\chi^2$ tests to statistical differences and repeated measures model assessed the weight gain. We estimated a multiple linear regression model to identify risk factors in weight loss. The significance level was 5%.
Results
Of 1947 patients with diagnostic of overweight or obesity, we analyzed 389, mean age 52 years ($\pm15.3$), 53% female. Overweight was the main reason for the consultation (63.8%). To all were prescribed a personalized meal plan, encourage physical activity and prescribed drugs to 10.8%. The mean follow-up was 12 months. The greatest weight loss, it was up to 12 months ($P<0.005$). At 3, 6 and 12 months, 66.8, 68.4 and 71.9%, respectively, lost weight. At 12 months, 39.5% lost more than 5% of their initial weight. The women lost more weight at 6 and 12 months ($P=0.045$). The reason for the consultation and number of visits has proven to be indicators of success in weight loss.
Conclusions
Most of the patients lost weight at all assessments and the bigger lost was at 12 months. Motivation and follow-up are indicative of greater success in weight loss.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1191

**Regulation of lipid droplet function in adipocytes: role of RAB18**

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Adipocytes are cells highly specialized in storing excess energy as neutral lipids in lipid droplets (LDs). These organelles act not only as mere fat reservoirs, but

also as active modulators of lipid storage and mobilization in response to hormonal signals. This role is accomplished through a plethora of proteins associated with the LD surface that spatially and temporally coordinate lipid metabolism and traffic on demand. Recently, we have shown that the LD-associated protein Rab18 is involved in insulin-mediated lipogenesis and in β-adrenergic-induced lipolysis in 3T3-L1 adipocytes. Given the involvement of the endoplasmic reticulum (ER) in the biogenesis, maintenance, and regression of LDs, herein we investigated whether Rab18 modulates LD mobilization and LD/ER relationship. Double immunocytochemistry and confocal microscopy revealed that both insulin and the β-adrenergic agonist isoproterenol increased Rab18 recruitment to LDs and the rapprochement of these organelles to ER membranes. However, unlike isoproterenol, insulin treatment induced Rab18-labeled LDs to contact specific regions of the ER membrane enriched in lipogenic enzymes (i.e. diacylglicerol-acyltransferase 2 (DGAT2)). Furthermore, disruption of ER traffic impaired insulin-induced Rab18 association with LDs, whereas isoproterenol effect was unaffected. These data suggest that, depending on the nature of the extracellular stimuli reaching the adipocytes, Rab18 is recruited to LDs from the ER membrane (upon insulin treatment) or, likely, from the cytosol (upon isoproterenol treatment). We also analyzed the role of microtubules in Rab18 association to LDs and LD/ER apposition. Thus, we found that microtubule depolymerization by nocodazole did not affect insulin- and isoproterenol-induced Rab18 mobilization towards LDs but increased LD rapprochement to the ER. Finally, we explored Rab18-interacting proteins by proteomic analysis. Altogether, our data suggest that Rab18 plays a role in the control of lipogenesis- and lipolysis-induced LD/ER association, probably through its interaction with the cytoskeleton.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1192

### Frequency of obesity, insulin resistance, cardio-metabolic risk factors and genetic variation in the genes encoding ADIPOQ and PPAR-γ in urban adults

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The aims of the study were to analyze the relationship among insulin resistance, obesity, dysglycemia, dyslipidemia and to evaluate the role of common variants in candidate genes for their ability to predict the metabolic syndrome and cardio-metabolic risk factors.

Subjects and methods

752 non-diabetic individuals (190 males and 562 females; age 35.05 + 4.47 years, BMI 26.72 + 5.92 kg/m$^2$) participating in the study were genotyped for variants in peroxisome proliferator-activated receptor gamma (PPAR-γ2 Pro12Ala) and in adiponectin (ADIPOQ 45T > G; 276C > T) genes. Fasting blood sample was examined: lipids (total cholesterol, HDL-C, LDL-C, TAG), adiponectin (Adp), insulin; HOMA-IR was calculated. Regression analyses were used to find the associations of single nucleotide polymorphisms with cardio-metabolic risk factors.

Results

We found higher frequency of abdominal obesity in females, but frequency of lipid disorders, prediabetes, arterial hypertensive and insulin resistance was higher in males.

The frequency of SNPs PPAR-γ Pro12Ala, ADIPOQ +45T > G (rs2241766), and +276G > T (rs 1501299) in the whole study population ($n$ = 752) and in the obesity subjects ($n$ = 368) is comparable (0.158 and 152; 0.064 and 0.058; 0.256 and 0.276, respectively).

In men T allele ADIPOQ rs 1501299 is consistently associated with obesity ($P$ = 0.002), abdominal obesity ($P$ = 0.04), prandial hyperglycemia ($P$ = 0.002); G allele ADIPOQ rs 1501299 plays a protective role for HDL cholesterol level ($P$ = 0.047).

In women the PPAR-γ2 Ala12 variant plays a protective role in hypercholesterolemia ($P$ = 0.01). T allele ADIPOQ rs2241766 is significantly associated with reduced risk of obesity ($P$ = 0.004).

Conclusion

The allelic variation of the genes encoding adiponectin and PPAR-γ is gender-dependently associated with obesity and cardio-metabolic risk factors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1193

### Melanocortin 4 receptor genetics in defining the morbid obesity in general population: common polymorphisms vs mutations

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Since the discovery of melanocortin 4 receptor (MC4R) as an important regulator of food intake it has been recognised as one of the major monogenic factors of obesity. However the mutations identified are usually very rare, not found in control subjects from the background population and can explain only a minor part of heritability (2–3%). Here we explore the role of non-synonymous mutations identified by MC4R sequencing in cohort of 380 severely obese individuals (BMI 39.3–67.9 kg/m$^2$) selected from Genome Database of Latvian Population comparing to 380 controls with normal BMI (18.5–24.9 kg/m$^2$). We also investigated the role of SNP rs17782313 located upstream of the MC4R that have been previously identified from GWAS in the same case-control samples. The genetic association revealed no correlation between rs17782313 and obesity or related traits. The sequencing of the MC4R coding region revealed four non-synonymous substitutions: V103I, S127L, V166I and I251L. Two subjects had double V103I and S127L mutations. Noticeably, V166I is a novel substitution that has not been reported before. S127L, V166I and double V103I/S127L mutant receptors had significantly decreased expression on cell surface compared to wt MC4R. Intriguingly, despite the low abundance in cell membrane the newly discovered V166I variant demonstrated higher cAMP response upon αMSH activation than wt receptor, while S127L mutation did not display any significant cAMP response. In opposite to MC4R we found that three SNPs in another obesity related fat mass and obesity-associated protein gene locus (FTO) are associated with obesity in our study group (the lowest $P$ value = 0.019). In conclusion, we show that non-synonymous mutations can only explain 1% of morbid obesity. Thus, neither the common SNP nor rare mutations are the major genetic cause of obesity in general population in opposite to FTO gene.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1194

### Effects of bezafibrate treatment on pancreatic remodeling and insulin resistance in ovariectomized C57BL/6 mice fed high-fat diet

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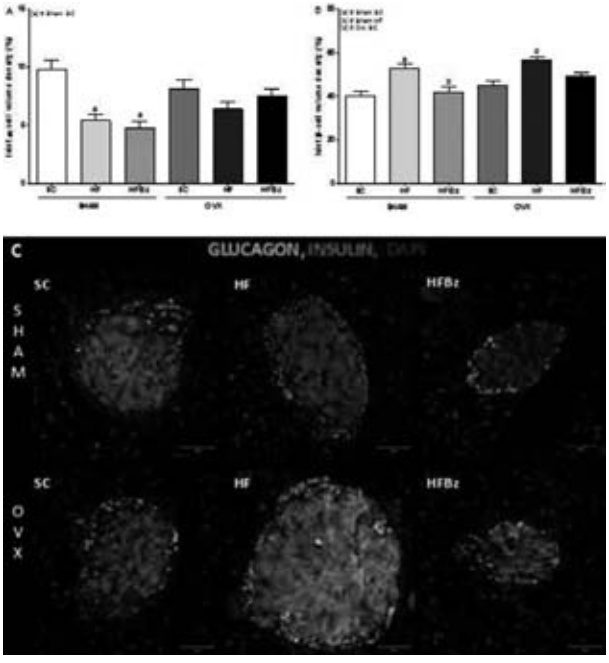
Introduction

Ovariectomized C57BL/6 mice are a good model to study the postmenopausal stage. It has been reported that high-fat diet (HFD) augments the body fat mass

and insulin resistance (IR) in animal models. Goal of this study was to investigate the combined effects of ovariectomy (OVX) and HFD on insulin sensitivity and pancreatic remodeling in C57BL/6 mice treated with bezafibrate.
Methods
Female C57BL/6 mice were subjected to surgical OVX and surgery without removal of the ovary (SHAM). Animals received standard chow diet (SC 10% lipids) or high-fat diet (HF 60% lipids). After 13 weeks of diet, the animals were subdivided in six groups ($n=5$), diet and/or bezafibrate: SHAM-SC, SHAM-HF, SHAM-HF-Bz, OVX-SC, OVX-HF and OVX-HF-Bz. After treatment of 5 weeks, the pancreas was removed and analyzed by morphometry, immunostaining and biochimical assay.
Results
OVX groups showed atrophied uterus compared to the SHAM groups, ensuring the success of surgically-induced menopause. Analyzing the effects of OVX and HF, the SHAM-HF and OVX-HF mice showed higher fasting glucose, plasma insulin, and HOMA-IR; increased body mass, islet hypertrophy, β-cells mass, and insulin immunostain; and decreased GLUT2 immunostain. Bezafibrate treatment reduced body mass, plasma insulin and HOMA-IR, and prevented islet hypertrophy.

**Table 1** Statistics results in C57BL/6 mice.

| Parameters | Groups | | | | | |
|---|---|---|---|---|---|---|
| | SHAM | | | OVX | | |
| Food intake (g)/day per mouse | 2.27±0.03 | 1.94±0.04a | 1.95±0.04 a | 2.21±0.04 | 1.91±0.03d | 1.89±0.04d |
| Body mass at 18 week (g) | 21.90±0.34 | 23.32±0.71 | 23.39±0.43 | 23.19±0.49 | 29.95±0.92bd | 26.52±0.53cde |
| Uterus mass (g) | 0.14±0.01 | 0.14±0.02 | 0.13±0.02 | 0.03±0.01a | 0.04±0.01b | 0.03±0.01c |
| Basal glucose (mmol/l) | 5.63±0.19 | 6.30±0.32 | 7.14±0.01a | 6.71±0.10 | 8.01±0.37bd | 8.21±0.30d |
| OGTT, AUC | 1518±568 | 1561±1390 | 1902±593 | 1719±793 | 2429±1403bd | 2294±983d |
| Serum insulin (μUI/l) | 11.86±0.76 | 10.23±0.77 | 9.91±0.77 | 15.07±1.08 | 19.37±1.19bd | 9.70±0.80de |
| HOMA-IR | 3.06±0.22 | 3.00±0.24 | 3.14±0.25 | 4.47±0.27 | 7.18±0.50bd | 3.57±0.36e |
| Pancreas mass (g) | 253.6±0.01 | 266.5±0.01 | 222.2±0.01 | 261.5±0.01 | 318.2±0.01bd | 282.0±0.01 |
| Vv islet (%) | 9.36±0.54 | 14.05±0.60a | 8.10±0.66b | 11.36±0.83 | 14.19±0.79 | 10.34±0.70e |
| Islet mass (mg) | 24.30±1.48 | 38.32±1.84a | 19.21±1.71b | 32.13±2.76 a | 44.02±2.45d | 29.16±2.08ce |
| b-cell mass (mg) | 17.51±2.18 | 24.54±2.06 | 10.37±2.00b | 20.54±2.27a | 20.63±2.55e | 20.63±2.55e |




Conclusion
The model of ovariectomy associated with HFD accentuated the effects of menopause, leading to the development of IR. Bezafibrate treatment reduces body mass, and also reduces plasma insulin and pancreatic islet hypertrophy in mice fed HFD.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1195

See OC5.1

## P1196

### Prediabetes in adult GH deficiency is associated with a substantial reduction in serum and adipose tissue expression levels of zinc-α2-glycoprotein

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Adult GH deficiency (GHD) is associated with prediabetes and low levels of GH are found in obesity. We hypothesized that zinc-α2-glycoprotein (ZAG), a novel adipokine reduced in obesity, is regulated by GH. ZAG serum and adipose tissue expression were measured in three study populations: i) cachectic (BMI=19.3 ±0.5 kg/m$^2$), lean/overweight (BMI=26.0±0.5 kg/m$^2$) and obese (BMI=36.0±1.6 kg/m$^2$) patients with chronic obstructive pulmonary disease (COPD, 62.5±1.1 years); ii) GHD adults ($n=16$, 30.6±1.1 years, BMI=27.1 ±1.2 kg/m$^2$) and 16 healthy matched controls; iii) patients with metabolic syndrome subjected to 6-months therapy with rhGH ($n=10$, 40–70 years, BMI=27–35 kg/m$^2$). Metabolic phenotyping included insulin sensitivity (EHC), body composition (MRI), OGTT and muscle lipid content ($^1$H-MRS). Adipose tissue samples were taken by needle biopsy. Adipocyte diameter was measured histomorphometrically. Gene expression was assessed by qRT-PCR, protein by immunoblotting and serum ZAG with ELISA. Compared to obese, cachectic COPD patients had fourfold increased ZAG in adipose tissue and serum ($P<0.01$). Both ZAG serum and mRNA levels correlated positively with circulating GH ($r=0.40$, $P<0.01$; $r=0.60$, $P<0.001$). Furthermore, serum and adipose tissue of prediabetic GHD adults displayed 71 and 62% decrease in ZAG mRNA and serum levels ($P<0.001$). Treatment with rhGH led to 1.8-fold increase in ZAG mRNA ($P<0.001$). ZAG protein decreased in response to euglycemic hyperinsulinemia. In addition, ZAG mRNA was positively associated with insulin sensitivity ($r=0.69$, $P<0.001$), serum adiponectin ($r=0.48$, $P<0.01$) and adiponectin mRNA ($r=0.49$, $P<0.01$). Negative correlations were found with fat cell size ($r=-0.56$, $P<0.001$) and intramyocellular lipids ($r=-0.61$, $P<0.05$). Our results clearly demonstrate the down-regulation of ZAG in serum and adipose tissue of prediabetic GHD adults as well as positive regulation of ZAG by rhGH, suggesting the regulatory role for GH independent on obesity. Associations of ZAG with insulin sensitivity, molecular adipose tissue phenotypes and intramyocellular lipids suggest the role for reduced ZAG in the development of metabolic disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1197

**Signalling properties of the melanocortin 3 receptor are modified by interaction with different ghrelin receptor variants in heterodimers**

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It is a widely accepted concept that G-protein-coupled receptors (GPCRs) are able to form homo- and heterodimers. The physiological relevance of these interactions is of utmost importance.
Recently, we demonstrated that due to co-expression of two key players of hypothalamic body weight regulation, the melanocortin 3 receptor (MC3R) and ghrelin receptor (GHSR), signalling of the MC3R is enhanced (hyperstimulation) as compared to activation of MC3R alone. By using two naturally occurring GHSR mutations (GHSR-A204E, GHSR-F279L) that silence the basal ligand independent activity we showed that the basally active conformation of GHSR is a crucial factor for modified signalling properties in MC3R/GHSR heterodimers.
The aim of this study was to confirm these previous findings by investigation of further naturally occurring GHSR-mutants in co-expression studies with MC3R.
We tested seven already published GHSR variants. Most of these mutations lead to a decreased ligand independent activity and/or to a decreased cell surface expression of GHSR. We measured Gs-induced cAMP accumulation of the co-expressed MC3R and the GHSR-mutants in COS-7 cells after stimulation with 1 μM α-MSH.
The co-expression of six tested GHSR-mutants with the MC3R and α-MSH challenge prevented MC3R hyperstimulation in contrast to the MC3R co-expressed with the GHSR-WT. Only one GHSR-mutant resemble the characteristic MC3R/GHSR hyperstimulation.
Taken together, by testing seven GHSR-mutants we here show that our initial finding of the importance of high basal GHSR activity in a heterodimeric constellation with MC3R is a general phenomenon and that specific GHSR variants almost always modulating MC3R signalling in heterodimers.
This study therefore gives general pathological implications and mechanistic insights into the role of GHSR and MC3R in the appetite regulation by interactive mutual effects on each other. Finally, our study support that elevated basal signalling activity is of high importance to understand the signalling network of GPCRs.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1198

**Metabolic signature of visceral and subcutaneous obese adipose tissue secretomes reveal altered tissular amino acid metabolism**

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Introduction
Obesity and its associated chronic comorbidities represent a worldwide health problem in epidemic increase. Recent researches have reported notable differences in the amino acids metabolism signatures between obese, mild insulin resistant but non-diabetic, and lean individuals. Moreover, amino acids metabolites are emerging as strong predictors in the development of diabetes in obesity. The contribution of the obese adipose tissue from various depots to the systemic altered amino acids pool is still under debate.
Goal
In the present study we applied a metabolomic approach to direct analyze the amino acids metabolites pattern of the secretomes of visceral and subcutaneous obese adipose tissue in obese subjects, without other major metabolic risk factors, as compared to lean controls.
Subjects and methods
Fat pads were isolated from obese ($n=8$) and non-obese ($n=8$) subjects during laparoscopic surgery. Subcutaneous (periumbilical) and visceral (omental) adipose tissue were incubated with serum-free medium to obtain their secretomes. Metabolite comparative profiling of the lean versus obese secretomes was performed by untargeted gas chromatography-mass spectrometry approach and results analyzed using standard tools.
Results
A significant increased release of alanine, 4-hydroxiproline and 2-ketoisocaproic acid was accounted for all the obese adipose tissue secretomes. Net visceral depot uptake of visceral obese adipose tissue of essential and branched-chain amino acids (BCAA: lysine, leucine; methionine and threonine) and glutamate was significantly diminished in obese as compared to the non-obese visceral adipose tissue secretomes.
Conclusion
This is the first translational metabolomic study reporting that obesity markedly affects the metabolic signature of adipose tissue secretomes. The highest alteration of the amino acids metabolite pattern is present in the visceral adipose fat depot secretome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1199

**Time-course of changes of GLUT4 expression in insulin-sensitive tissues in an animal model of metabolic syndrome**

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Background
Reduced GLUT4 expression in insulin-sensitive tissues, insulin resistance, inflammation, nonalcoholic steatohepatitis (NASH) and hypertension are pathophysiologic changes expected in metabolic syndrome animal models.
Aims
To fully characterize the development and maintenance of metabolic syndrome features over time (GLUT4 in insulin-sensitive tissues, insulin resistance, inflammation, blood pressure and liver changes) in spontaneously hypertensive rats (SHR) treated with monosodium glutamate (MSG).
Methods
Spontaneously neonate hypertensive rats (18/group) were treated with MSG (MetS) during 9 days, and compared with Wistar-Kyoto (C) and saline-treated SHR (H). Insulin resistance (glucose decay constant rate, kITT), blood pressure (BP), C-reactive protein (CRP), interleukin 6 (IL6), TNF-α, adiponectin, GLUT4 protein (heart, white adipose tissue and gastrocnemius), liver histology and immunohistochemistry for IL1β were evaluated at 3, 6 and 9 months of age.
Results
MetS rats were more insulin resistant ($P<0.001$ for all ages) and had higher BP ($P<0.05$ for all ages) as compared to C. At 6 months, CRP, IL6 and TNF-α were higher ($P<0.001$ for all comparisons) in MetS rats vs H; adiponectin was lower in MetS at 9 months (MetS: $32\pm2$, H: $42\pm2$, C: $45\pm2$ pg/ml; $P<0.001$). GLUT4 protein was reduced in MetS as compared to C: in the heart and gastrocnemius, GLUT4 was 30–40% lower at 3-mo, and it was maintained at 6- and 9-mo. In white adipose tissue, GLUT4 was reduced by ~50% at 3-mo, and at 6- and 9-mo

it was still 20% lower. Histological analysis showed hepatocyte ballooning and fibrosis in the MetS group at 9-mo, and increased IL1β staining when compared with C and H at all ages.
Conclusions
Spontaneously hypertensive rats treated with MSG presented and maintained all metabolic syndrome characteristics, including insulin resistance, high BP and inflammatory marker levels, NASH characteristics and also reduced GLUT4 content in insulin-sensitive tissues.
GLUT4 protein expression at 3, 6 and 9 months of age. The western blot bands and their respective quantitative analyses are represented. C: Wistar-Kyoto rats that did not receive any treatment; H: spontaneously hypertensive rats that did not receive any treatment; MetS: spontaneously hypertensive rats that received MSG during the neonatal period. $n=5$ in all groups. Two-way ANOVA panel A: group, time and interaction ($P<0.001$); Panel B: group ($P=0.006$), time ($P<0.001$) and interaction ($P=0.003$); Panel C: group, time and interaction ($P<0.001$), followed by the Bonferroni's *post hoc* test: ***$P<0.001$ vs. C; $^{\dagger}P<0.05$ and $^{\dagger\dagger\dagger}P<0.001$ vs H at same time. The time course changes inside groups are also showed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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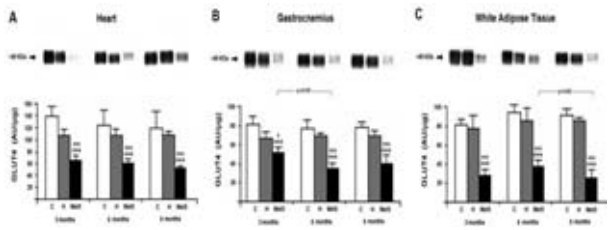


## P1200

**Acute ghrelin effects on depressive-like behavior in bulbectomyced mice: a possible mechanism of action**

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Ghrelin (Ghr) is an orexigenic peptide investigated by Lutter and col. 2008 for its potential role in the development of depressive symptoms induced by chronic stress, suggesting that Ghr defends against depressive-like symptoms. Rodent bilateral olfactory bulbectomy (OB) is an animal model that appears to fulfill many of the criteria required for the study of depression; hence, Ghr could be an alternative therapeutic tool for depression therapy. We study the possibility that Ghr could reverse some depressive-like behaviors induced by OB in mice, using the tail suspension test (TST), a test predictive of antidepressant activity, and Ghr effect on mood regulatory hormones. Adult female Albino's Swiss mice were divided in sham and OB, and subsequently infused with intracerebroventricular (ICV) saline (S) or Ghr (0.03 or 3.0 nmol/μl). After the TST was applied, mice were sacrificed and the hypothalamus was dissected in order to study the mRNA expression of genes related to mood using real time PCR.
The OB animals treated with S (OB-S) presented an increase on immobility time compared to sham ($P<0.05$), but the acute infuse of Ghr 3.0 nmol/μl induced a decrease on immobility time ($P \leq 0.05$). The OB infused with Ghr 0.3 nmol/ul showed higher expression of delta opioid receptor (DOR) compared to OB-S ($F=5.484$, $P=0.009$) and sham that received the same dose of Ghr ($F=8.243$, $P=0.0072$). Similar effect was observed in the expression of kappa ($P<0.05$) and Mu ($P<0.01$) opioid receptor. The results show that Ghr 3.0 nmol/μl could reverts the immobility response in OB mice and provide information about the molecular mechanisms of this effect, showing its potential as antidepressant drug.
Declaration of interest
I declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1201

**Association of the obesity/type 2 diabetes-related genetic variants with visceral fat acccumulation in obese, overweight and lean subjects**

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Introduction
It has been recently suggested the genetic predisposition to obesity leads to increased risk of type 2 diabetes by its obesity predisposing effect. Surprisingly genetic loci that contribute to the development of obesity identified by genome-wide association studies (GWAS) account for only 2% of the variance in BMI. Visceral fat accumulation has an important role in increasing the risk of developing metabolic disorders, such as type 2 diabetes, dyslipidemia and CVD, but data concerning the genetic background of body fat distribution are limited. The aim of our study was to analyze whether there are specific genetic factors that influence the risk of visceral fat accumulation.
Description of methods
We genotyped 64 SNPs within 40 genes associated with obesity and/or type 2 diabetes (identified by GWAS) in 468 overweight/obese patients and 245 healthy volunteers with normal weight (302 men and 411 women), who underwent anthropometry (BMI, WHR) and body composition analysis: body fat percentage, visceral (VAT) and subcutaneous abdominal adipose tissue (SAT) by multi-frequency bio-impedance method.
Results and conclusion
The association of visceral fat content and VAT/SAT ratio with the distribution of genotypes of the following SNPs: MC4R rs1350341 ($P=005$; $P=0.015$), MC4R rs17782313 ($P=0.035$, $P=0.002$), PPARG rs1801282 ($P=0.02$, $P=0.0019$); BDNF rs6265 ($P=0.034$, $P=0.006$) and SH2B1 rs7498665 ($P=0.042$, $P=0.0038$) has been found in the studied subjects. Our results suggest that there are VAT-specific genetic factors that influence the risk of visceral fat accumulation. The identified genetic variants may help to understand the mechanisms of body fat distribution and serve as early biomarkers of increased risk of metabolic disorders development in overweight/obese patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1202

See S70.5

## P1203

**Loss of hypothalamic-pituitary-adrenal axis improves obese phenotype and restores hypothalamic agouti-related protein mRNA in leptin-deficient ob/ob mice**

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Purpose
Glucocorticoid excess causes obesity and hyperphagia (e.g. Cushing syndrome), but the molecular mechanism of glucocorticoid-induced hyperphagia is not fully understood. To clarify this point, we investigated here the phenotype of leptin and Corticotropin-releasing hormone (CRH) double knockout mice (DKO).
Methods
Leptin-deficient ob/ob mice were bred with CRH-deficient mice for generation of DKO mice. We examined body weight, food consumption and hypothalamic appetite-related neuropeptide mRNA levels [neuropetide Y (NPY), proopiomelanocortin (POMC) and Agouti-related protein (AgRP)] by quantitative real time PCR, in wild-type littermates (WT), ob/ob and DKO. We also examined the effect of glucocorticoid replacement (corticosterone pellet implantation for 4 weeks) on DKO.
Results
Circulating glucocorticoid levels were markedly high in ob/ob but low in DKO (CORT: WT 4.2±3.5, ob/ob 206.1±48.1, DKO 0.0±0.0 ng/ml). At 9 weeks old, body weight and food intake in DKO were decreased compared with ob/ob, but still increased compared with WT (body weight: WT 25.4±0.3, ob/ob 42.7±1.6, DKO 35.2±0.5 g). The changes of hypothalamic appetite-related neuropeptide levels observed in ob/ob, such as increased in NPY and AgRP mRNA and decreased in POMC mRNA, were attenuated in DKO, especially, hypothalamic AgRP mRNA were dynamically changed (AgRP/GAPDH: WT 100.0±6.7, ob/ob 509.4±33.2, DKO 90.8±8.3). Finally, glucocorticoid replacement on DKO caused to increase food intake and hypothalamic AgRP mRNA (AgRP/GAPDH: WT 100.0±7.8, ob/ob 521.3±59.5, DKO 94.2±8.8, DKO +CORT 243.4±20.0), but there were no alteration in NPY or POMC.
Conclusion
These results suggest that ob/ob mice require glucocorticoid excess and related changes in hypothalamic neuropeptides (NPY, POMC and AgRP) to maintain their body weight and appetite. Because hypothalamic AgRP expressions are quite parallel to circulating glucocorticoid levels in the present study, hypothalamic AgRP is considered as a key molecule in glucocorticoid-induced hyperphagia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1204

**Different metabolic response to intagastric balloon treatment between non-morbid and morbid type of obesity**

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BioEnterics Intragastric Balloon (BIB) is a non-invasive procedure which proved to be effective in short-term treatment of obesity. It's effect on alterations in metabolic parameters is poorly understood. Since our previous study showed different ghrelin and leptin response between groups of morbidly (M) and non-morbidly (NM) patients after BIB placement, we aimed to investigate the alteration dynamics in metabolic parameters between these two groups.
Our prospective single-center study included 44 Caucasians treated with BIB for six months, with age range of 20–59, and divided to NM or M type of obesity based on body mass index (BMI) cutoff of 40 kg/m$^2$. Control blood samples were collected at 1, 3, 6 and 12 months after BIB placement. Serum glucose, insulin, C-peptide, glycated hemoglobin, GH, uric acid, lipidogram and homeostatic model assessment 2 (HOMA2) were determined.
Significant differences were observed in anthropometrics without differences between genders or comorbidities. The baseline BMI for NM vs M was 37.65 vs 44.60 kg/m$^2$. Weight loss was statistically different ($P<0.001$) between the studied groups with a median control weight at 6 months of 33.05 vs 39.65 kg/m$^2$. Fasting insulin, HOMA2, glycated hemoglobin and uric acid decreased to similar values in both groups during sixth month, but significantly greater decrease was observed in NM group during first and third month. GH increased substantially in both groups but significantly greater increase occurred in NM group during third month. Fasting serum glucose, C-peptide, cholesterol and triglycerides slightly decreased but without the difference in dynamics.
To our knowledge this is the most detailed study on metabolic parameters in obesity treatment. The results suggest a potential pattern of individualization between NM and M obese patients which could influence future indications for BIB use. Additionally, further studies are needed in order to enlighten the pathophysiologic mechanisms of obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1205

**Metabolic and hormonal consequences of circadian rhythm disruption in Bmal1 null mice**

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There is compelling evidence that shiftworkers are at risk of developing metabolic disturbances, diabetes and obesity as a result of disruption to circadian rhythms, but the mechanisms by which this may occur are not clear. The *Bmal1* null mouse, which lacks both central and peripheral tissue rhythms, was used in this study to address the role of circadian rhythmicity in metabolic homeostasis. Male Bmal1 null mice and wild-type litter mates (2 months) were killed 4 hourly over 24 h and blood, liver and adipose tissue collected. Separate groups were subjected to glucose, pyruvate and insulin tolerance tests. Male and female Bmal1 null mice fed a 22% fat (w/w) diet were killed at 2 months and blood and tissues collected.
Across 24 h plasma insulin was lower and adiponectin and leptin were higher in *Bmal1* null mice compared to wild-type mice while glucose and NEFA were unchanged. Liver *pfkfb3* mRNA in *Bmal1* null mice was decreased, but expression of *per2*, *gck*, *pck1* and *adipor2* mRNA was unchanged and the rhythm of *per2*, *gck*, and *pfkfb3* mRNA absent. In adipose tissue, *per2*, *adipoq*, *retn* and *nampt* mRNA expression was decreased in *Bmal1* null mice and the rhythm of expression of *per2*, *adipoq* and *nampt* mRNA absent.
*Bmal1* null mice had normal glucose and insulin tolerance, but both male and female *Bmal1* null mice had *improved* pyruvate sensitivity. *Bmal1* mice of both sexes weighed less than wild type mice, but had larger epigonadal and retroperitoneal fat depots, although they responded similarly to the high fat diet with respect to increased adiposity and hormone changes.
These studies provide further evidence for a role of circadian rhythmicity in the glucose/insulin and adipoinsular axes. Disruption of tissue rhythmicity in shiftwork, in conjunction with poor diet and sleep loss may contribute to the health burden experienced by shiftworkers.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1206

**Aldosterone increases oxidative stress in differentiated, but not in undifferentiated 3T3-L1 adipocytes via activation of mineralocorticoid receptors**

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In the cardiovascular system, aldosterone promotes inflammation, tissue remodeling and endothelial dysfunction. In diet-induced obesity, lipid accumulation involves acceleration of preadipocyte differentiation into mature adipocytes. Mutually exerted control mechanisms may exist between aldosterone secretion and fat: circulating aldosterone is increased in proportion to fat mass in humans and fat tissue expresses mineralocorticoid receptors (MR). Here we tested whether or not aldosterone affects oxidative stress in 3T3-L1 cells, a rodent line of preadipocytes which undergoes differentiation to mature fat cells under the

combined influence of serum, dexamethasone and insulin. First, both undifferentiated and differentiated 3T3-L1 cells expressed MR as quantified by Real Time PCR. Second, aldosterone (at a physiological concentration-1 nmol/l) had no effect on cell differentiation as assessed by the expression of fat cell differentiation markers, including AP2 and PPARγ. Third, aldosterone (1 nmol/l) induced a ∼3.5 increase in the formation of intracellular reactive oxygen species (ROS) as determined by oxidative conversion of cell-permeable chloromethyl-2′,7′-dichlorodihydroflurescein diacetate to fluorescent dichlorofluorescein (DCF) in both differentiating and fully differentiated adipocytes but not in undifferentiated 3T3-L1 preadipocytes. This effect was fully blocked by the specific MR antagonist eplerelone (100 nmol/l). We identified three isoforms of lipoxygenase (LO), dioxygenase enzymes which incorporate molecular oxygen into unsaturated fatty acids such as arachidonic acid and linoleic acid in these cells, platelet 12(S)-LO, leukocyte type (12/15)- and an epidermal LO, of which the expression of the platelet and leukocyte type increased with aldosterone treatment. One LO product, 12 hydroxyeicosatetraenoic acid (12HETE) was able to increase ROS formation in differentiated 3T3-L1 cells (X2-3 folds). These results suggest that aldosterone can increase oxidative stress in differentiated, but not undifferentiated adipocytes, indicating that fat accumulation in mature fat cells predisposes adipose tissue to the pro-oxidative effect of aldosterone, and perhaps serves as a means to increase ROS through oxidation of fatty acids such as 12HETE.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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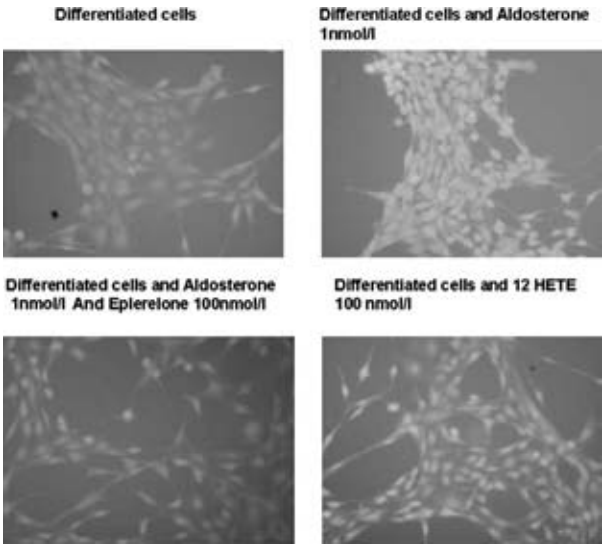


## P1207

### Testosterone protects from metabolic syndrome-associated prostate inflammation: an experimental study in rabbit

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Metabolic syndrome (MetS) and benign prostatic hyperplasia (BPH)/lower urinary tract symptoms (LUTS) are often associated. One of their common denominators is hypogonadism. However, testosterone supplementation is limited by concerns for potential prostatic side effects. The objective was to determine whether MetS-associated prostate alterations are prevented by testosterone supplementation. We used a previously described animal model of MetS, obtained by feeding male rabbits a high-fat diet (HFD) for 12 weeks. Subsets of HFD rabbits were treated with testosterone or with the farnesoid X receptor agonist INT-747. Rabbits fed a standard diet were used as controls. HFD-animals develop hypogonadism and all the MetS features: hyperglycemia, glucose intolerance, dyslipidemia, hypertension, and visceral obesity. In addition, HFD-animals show a prostate inflammation. Immunohistochemical analysis demonstrated that HFD-induced prostate fibrosis, hypoxia, and inflammation. The mRNA expression of several proinflammatory (IL8, IL6, IL1β, and TNFα), T lymphocyte (CD4, CD8, Tbet, Gata3, and ROR γt), macrophage (TLR2, TLR4, and STAMP2), neutrophil (lactoferrin), inflammation (COX2 and RAGE), and fibrosis/myofibroblast activation (TGFβ, SM22α, αSMA, RhoA, and ROCK1/-ROCK2) markers was significantly increased in HFD prostate. Testosterone, as well as INT-747, treatment prevented some MetS features, although only testosterone normalized all the HFD-induced prostate alterations. Interestingly, the ratio between testosterone and estradiol plasma level retains a significant, negative, association with all the fibrosis and the majority of inflammatory markers analyzed. These data highlight that testosterone protects rabbit prostate from MetS-induced prostatic hypoxia, fibrosis, and inflammation, which can play a role toward the development/progression of BPH/LUTS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1208

### GPR83 in interaction with GHSR and MC3R: lessons from *in vitro* studies

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GPR83 is an orphan G-protein coupled receptor (GPCR) and is expressed in thymus and brain. Within the brain GPR83 mRNA is identified at high levels in the hypothalamic arcuate nucleus that is known to control for body weight regulation.

Expression of GPR83 in the arcuate nucleus and the protection of GPR83 knock-out mice against diet induced obesity prompted us to investigate GPR83 signaling properties as well as potential interaction with the melanocortin 3 receptor (MC3R) and the growth hormone secretagogue receptor (GHSR). Both receptors are also localized in the arcuate nucelus and they are known to be key-players of body weight regulation. Recently we demonstrated that MC3R and GHSR are able to form heterodimers which modulates mutually the signaling properties of both receptors.

In this study investigation of cAMP accumulation and IP3 formation *in vitro* revealed no basal activity of GPR83 in Gs nor Gi, but significant basal activity in Gq/11 mediated signaling. Due to unknown GPR83 ligands we designed and tested a potential constitutively active mutant in transmembrane helix 6 and confirmed thereby activation of Gq/11 signaling. To test for GPR83/MC3R and GPR83/GHSR heterodimerization a sandwich ELISA as well as Yellow fluorescent protein complementation assay were utilized and both methods confirmed interaction of GPR83 with MC3R or GHSR *in vitro*. Functional characterization of the GPR83/MC3R and GPR83/GHSR heterodimers in coexpression studies revealed a modification of signaling properties compared to monomers or homodimers: down-regulation of GHSR signaling in response to ghrelin and up-regulation of MC3R in response to its ligands alpha- and gamma-MSH.

In conclusion, GPR83 might be a new important key-player in hypothalamic body weight regulation and could therefore represent a value target to modulate energy metabolism. This receptor couples intracellularly to Gq and is able to modify by formation of heterodimers the signaling capacities of MC3R and GHSR.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1209

**Metabolic effects of 3-iodothyronamine in hepatocytes**

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3-iodothyronamine (T1AM) is an endogenous thyroid hormone derivative which produces profound metabolic effects such as a shift in fuel substrate utilization from carbohydrates to lipid. Thus, in the present work we investigated the effects of T1AM on hepatic glucose metabolism.

To assess metabolite release, human hepatocellular carcinoma cells (HepG2) were exposed for 4 h to different concentrations of exogenous T1AM (0.1, 1 and 10 μM) in glucose production buffer (DME base containing pyruvate and lactate). Cell culture medium was then collected and glucose and ketone body (acetoacetate and β-hydroxybutyrate) levels were evaluated. In addition, isolated rat liver preparations were perfused either with Krebs-Henseleit buffer or glucose production buffer containing 1 μM T1AM. The effluent perfusate was then collected for 60 min at 5 min intervals to measure the release of glucose and ketone bodies (acetoacetate and β-hydroxybutyrate).

In HepG2, only infusion with 1 μM T1AM induced a significant increase in glucose production ($9.11 \pm 0.37$ vs $7.48 \pm 0.14$ μg/mg of total proteins in cell lysate, $P < 0.01$), and a significant decrease in acetoacetate release ($252.4 \pm 10.5$ vs $286.8 \pm 7.2$ nmol/ mg of total proteins in cell lysate $P < 0.05$).

Liver perfusion with glucose production buffer in the presence of 1 μM T1AM also showed a significant increase of glucose production ($0.555 \pm 0.062$ vs $0.369 \pm 0.043$ mg/min per g $P < 0.05$), while infusion with Krebs-Henseleit buffer did not produce any significant change in glucose metabolism.

In conclusions our preliminary data suggested that T1AM stimulated gluconeogenesis and inhibited ketogenesis under conditions of glucose deprivation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1210

**The adipokines TNF-α and IL6 predicted incident cancer development in a Chinese community-based cohort**

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Adipose tissue is recognized as an active endocrine organ. Cytokines released from adipocytes induce chronic low-grade inflammation, which is linked to the development of cardiovascular diseases and malignancy.In this study, we examined the relationship between baseline adipokines level and incident cancer risk in a community-based cohort of Chinese subjects. Subjects were recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study 2(CRISPS 2) cohort. Those with known malignancy were excluded. Fasting blood forglucose, insulin, lipids, the adipokines interleukin-6 (IL6), soluble tumor necrosis factor receptor 2 (sTNFR2, as a surrogate marker of tumor necrosis factor-alpha), adiponectin and adipocyte-fatty acid binding protein (A-FABP) levels was collected at baseline. Incident cancer cases were identified after a median follow up of 9.6 years. 1897 subjects were included in the final analysis. During the study period, 94 subjects developed cancers. Compared to subjects with no cancer, subjects who developed cancer were older at baseline ($61.0 \pm 11.5$ vs $51.9 \pm 11.8$, $P < 0.001$), had a higher proportion of males (59.6 vs 45.9%, $P = 0.009$), central obesity (38.3 vs 29.6%, $P = 0.008$), diabetes (27.7 vs 14.8%, $P = 0.036$), hypertension (36.2 vs 26.7%, $P = 0.045$) and dyslipidemia (71.9 vs 60.6%, $P = 0.013$). They had a higher serum level of IL6 (0.78 vs 0.58 pg/ml in male, 0.65 vs 0.54 pg/ml in female, $P < 0.001$), sTNFR2 (2419.2 vs 1991.7 ng/ml in male, 2051.7 vs 1816.0 ng/ml in female, $P < 0.001$) and A-FABP (21.0 vs 19.0 ng/ml in male, 24.2 vs 27.9 ng/ml in female, $P = 0.02$). Adiponectin level between the two groups was not significantly different. After adjustment for conventional risk factors, only baseline IL6 (HR 1.51, 95% CI 1.16–1.97) and sTNFR2 (HR 2.36, 95%CI 1.16–4.81) predicted incident cancer development. Our data supported the hypothesis that chronic low grade inflammation caused by obesity could increase the risk of malignancy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1211

**ASP injection promotes macrophage infiltration and M1 polarization in obese adipose tissue**

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Recent integrative approaches showed that adipose tissue play a major endocrine role. Molecules secreted by the expanding adipose tissue modulate systemic inflammation and contribute heavily to the pathology of obesity co-morbidities. Acylation stimulating protein (ASP) is an adipokine that has been linked with energy metabolism and lipid metabolism. The ASP receptor, C5L2, is widespread within tissues in the organism, yet ASP effects outside the adipose tissue remain explored. Both ASP and its receptor, C5L2, are also linked to immunity and inflammation. We hypothesized that ASP could play an inflammatory role in addition to its metabolic effects and investigated ASP acute effects in mice.

In the present study, we show that acute ASP injection influences substrate partitioning. ASP increases glucose uptake in the skeletal muscle and adipose tissue and decreases liver glucose absorption. A more rapid glucose clearance is seen after ASP injection, with lower associated insulin levels. Insulin sensitivity is left unaltered by ASP treatment.

In addition, we show that macrophage infiltration within the adipose tissue, skeletal muscle and liver is mildly increased by ASP injection. While anti-inflammatory M2 macrophage tissue content is unaltered, pro-inflammatory M1 macrophages population is increased. ASP also increases the plasmatic concentration of several circulating adipocytokines including IL6, TNF-a and MIP-1a.

Our results suggest that ASP has inflammatory effects in addition to its metabolic role. Plasma ASP concentration is elevated in obesity, type 2 diabetes and cardiovascular diseases. A better understanding of the inflammatory role of ASP in these pathologies could potentially set the basis for the development of a therapeutic avenue based on ASP neutralization.

Inflammation mediators

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This work was supported, however funding details unavailable.

**Table 1**

| Treatment | Control($n=8$) | rASP($n=7$) |
|---|---|---|
| IL6(pg/ml) | $1.8 \pm 0.9$ | $22.8 \pm 3.7^{\S}$ |
| IL10 (pg/ml) | $6.8 \pm 2.3$ | $18.6 \pm 4.8^{*}$ |
| G-CSF (pg/ml) | $38.8 \pm 7.7$ | $70.2 \pm 12.9^{*}$ |
| GM-CSF (pg/ml) | $14.3 \pm 2.5$ | $29.3 \pm 5.9^{*}$ |
| KC (pg/ml) | $65.3 \pm 12.2$ | $403.4 \pm 95.1^{\ddagger}$ |
| MIP1-a(pg/ml) | $3.2 \pm 0.7$ | $6.76 \pm 1.2^{\dagger}$ |
| TNF-a (pg/ml) | $39.6 \pm 4.4$ | $105.0 \pm 24.1^{\dagger}$ |

Plasma inflammation mediators of control mice vs rASP injected mice. Inflammation mediators levels were compared by t-tests where $^{*}P = 0.08$, $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$, $^{\S}P < 0.001$.

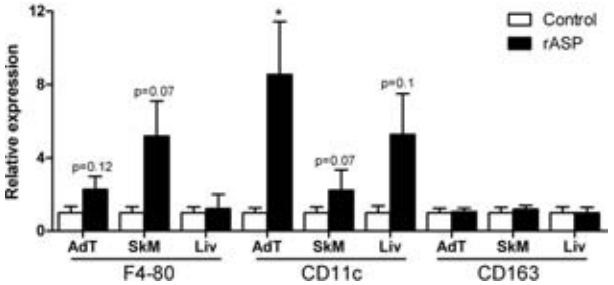


**Figure 1** Macrophage total, M1 and M2 markers. F4/80 represents total macrophage content. CD11c represents M1 macrophage content. CD163 represents M2 macrophage content. AdT, adipose tissue; SkM, skeletal muscle; Liv, liver. * $P < 0.05$.

## P1212

**Regulation of adiponectin by free fatty acids in lean and obese subjects**

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Objective
To evaluate the effects of free fatty acids (FFAs) on the regulation of plasma and adipose tissue mRNA adiponectin expression in lean and obese subjects.
Methods
Seven obese men ($31.3 \pm 1.1$ kg/m$^2$) and eight lean men ($21.3 \pm 0.4$ kg/m$^2$) were studied. Subjects received an Intralipid 20% infusion over 180-min with sodium heparin. Blood sampling and abdominal subcutaneous adipose tissue biopsy were taken before and after.
Results
The FFA concentration was increased six fold after Intralipid/heparin infusion in both lean and obese groups. Adipose tissue adiponectin mRNA was significantly reduced by Intralipid/heparin infusion ($38.0 \pm 7.7$ vs $21.1 \pm 3.0.0$, $P<0.05$), and plasma level of adiponectin was reduced from 5.72 to 5.37 which, however, was a non-significant reduction ($P=0.16$). If the two groups were separated, in the lean subjects, adiponectin mRNA was significantly lower after Intralipid/heparin infusion ($48.3 \pm 12.5$ vs $23.2 \pm 5.0$, $P<0.05$), whereas there was no difference in plasma adiponectin before and after ($5.89 \pm 0.6$ vs $5.84 \pm 0.8$, $P=0.88$). In obese subjects, plasma adiponectin concentration was significantly lower after Intralipid/heparin infusion ($5.5 \pm 0.8$ vs $4.8 \pm 0.8$, $P<0.05$), whereas there was no difference in adiponectin mRNA expression ($26.0 \pm 6.3$ vs $18.7 \pm 3.1$, $P=0.24$).
Conclusions
Albeit the other data on the effect of FFAs on adiponectin concentrations are not consistent, we could get that FFAs suppress the expression of adiponectin in lean and obese human. The issue of possible FFA-induced regulation of adiponectin warrants further investigations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1213

**SUR2a gene is upregulated in skeletal muscle insulin resistance of obese rats: correlation with GLUT4 gene**

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Objective
The aim of this study was to investigate whether SUR2A (sulfonylurea receptor type 2A, ABCC9) MEF2a (myocyte enhancer factor-2a) and HIF1a (Hypoxia-inducible factor-1) can influence glucose uptake in oxidative muscle soleus by regulating GLUT4 expression.
Methods
Insulin resistance was induced in male Wistar rats by inducing obesity with monosodium glutamate (MSG). At the age 3 months, half control and MSG-treated animals started receiving 0.1 mg/kg/day of glimepiride in the drinking water, and distributed in 3 experimental groups: control rats (C), MSG-obese rats (O) and MSG-obese rats treated with glimepiride (OG). Animals were submitted to insulin tolerance test (ITT) and samples of soleus muscle were excised for quantification of gene expression by Real Time RT-PCR and GLUT4 protein.
Results
OG rats presented a ~51% increase in SUR2A mRNA content in comparison to O and C rats ($P<0.001$). O rats showed 16% increase in GLUT4 mRNA content ($P<0.01$ vs C), without any increase in GLUT4 protein. After glimepiride treatment, OG rats increased GLUT4 mRNA (21%, $P<0.01$ vs C) and protein (131%, $P<0.01$ vs C; and 73%, $P<0.05$ vs O). No significant differences ($P>0.05$) in mRNA expression of MEF2a and HIF1a were found.
Conclusion
We conclude that the glimepiride increased the GLUT4 expression in soleus of the insulin resistant animals which could be associated to SUR2A genes expression.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P1214

**Circulating oxidized LDL in patients with visceral obesity**

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Background
Visceral obesity has been associated with systemic oxidative stress, measured by oxidized LDL (oxLDL).
Aim
Investigate circulating oxLDL levels in patients with visceral obesity, and its relationship with metabolic abnormalities found in visceral obesity.
Methods
We evaluated blood pressure (BP), waist circumference (WC), fasting glucose, total cholesterol, LDL, HDL, triglycerides, ApoA1, ApoB, ApoCIII, Lp(a) and oxLDL in 100 asymptomatic non-smoking patients (56 men/44 women) aged 50–60 years, without cardiovascular disease.
Subjects were grouped as it follows: i) WC < 102 cm in men and WC < 88 cm in women; ii) WC ≥ 102 cm in men and WC ≥ 88 cm in women. Data processed in SPSS 16.0.
Results
Subjects with increased WC had higher systolic BP (women: 135+/−24 vs 146+/−27 mmHg, $P<0.05$; men: 134+/−22 vs 145+/−25 mmHg, $P<0.05$), higher levels of triglycerides (women: 129.5+/−54.0 vs 172.9+/−13.0 mg/dl, $P<0.05$; men: 132.2+/−18.0 vs 183.6+/−76.0 mg/dl, $P<0.01$) and ApoCIII (women: 8.8+/−4.3 vs 12.3+/−9.9 mmHg, $P<0.01$; men: 9.4+/−5.3 vs 14.4+/−7.4 mmHg, $P<0.01$), and lower HDL (women: 56.8+/−11.8 vs 47.3+/−13.4 mg/dl, $P<0.05$; men: 44.5+/−11.1 vs 36.8+/−10.4 mg/dl, $P<0.05$) and ApoA1 (women: 170.0+/−22.6 vs 152.6+/−27.6 mg/dl, $P<0.05$; men: 144.6+/−22.3 vs 133.2+/−24.2 mg/dl, $P<0.05$). There was no significant difference ($P>0.05$) between the groups in fasting glucose, LDL, ApoB and Lp(a) levels; however, those with increased WC had higher levels of oxLDL (women: 59.8+/−14.7 vs 67.8+/−14.8 mg/dl, $P<0.05$; men: 61.3+/−21.1 vs 69.0+/−19.5 mg/dl, $P<0.05$).
OxLDL was positively correlated with WC ($r=0.86$, $P<0.01$), systolic BP ($r=0.27$, $P<0.05$), plasma triglyceride ($r=0.29$, $P<0.05$), ApoCIII ($r=0.38$, $P<0.05$), LDL ($r=0.51$, $P<0.01$) and ApoB ($r=0.54$, $P<0.01$); and negatively correlated with HDL ($r=-0.26$, $P<0.01$) and ApoA1 ($r=-0.20$, $P<0.01$).
Conclusion
The study showed that high WC was strongly associated with high levels of oxLDL.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P1215

**Leptin modulates brain neural activity associated with feeding behavior in patients with lipodystrophy**

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Introduction
Markedly decreased plasma leptin concentrations in patients with lipodystrophy commonly lead to overeating. Leptin-replacement therapy improves feeding behavior in these patients. The aim of the present study is to clarify neural networks influenced by leptin signals for appetite regulation in the patients with lipodystrophy.
Methods
We measured neural responses to visual food stimuli by use of functional magnetic resonance imaging (fMRI) and investigated subjective feeling on appetite under both fasting and postprandial conditions in ten patients with or without leptin-replacement therapy and age- and sex-matched ten healthy subjects.
Results
In fMRI analysis, significant difference of food-related neural activity between controls and patients was detected in many regions under postprandial condition,

while only in a few area under fasting condition. Leptin increased neural activity in a region involved in satiety and suppressed in regions involved in hunger in patients under postprandial condition, while altered only in a few areas under fasting condition. In subjective feeling, there was no apparent difference of hunger feeling under fasting condition, while postprandial satiety feeling in patients was significantly reduced compared to controls, which was effectively increased by leptin.
Conclusion
These findings may suggest that improvement of feeding behavior by leptin is associated with modulation of neural processing in the brain regions involved in energy homeostasis and appetite regulation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1216

**Age variations in adiposity and body fat composition among spanish women**

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Objective
To characterize the age variations of fatness pattern in Spanish women
Research methods and procedures:
A cross-sectional study of 2226 Spanish women (16 to 74 years old) women was undertaken to study age variations in adiposity, body composition, obesity and central fat distribution. The women were divided into seven groups: Group I (G I, 15–24 years), Group II (G II, 25–34 years), Group III (G III, 35–44 years), Group IV (G IV, 45–54 years), Group V (G V, 55–64 years), Group VI (G VI, 65–74) and Group VII (G VII, > 74 years). Body composition measures were obtained, and regional fat and lean mass patterns were characterized with the use of electrical bioimpendance (BIA) (Body Composition Analyzer BC-418MA TANITA). Significant differences were assessed with Student's t test and ANOVA adjusting for age and BMI.
Results
A significant increasing age trend was observed in adiposity and body fat composition measures. Women in G I had significantly lower means compared with those in G V and above. However, there was no significant age trend in lean mass measurements and a significant decrease was observed in group VII. The results revealed that significantly more women in G I-G III (45.8%) were normal weighted (BMI 18.5 to 24.9), while significantly more women in G IV and above were overweighed (BMC $\geq$ 25) and obese (BMI $\geq$ 30). Age had significant positive correlations with measures of adiposity and body fat composition. This significant positive impact of age remained even after controlling for the effect of BMI.
Conclusion
In conclusion, the present investigation revealed that among Spanish women there is a significant positive age trend in adiposity and body fat composition, which is independent of overall adiposity (BMI). However, with ageing, muscle trends to decrease. Furthermore, with increasing age, there is a trend of increasing levels of overweight and obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1217

**Serum liver fatty acid binding protein levels correlate positively with obesity and insulin resistance in chinese young adults**

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Clear evidence on the specific impact of fatty acid binding proteins (FABPs) on cell biology and lipid metabolism in complex systems had been lacking until FABP-deficient mice models were created. Surprisingly, no change in appearance, gross morphology or viability was observed in FABP1-deficient mice. However, metabolic parameters in mice upon exposure to high-fat/cholesterol diet differed between studies. Therefore, liver fatty acid-binding protein (also called FABP1) plays an inconclusive role in adiposity. We investigated the association of serum FABP1 levels with obesity and insulin resistance in Chinese young people lower than 30 years old. Cross-sectional analysis including 200 obese and 172 normal-weight subjects matched for age and sex, anthropometric measurements were performed and serum FABP1 and biochemical characteristics were measured. Insulin resistance was determined by homeostasis model assessment of insulin resistance (HOMA-IR) and by the insulin sensitivity index (Si). FABP1 levels in obese subjects were significantly higher than those in normal-weight subjects ($P<0.001$) and the significance remained after adjustment for age, gender, alanine aminotransferase and aspartate aminotransferase ($P<0.001$). Serum FABP1 levels were significantly correlated with many metabolic-related indices, with BMI and triglycerides as the independent determinants. FABP1 levels remained an independent risk factor of insulin resistance assessed by HOMA-IR ($\beta=0.119$, $P=0.006$) and Si values lower than 0.990 (OR = 2.107 per S.D. unit, 95% CI [1.230–3.611], $P=0.007$) after adjustment for age, sex, BMI, waist circumference, systolic blood pressure, and fasting blood glucose. Serum FABP1 correlate positively with obesity and insulin resistance in Chinese young adults. Our data supports the fact that FABP1 might be an important mediator participating in fatty acid metabolism and energy balance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1218

**Gender differences in measurements of total and regional body composition in preschool children**

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Background
Generally, abdominal adipose tissue correlates stronger to decreased insulin sensitivity than what BMI does. In adults, males have more visceral adipose tissue (VAT) and less subcutaneous adipose tissue (SAT) than females. Comparisons between measures of body composition from magnetic resonance imaging (MRI), dual x-ray absorptiometry (DXA) and anthropometry have been studied previously; however not in preschool children.
Aim
This study compares abdominal and whole-body measures of body composition from MRI, DXA and anthropometry in preschool children and examined whether there are differences in the abdominal fat distribution between genders.
Methods
One-hundred-five children (57 boys, 48 girls) were recruited. Body composition was measured using a GE Lunar Prodigy DXA. Weight, height and waist circumference (WC) were recorded. In a subgroup of 33 boys and 18 girls, MRI was performed using sixteen T1-weighted slices over the entire abdomen. SAT and VAT volumes were measured using semi-automated segmentation.
Results
BMI, WC (cm), WHtR did not differ between genders. Total FM% and truncal FM% measured with DXA differed between genders (21 and 17% in girls versus 17 and 13% in boys, respectively ($P<0.01$)), but this was not the case in the subgroup. Total LM (kg) and truncal LM (kg) was increased in boys compared with girls (11.7 kg and 6.5 kg in girls, and 12.7 kg and 7.1 kg in boys, ($P<0.001$)). This gender difference was also seen in the subgroup ($P<0.01$). Boys had significantly more VAT than girls (0.17 (L) vs 0.10 (L), $P<0.001$). Abdominal SAT measured with MRI correlated to truncal fat mass measured with DXA ($r=0.91$, $P<0.001$) while VAT did not. Neither BMI nor WC correlated to VAT. Conclussion: DXA and anthropometric fat measures correlate stronger to MRI measures of SAT than of VAT. From these data boys seem to have more VAT than girls already at this young age.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1219

**Comparison of serum high-molecular-weight (HMW) adiponectin and homeostasis model assessment of insulin resistance (HOMA-IR) as markers for metabolic syndrome (MS) in estonian adult population**

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Introduction
The MS is a cluster of metabolic risk factors which are closely associated with insulin resistance and hypoadiponectinemia. The HMW adiponectin has been acknowledged as a predictive marker for MS. The prevalence of MS increases worldwide. Recently, the prevalence of MS among Estonian adults was estimated to be 26%. In the current study, we aimed to compare the utility of HMW adiponectin and HOMA-IR for the identification of MS.
Methods
Our study was a cross-sectional, population-based sample of the general population in Estonia aged 20–74 years and included 458 subjects (191 men). MS was diagnosed by National Cholesterol Education Program Adult Treatment Panel III criteria. Insulin resistance was estimated using the HOMA-IR. HMW adiponectin was measured by ELISA. The areas under the receiver operator characteristic curves (AUROC) were calculated to compare the ability of HMW adiponectin and HOMA-IR to identify subjects with MS.
Results
The median (interquartile range) HMW adiponectin level (μg/ml) was significantly lower among subjects with MS compared with subjects without MS in both genders: in men 2.1 (1.3–3.0) vs 2.8 (1.7–4.3) respectively ($P=0.002$) and in women 3.1 (2.1–4.8) vs 5.1 (3.5–6.9), respectively ($P=0.0001$). Subjects with MS had significantly higher HOMA-IR ($\pm$S.D.) compared with subjects without MS: $2.9\pm2.0$ vs $1.1\pm1.0$, respectively ($P=0.0001$). The AUROC for HMW adiponectin and HOMA-IR to predict the presence of MS were in men 0.64 vs 0.864 respectively ($P=0.0001$) and in women 0.702 vs 0.839 respectively ($P=0.0008$). After adjustment for age and BMI the AUROC for HMW adiponectin and HOMA-IR were in men 0.833 vs 0.88 respectively ($P=0.02$) and in women 0.897 vs 0.907, respectively ($P=0.5$).
Conclusions
The lower HMW adiponectin level and higher HOMA-IR are clearly associated with MS. HOMA-IR was superior to HMW adiponectin to predict the presence of MS in Estonian adult population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1220

**Local inflammatory response in adipose tissue dysfunction**

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Chronic low-grade inflammatory condition is associated with metabolic pathologies such as obesity. However, the role exerted by local inflammation occurring in different fat depots, such as subcutaneous (SAT) versus visceral (VAT) adipose tissue (AT), is still under evaluation. Aim of the present study was to characterize the inflammatory state in adipose tissue of obese (OB: $n=58$, mean$\pm$S.D. BMI$=47\pm9$) vs normoweight (NOR: $n=28$, mean$\pm$S.D BMI$=24\pm2$) patients subjected to bariatric or general abdominal surgery at our University Hospital.
Blood, VAT and SAT biopsies were collected at surgery for analyses. Cytofluorimetric immunophenotyping of blood cells and stromal vascular fraction obtained from AT revealed a higher presence of gammadelta lymphocytes in blood and AT from OB vs NOR. Moreover, TaqMan analysis of gene expression confirmed a significant increase in specific inflammatory markers in AT of OB (n = 38) vs NOR ($n=26$), being VAT from OB interested by a significantly worse inflammatory state characterized by M1 macrophage polarization (CD40+SE expression fold increase$=3.76+0.68$ in VAT of OB/NOR, $P<0.005$; CD206+SE expression fold increase$=0.39+0.04$ in VAT of OB/NOR, $P<0.05$) and decreased regulatory components (IL10 VAT/SAT$\pm$SE expression$=10.6\pm3.9$ in NOR and $1.6\pm0.4$ in OB, $P<0.05$; foxp3/CD3$\pm$SE expression: $27.7\pm12.3$ SAT and $5.5+1.5$ VAT of NOR, $17.4+1.8$ SAT and $3.2+0.5$ VAT of OB; $P<0.05$ VAT NOR vs OB and $P<0.001$ OB SAT vs VAT). This condition was associated with a reduced VAT functionality in OB vs NOR evaluated by adiponectin expression (adiponectin$\pm$SE expression: 29.1E6$\pm$10.1E6 SAT and 18.0E6$\pm$.9E6 VAT in 17 NOR, 10.2E6$\pm$1.7E6 SAT and 6.5E6$\pm$1.1E6 VAT in 26 OB; $P<0.01$ SAT and VAT NOR vs OB, $P<0.05$ OB SAT vs VAT).
In conclusion, these data suggest that obese AT is characterized by a local inflammation associated with functional metabolic alterations, more evident in VAT than SAT, which may play a pivotal role in the development of metabolic pathologies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1221

**Effect of two different types of bariatric surgery on metabolic profile in obese type 2 diabetic women**

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Introduction
Bariatric surgery has been proved to be the most effective treatment for type 2 diabetes mellitus (T2D). Post-operative improvement in insulin sensitivity seems to be partly independent of weight loss and may be affected by changes in incretin secretion. Previous studies reported that the glucose-dependent insulinotropic polypeptide (GIP)-induced enhancement of fat stores may lead to increased insulin resistance. We determinated the effect of laparoscopic adjustable gastric banding (LAGB) and biliopancreatic diversion (BPD) on glucose profile and on secretion of GIP in response to meal test.
Methods
Our cohort included 23 obese T2D women who underwent bariatric surgery: 11 BPD (age:$50\pm6.2$y; BMI:$47\pm7.8$), 12 LAGB (age:$54\pm9.5$y; BMI:$44\pm7.3$). Meal test (300 ml protein drink) and euglycaemic hyperinsulinaemic clamp were performed before, 1 month after surgery and at time when 15% weight loss was achieved. Investigated parameters: BMI, insulin resistance/sensitivity, and GIP. Statistical analysis: ANOVA with repeated measures.
Results
We confirmed that BPD had more significant impact on weight loss than LAGB ($P=0.0002$). Both surgeries significantly reduced HbA1c ($P=0.001$), but in the long-term perspective, BPD seems to be more efficient with regard to HbA1c reduction ($P=0.06$). In response to surgery insulin resistance measured by HOMA-IR significantly decreased ($P=0.0001$) while insulin sensitivity measured by clamp increased ($P=0.0002$) but there was no interaction with the type of surgery. No difference in fasting levels of GIP between the study groups was observed. On the other hand, stimulated levels of GIP after the meal test were significantly reduced in BPD group in comparison with LAGB group ($P=0.003$).
Conclusion
Both BPD and LAGB positively affected insulin sensitivity. However, a better glucose control was observed with 15% body weight reduction after BPD. Decreased GIP levels revealed during the meal test may contribute to the positive changes in glucose metabolism in patients who underwent BPD.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1222

**Visceral-to-subcutaneous fat ratio using fat measurement CT is essential for the assessment of risk for diabetes, hypertension and early atherosclerosis**

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Background and aims
Obesity is an important risk factor for metabolic and cardiovascular disease. Although body mass index (BMI) and waist circumference are convenient and economic methods for assessment of obesity, those could not fully reflect risk for diabetes, hypertension and early atherosclerosis.
Materials and methods
We cross-sectionally analyzed anthropometric and laboratory data of 868 Korean adults (age 53.2±11.2 years) which were recorded during regular health check-ups. Visceral fat area was determined by computed tomography, and brachial-ankle pulse wave velocity was measured as an indicator of arterial stiffness. Visceral fat obesity (VFO) was defined with our own reference value of visceral-to-subcutaneous fat ratio (VSR; median value of subjects without hypertension, diabetes, or cardiovascular disease, ≥0.74 for men and ≥0.4 for women).
Results
Subjects with generally or abdominally not obese but VFO were older and higher proportion of men, (higher prevalence of hypertension and diabetes only in abdominally obese analysis), lower BMI, waist circumference, fat mass, total and subcutaneous FA but higher visceral FA and VSR (and baPWV only in abdominally obese analysis) than those with generally or abdominally obese without VFO.
Logistic regression analysis including sex, age, BMI, waist circumference, PBF and VSR quartile showed that higher quartile of VSR was 1.7 times and 2.6 times independently increased risk for type 2 DM and 1.7 times increased risk for hypertension. Logistic regression analysis including sex, age, BMI, waist circumference, PBF, VSR quartile, hypertension and diabetes showed that higher quartile of VSR was 2.1 times independently increased risk for the presence of arterial stiffness.
Conclusion
These results suggest that measurement of visceral-to-subcutaneous fat ratio would be useful for assessment of cardio-metabolic risk to identify individuals who need more intensive intervention to prevent the metabolic diseases and atherosclerosis in overweight or obese subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1223

**Effect of obesity on vitamin d and parathormone levels**

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Aim
In this study our aim was to investigate the relationship between obesity and vitamin D and hyperparathyroidism.
Material and Method
127 healthy persons included in this study. Study group were divided into four groups according to body mass index (BMI). Group 1 ($n=35$) BMI 18.5–24.9 kg/m$^2$, Group 2 ($n=35$) BMI 25–29.9 kg/m$^2$, Group 3 ($n=35$) BMI 3.0–34.9 kg/m$^2$, Group 4 ($n=22$) BMI 35–40 kg/m$^2$. Anthropometric measurements were performed. Serum calcium (Ca), phosphorus (P), parathormone (PTH), 25(OH) D levels were evaluated.
Results
Negative linear relationship was found between BMI and serum Ca and Vit D (respectively, $P=0.040$ $r=-0.181$, $P=0.016$ $r=-0.214$). There was no significant relationship between BMI and PTH levels ($P>0.05$). A significant reduction was observed in vitamin D levels while waist circumference increases ($P=0.049$). There was no significant decrease in Serum Ca level ($P>0.05$). Significant decrease was observed in vitamin D and serum Ca while body fat mass increases (respectively, $P=0.026$, $P=0.021$). Serum Ca levels of group 2 was significantly higher than group 3 and 4 (respectively, $P=0.049$, $P=0.000$). vitamin D levels in group 4 was significantly lower than group 2 (respectively, 18.96±9.88 μg/l, 30.06±18.48 μg/l) ($P>0.05$). There was no significant difference in PTH levels between groups.
Conclusion
As a result of our study, we determined low vitamin D and calcium levels in obese patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1224

**Limited effects of leptin and adiponectin on proliferation and protein metabolism of porcine myoblasts under serum-free culture conditions**

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Cross-talk between adipose tissue and skeletal muscle via endocrine and paracrine mechanisms may be important in determining nutrient partitioning and lean-to-fat ratio. Such interactions could be mediated in part by adipokines secreted from adipose tissue. Little is known, however, about the role of adipokines in influencing growth and development of skeletal muscle.
The aim of this study was to determine the direct effects of leptin and adiponectin on the growth of porcine skeletal muscle cells *in vitro*. Adiponectin receptors (ADIPOR1, ADIPOR2) were abundant at mRNA and protein level in proliferating and differentiating myoblast cultures derived from *semimembranosus* and *semitendinosus* muscles of newborn piglets, whereas the long-form leptin receptor (LEPR) expression was close to the detection limit. In serum-free medium (SFM) adiponectin (10, 20, 40 μg/ml) attenuated the proliferation of porcine myoblasts, measured as [$^3$H]-thymidine incorporation and live cell monitoring in response to 24-h and 48-h exposure, in a dose-dependent manner. This effect resulted from suppressed basic fibroblast growth factor (bFGF)-mediated stimulation of DNA synthesis that remained unchanged by adiponectin in serum-free medium (SFM) without bFGF. No effects of leptin (5, 10, 20, 40, 80 ng/ml) on myoblast proliferation in SFM were detectable. Neither leptin nor adiponectin altered protein synthesis and degradation, recorded as incorporation or release, respectively, of [$^3$H]-phenylalanine, in differentiating porcine myoblasts cultured in SFM. The results on receptor abundance suggest that porcine skeletal muscle cells may be sensitive to adiponectin and leptin. However, except via inhibitory interaction of adiponectin with bFGF, these adipokines appear not to affect *in vitro* proliferation and protein metabolism of porcine muscle cells directly under serum-free culture conditions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1225

**Effect of secreted frizzled-related protein 2 (sFRP2) from adipose tissue on pancreatic cell function**

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The detrimental effect of excessive obesity on insulin resistance is well established. The expansion of adipose tissue is dependent on two processes: adipogenesis and angiogenesis and the Wnt signalling pathway has been reported to affect both. In adipose tissue the Wnt signalling pathway functions in a converse manner: increasing commitment of mesenchymal stem cells to preadipocytes and inhibiting differentiation of preadipocytes to mature adipocytes by decreasing expression of CEBPα and PPARγ.
One of the antagonists of the canonical Wnt signalling pathway is secreted frizzled-related protein 2 (sFRP2) however, recent studies have identified sFRP2 as a novel factor stimulating angiogenesis via a non-canonical, calcineurin/NFAT signalling pathway.
Expression of sFRP2 and Wnt receptors, Fzd1 and Fzd2, mRNA has been reported in preadipocytes and Wnt signalling is also known to be involved in pancreatic islet function, insulin production and secretion. We hypothesised that sFRP2 secreted from adipose tissue could provide a link between obesity and

diabetes through a deleterious action on beta cells/ insulin secretion.
Human omental (OM) and subcutaneous (SC) adipose tissues expressed high levels of sFRP2 mRNA with more in OM than SC adipocytes (3.6-fold increase, $n=8$, $p=0.003$). In mouse, sFRP2 mRNA was present in gonadal and subcutaneous fats but absent in liver, pancreas and pancreatic α- and β-cell lines. All mouse tissues and cells studied were positive for Wnt signalling receptors Fzd1 and Fzd2. Proliferating mouse pancreatic α- and β-cells treated with recombinant mouse sFRP2 protein (10 ng to 200 ng/ml) for 4 days, showed a dose-dependent decrease in proliferation rates in β-cells (10 ng/ml: 96%, 100 ng/ml: 84%, 200 ng/ml: 72 vs 100% control, $P<0.01$) but not α-cells or the human preadipocyte cell line, ChubS7.
We postulate that secreted sFRP2 from expanding adipose tissue could have a harmful effect on pancreatic beta cell function and contribute to the pathogenesis of insulin resistance in human obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1226

**Curcumin inhibits the upregulation of cathepsin L by palmitate in fat**

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Objective
Cathepsin L can control adipogenesis and relate to acute coronary syndrome. However, it is not clear whether cathepsin L can be affected by saturated fatty acid and decrease by curcumin. We examined the hypothesis that palmitate upregulates cathepsin L expression in adipose tissue and curcumin can be block that effect.
Methods
3T3-L1 cells were fully differentiated for 8 to 10 days and treated with palmitate, LPS, IL6, TNF-α, IL6 neutralizing Ab, curcumin. Six-week-old C3H/HeN with normal TLR4 and C3H/HeJ with TLR4 mutation got LPS injection ip (1 mg/kg) or were fed with high fat diet for 13 weeks and sacrifice to harvest epididymal fat.
Results
Real-time PCR revealed that cathepsin L expression was upregulated by palmitate. But there was no additional effect when we use palmitate-treated RAW264.7 cells' media instead of direct treatment to 3T3-L1 cells. Cathepsin L was upregulated after treatment of TNF-α or IL6 like palmitate. IL6 neutralizing Ab and curcumin attenuated this effect of palmitate. Cathepsin L increased in both kinds of mice fed high fat diet compared with chow diet. But we cannot find similar pattern at mice which got LPS ip injection.
Conclusions
We concluded that cathepsin L expression in fat are upregulated by palmitate via inflammatory cytokines, not TLR4, thereby might have a critical role in developing acute coronary syndrome of metabolic syndrome and there are no additional effects of infiltrating macrophages. In addition, curcumin can be a candidate of drug which can amilorate the metabolic syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1227

**Preventing childhood obesity by community based intervention approaches**

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Introduction
EPODE is a coordinated, capacity-building approach for communities to implement effective and sustainable strategies to prevent childhood obesity.
Methods
At central level, a coordination team using social marketing and organizational techniques trains and coaches a local project manager nominated in each EPODE town by the local authorities. The local project manager is also provided with tools to mobilize local stakeholders through a local steering committee and local networks. The added value of the methodology is based on a strong scientific input, institutional endorsement, evidence-base and social marketing techniques, sustainable resources, brand dynamics and evaluation. EPODE Monitoring and Evaluation practices to date include not only outcome but also process and output indicators at central, local, setting, child levels.
Results
The EPODE methodology is now implemented in more than 300 towns in six countries (Spain, Belgium, Greece, France, South Australia, Mexico) and concerns more than five million people. At child level the prevalence of overweight and obesity in children aged 5 to 12 is monitored. In the 8 French pilot towns, the response rate is high (95%). The prevalence of children overweight including obesity decreased in these pilot towns between 2005 and 2009 (from 20.6 to 18,8%, $P<0.0001$). From 2008, the EPODE European Network project is enriching the EPODE methodology and facilitating the implementation of similar initiatives in other European countries.
Conclusion
Childhood obesity is a complex issue and needs a multistakeholder involvement at all levels to foster healthier lifestyles in a sustainable way. The EPODE methodology contributes to this approach.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1228

**Reduction of body weight and waist circumference after long-term treatment of hypogonadal men with testosterone undecanoate**

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Introduction
Obesity is associated with reduced testosterone, and low testosterone induces weight gain. This study analysed the effects of normalization of serum testosterone in mainly elderly, hypogonadal men.
Methods
Prospective registry study of 252 men (mean age 60.6±8.0 years), with testosterone levels between ≤ 3.5 ng/ml. They received parenteral testosterone undecanoate 1000 mg at day 1, week 6 and every 12 weeks thereafter for up to 66 months.
Results
After 60 months the following changes were observed: weight declined from 106.28±16.98 to 90.07±9.51 kg, waist circumference from 107.23±9.15 to 98.46±7.39 cm and body mass index from 33.95±5.56 to 29.17±3.09 kg/m$^2$. All changes were progressive over 60 months and statistically significant vs baseline and vs previous year ($P<0.0001$). In addition, total cholesterol declined from 281.97±39.86 to 188.12±11.31, LDL from 163.75±41.62 to 109.84 ±35.41 and triglycerides from 276.38±51.57 to 189.78±11.33 mg/dl. The decline was progressive over 24 months and statistically significant vs baseline and vs previous year ($P<0.0001$) until a plateau was reached after 2 years. HDL declined from 62.06±27 to 52.45±16.82 mg/dl after 60 months, however, with fluctuations. There was a statistically significant increase vs baseline at 24 months ($P=0.0254$) and a decrease vs baseline at 36 months ($P<0.0001$) with a plateau after 36 months.
Conclusions
Raising serum testosterone to normal resulted in continuous loss of body weight, waist circumference and BMI. These improvements were progressive over the full 5 years of the study. Levels of total cholesterol, LDL and triglycerides decreased over the first 2 years. HDL showed some fluctuations but means remained above 47 mg/dl. Long-term administration of testosterone to middle-aged, hypogonadal men improves body composition and lipid profile.
Declaration of interest
I fully declare a conflict of interest. Details below:
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## P1229

**Connection of adipocitokines and inflammatory markers in obese woman: influence of body weight reduction**

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Fat tissue is highly active tissue, both metabolically and as endocrine tissue, which produced large number of bioactive peptides, known as adipocitokines. They seem to contribute in pathogenesis of obesity, insulin resistance, diabetes mellitus type 2, hypertension, hyperlipidemia, and vascular disease. Adipocitokines and low grade inflammation could be connection between obesity and coexisting diseases. This is the reason we believe that body weight reduction could have positive effects on decreasing the risk for those diseases. The aim of this study is to evaluate the effect of hypo caloric diet and body weight reduction on serum levels of leptin, adiponectin and inflammatory markers in obese woman and their correlations. Methodology and material: We included in the study 90 obese women, with body mass index (BMI) $36.43 \pm 5.42$; waist circuference (WC) $103.12 \pm 14.33$; weist circuference/hip circuference ratio (WC/HC) $0.89 \pm 0.08$. In all subjects we determined serum levels of leptin,adiponectin, CRP and fibrinogen before and after six months of hypo caloric diet and body weight reduction. Results: Average weight loss was $8.73 \pm 1.98$ kg or $8.64 \pm 1.96\%$ ($P < 0.001$). That results in significant decreasing of anthropometric parameters, BMI, WC and WC/HC ratio, ($P < 0.001$). Serum levels of leptin, CRP and fibrinogen decreased and serum level of adiponectin increased, all on maximum level of statistical significance ($P < 0.001$). There is statistically significant positive correlation between anthropometric parameters and inflammatory markers, before and after body weight reduction. There is positive correlation between leptin, and negative correlation between adiponectin and anthropometric parameters. Before and after body weight reduction there is statistically positive correlation between leptin and negative correlation between adiponectin and serum levels of CRP and fibrinogen. Conclusion: Our results suggests that changes in adipocitokines and state of low grade inflammations are connected and that body weight reduction has significant positive effect on all parameters, what emphasizes the importance of restricted dietary regime as therapy of obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1230

**Proteomic analysis identifies adiponectin as a key plasma protein associated to vitamin D deficiency in pediatric obesity**

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Key circulating molecules that link the emerging role of vitamin-D to pediatric obesity and its co-morbidities remain unclear. To identify potentially involved regulators, this study analyzed plasma proteoma from obese children dichotomized by 25OH-vitamin D (25OHD) levels and upon vitamin-D3 therapy in 25OHD deficient subjects.

A total of 42 obese children (M/F = 18/24, bmi > 95° centile) were selected and divided according to 25OHD levels into deficient (G1; $n = 18$; 25OHD < 14.5 ng/ml) or normal 25OHD (G2; $n = 24$; > 30 ng/ml). Eight subjects from G1 underwent a 12mo treatment with 400 UI/die vitamin-D3. Proteomic analyses by 2D-electrophoresis with different IPG ranges were performed at baseline with spots quantitated by PDQuest.

2D-electrophoresis with IPG3-10 identified 28 spots significantly different between groups ($P < 0.05$). Among these, 57% were localized within PI3-6. Analysis restricted to this range revealed 20 spots significantly different between groups ($P < 0.05$). Overall, 20% of IPG3-6 associated spots were downregulated in G1 compared to G2. Adiponectin was selected for confirmational studies due to strong correlations with obesity and its co-morbidities, as well as its localization within a susceptibility gene loci. WIB analysis of 2D-gels showed down-regulation of adiponectin in 25OHD-deficient subjects. Adiponectin expression was lower in plasma from G1 than G2 ($7187 \pm 383$ vs $8594 \pm 587$ AU; $P < 0.035$) and increased following 12mo vitamin-D3 treatment in 80% of G1 subjects by an average of 8% ($P < 0.02$). Analysis of HMW adiponectin, a surrogate indicator of insulin sensitivity, demonstrated significantly lower levels in G1 subjects ($697.1 \pm 127.7$ vs $1270.5 \pm 198$ AU; $P = 0.015$), which improved upon vitamin-D3 therapy ($583.3 \pm 162.1$ vs $1412.1 \pm 251.7$ AU; $P < 0.02$).

Our proteomic approach to identify key circulating molecules that link 25OHD levels to pediatric obesity, has demonstrated that vitamin-D deficiency is associated with an altered adiponectin expression, specifically the HMW form, which can be significantly improved by vitamin-D3 administration. Direct effects of vitamin-D on adiponectin production in the adipose tissue remain to be elucidated.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1231

**Plasma adipokines and improvement of insulin sensitivity during multi-phase long-term dietary intervention**

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Context

Weight-reducing dietary intervention (DI) promotes an improvement in insulin sensitivity (IS) since early stage of the diet. The diet-induced changes of plasma levels of cytokines and chemokines that are expressed in adipose tissue (adipokines) have been suggested to play a role.

Aim

To examine relationship between the diet-induced changes of IS (assessed using homeostatic model assessment of insulin resistance (HOMA-IR)) and those of plasma adipokines during different time-points of a 6 months multi-phase DI.

Subjects and methods

obese women (BMI $34.8 \pm 3.8$ kg/m$^2$) were followed during 6 months DI that consisted of a 28 days very-low-calorie-diet (VLCD) and subsequent 5 months weight stabilization phase (WS) including 2 months low-calorie-diet and subsequent 3 months weight maintenance diet. Plasma sampling for analysis of a number of adipokines (serum amyloid A (SAA), haptoglobin, interleukines $-6, -8, -1\beta$, tumor-necrosis-factor-$\alpha$, monocyte-chemoattractant-protein-1, plasminogen-activator-inhibitor-1) using Luminex technology and of relevant hormones and metabolites were performed before the diet and at the the end of each phase.

Results

Body weight (BW) and HOMA-IR decreased during VLCD and during the entire DI (BW: $95.9 \pm 12.5$ vs $88.5 \pm 12.0$ ($P < 0.001$) vs $85.3 \pm 12.4$ ($P < 0.001$), HOMA-IR: $2.9 \pm 1.7$ vs $1.6 \pm 0.8$ ($P < 0.001$) vs $1.7 \pm 0.9$ ($P < 0.001$) respectively). The pre-diet levels of plasma adiponectin correlated positively with HOMA-IR decrease during the early stage of the diet, i.e. during VLCD. The decrease of plasma SAA and haptoglobin levels during VLCD correlated positively with the increase of HOMA-IR during the entire DI. No such correlations were found for other examined adipokines.

Conclusion

Among the adipokines examined, the pre-diet level of plasma adiponectin and the response (decrease) of acute phase proteins (SAA and haptoglobin) during the early stage of the DI appear as determinants of the diet-induced improvement of IS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1232

**Obesity the first year of life: influence of glycemia at pregnancy, breastfeeding and sleep**

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Introduction

Pre- and postnatal factors have an influence on obesity the first year of life. Among them maternal glucose levels, breastfeeding, ablactation and recently sleep duration are under study in diverse populations.

Methods

In a prospective study, we recruited 139 pregnant women and obtained fasting and post-load (75 g) glucose levels. We studied children robustness

(waist girth/height) at 6 months and one year of age, and the influence of altered fasting and post-load glucose levels at 6 months of pregnancy, breastfeeding, ablactation, and duration of sleep, leptin and insulin levels.
Results
Altered glucose levels (fasting $\geq 100$, $< 126$ mg/dl or post-load $\geq 140, < 200$) were found in 21 women. Their children, compared with those from mothers without altered glucose did not have significantly different anthropometric measures at birth, at 6 months or at 1 year of age. At 6 months leptin levels were associated with the mother's fasting glucose at pregnancy ($P < 0.000001$). At this time, duration of breastfeeding was negatively correlated with waist girdle/height index ($P < 0.00001$) and with leptin ($P < 0.00002$) and insulin levels ($P < 0.000001$), but these associations were not maintained at 1 year of age. Ablactation was associated with insulin levels at 6 months ($P < 0.00007$) and with the waist girdle/height index at one year of age ($P < 0.0001$). We interpreted that the effects of breastfeeding and early ablactation under our conditions have protective effect against obesity. Duration of sleep was associated with the robustness index negatively at 1 year of age ($P < 0.002$), but positively at 6 months ($< 0.0044$). These results suggest that sleep duration the first 6 months of age is related to different metabolic influences than at later months.
Conclusions
We concluded that limited alteration of glucose levels at pregnancy may influence children's adiposity. Breastfeeding, ablactation and sleep have influences on children robustness during the first year of life.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1233

### Anthropometric, hormonal and nutritional correlates of epicardial fat in obese women

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Background and aim
Epicardial fat (EF) is a metabolically active organ as well as visceral fat (VF). EF has been recognized as a potential additional marker of cardiac risk in obesity and type 2 diabetes. This study wants to assess the relation between EF and VF and the correlation with nutritional and hormonal parameters.
Material and Methods
Fifty-nine overweight/obese women were enrolled in the study. All women underwent anthropometric measurements, nutritional assessment and oral glucose tolerance test; HOMA-IR and ISIcomposite were calculated to assess insulin resistance and sensitivity respectively. Twenty-four hours urine were collected for urinary free cortisol determination (UFC/24 h). They underwent echocardiography for detection of EF. A subgroup of 27 obese women with stress related obesity (SRO) was also examined. They were characterized by rapid weight gain after a stressful event exposure (Vicennati V. et al, 2009).
Results
BMI was $33.4 \pm 3.28$ kg/m$^2$ and waist circumference $104 \pm 8.8$ cm. EF was $133 \pm 62$ cm$^2$. EF was positively and significantly correlated with BMI ($P = 0.001$), waist circumference ($P = 0.000$), ISIcomposite ($P = 0.025$), HOMA-IR ($P = 0.05$). No significant relationship was present between EF and total testosterone and UFC/24 h. EF was also positively and significantly correlated with dietary lipid intake ($P = 0.009$), satured fatty acids ($P = 0.017$), polyunsaturated ($P = 0.018$) and monounsaturated fatty acids ($P = 0.002$). EF was also significantly correlated with dietary cholesterol ($P = 0.0461$). In a multiple correlation model, only waist circumference persisted significant ($P = 0.036$). In the SRO group, EF ($120 \pm 54$ cm$^2$) was positively correlated to BMI ($P = 0.017$), HOMA-IR ($P = 0.012$), UFC/24 h ($P = 0.018$). In a multiple regression model, only waist circumference persisted significantly correlated to EF ($P = 0.03$).
Conclusion
These findings suggest that EF represent a target visceral fat depots. Further studies should investigated the specific relationship between EF and the endocrine and metabolic milieu in a larger population with different obesity phenotypes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1234

### How informative is visceral fat measurement by bioelectrical impedance?

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Although obesity is a powerful risk factor for developing metabolic syndrome (MetS), it is not present in all obese individuals. Increased visceral adipose tissue is the hallmark of this syndrome. In this cross sectional survey abdominal bioelectrical impedence was used to measure the visceral adipose tissue (VAT) and trunk fat percentages (TF%) in participants. A total of 286 patients (196 female and 90 males) were enrolled in the study. In both sexes VAT was significantly positively correlated with body mass index, waist circumference, TF%, HOMA IR, fat percentage, fasting plasma glucose and triglycerides. Strongest correlations were between VAT and TF%, VAT and device measured waist circumference and between VAT and manual waist circumference ($r = 0.95$, $r = 0{,}94$, $r = 0.92$ respectively in female and $r = 0.99$, $r = 0{,}93$, $r = 0.94$ respectively in male participants). The mean VAT and TF% in MetS(+) groups were significantly higher than patients in MetS(−) groups in both sexes. The areas under the ROC curves were 0.730 (95% CI: 0.661–0.791) for female VAT and 0.702 (95% CI: 0.654–0.749) for male VAT in predicting MetS. We suggest that using such a device in clinical practice is an alternative technique for examining and follow up of patients with MetS.
Sensitivity and spesificity of VAT for predicting MetS at certain cut off values in male and female

**Table 1**

| VAT | Male Senstivity | Male Spesifisity | Female Senstivity | Female Spesifisity |
|---|---|---|---|---|
| 7.250 | | | 100 | 29 |
| 9.750 | | | 90 | 50 |
| 11.750 | | | 57 | 69 |
| 14.750 | 92 | 28 | 25 | 90 |
| 16.000 | 90 | 40 | | |
| 19.750 | 70 | 53 | | |
| 30.250 | 15 | 90 | | |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1235

### A selective androgen receptor modulator, S42 possesses beneficial metabolic effects

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Androgen is an important factor for determining body composition in males. Abdominal obesity is inversely correlated with serum androgen levels in men. Androgen receptor (AR) null male mice revealed late-onset visceral obesity, accompanying energy balance abnormality. We previously reported a novel synthetic steroid, S42 as a promising candidate of a selective androgen receptor modulator (SARM). When orchiectomized Sprague-Dawley rats were administered with S42 for 3 weeks, the muscle weight of the levator ani was increased as markedly as that induced by dihydrotestosterone (DHT), but the weight of the prostate was not elevated at any doses in contrast to DHT. In addition, although the plasma triglyceride level was unaffected by DHT, it was significantly reduced by S42. Taken together, S42 works as an AR agonist in muscle and as an AR antagonist in the prostate, accompanying beneficial potentials on lipid metabolism. We then administered S42 (10 mg/kgBW) by intraperitoneal injection in high-fat diet induced obese mouse model for 16 weeks and found that S42 significantly decreased the body weight (BW) compared with the placebo administration without affecting the food intake. The BW reduction was accompanied by the marked reduction of visceral fat determined by the CT analysis. The aggravated insulin sensitivity by high fat diet was also improved by the S42 treatment but not by placebo. A similar effect of S42 was also observed when S42 was administered

to ob/ob mouse for 4 weeks. S42 could reduce the BW with marked reduction of visceral fat in ob/ob mouse. These results suggest that S42 have some beneficial metabolic effect on fat accumulation and insulin sensitivity *in vivo*.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1236

**Effect of orlistat in Russian adolescents with metabolic syndrome: a randomized controlled trial**

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Background
Metabolic syndrome (MS) in children is associated with future risk of developing type 2 diabetes mellitus and cardiovascular disease in adults. In the pediatric population lifestyle interventions alone are often insufficient.
Objective
To evaluate the efficacy and safety of orlistat in obese adolescents with MS.
Design
A 6 month open, comparative, randomized study of 60 obese adolescents (aged 12–17 years; mean body mass index (BMI) 32 kg/m$^2$) with MS. The main group ($n=30$) was treated with a hypocaloric diet, aerobic exercises and orlistat 120 mg three times daily for 6 months. The control group ($n=30$) was subjected to the same diet and exercise regimen without the orlistat application. Changes in BMI, waist circumference, body composition, blood pressure, fasting serum lipid and glycemic parameters were evaluated.
Results
BMI decreased by 3.09 kg/m$^2$ in the orlistat group and only by 1.11 kg/m$^2$ in the control group ($P=0{,}033$). The orlistat group compared with the control group also had a significant reduction of body fat ($-6600{,}0$ vs $-2235{,}5$ g, $P=0.011$), waist circumference ($-6.50$ vs $-2.50$ cm, $P=0.019$), LDL-cholesterol ($-0.33$ vs $-0.20$ mmol/l, $P=0.024$) and total cholesterol levels ($-0.25$ vs $-0.05$ mmol/l, $P=0.025$). HDL-cholesterol, triglyceride levels and blood pressures improved similarly within both groups; comparisons between groups, however, were not statistically significant. After 6 months of orlistat therapy 68% of the adolescents achieved clinically meaningful weight loss. In the control group only 41% of patients reduced their weight by more than 5%. Orlistat was well tolerated by patients. Most adverse events were mild and can be attributed to the expected adverse reactions.
Conclusion
The study demonstrated significant efficacy and safety when orlistat was combined with the complex therapy in obese adolescents with MS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1237

**Extreme obesity partly blunts the discriminative metabolic effects of polycystic ovary syndrome (PCOS)**

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The epidemic of obesity and diabetes is increased worldwide, and both are more prevalent in PCOS than non-PCOS ovulatory women. In the general population, the risk of cardiovascular mortality is at least doubled in women with extreme obesity (BMI $>40$ kg/m$^2$). To explore if PCOS adds further metabolic detriment to extreme obesity, this prospective study compared the metabolic profiles, OGTT-derived insulin sensitivity and body composition between 30 adult PCOS and 30 non-PCOS women with extreme obesity (age, $27.8\pm5$ vs $27.7\pm7$ years; BMI, $44.8\pm4.4$ vs $43.7\pm3$ kg/m$^2$; WHR, $0.87\pm0.1$ vs $0.89\pm0.1$) seen consecutively at the IRCSS Istituto Auxologico Italiano for workup of obesity. None had a history of cardiometabolic disorders and PCOS was diagnosed by Rotterdam and AE-PCOS criteria. PCOS and non-PCOS women had similar levels of glucose and insulin, total or fractionated cholesterol and C-reactive protein. HbA1c ($5.5\pm0.4$ vs $5.6\pm0.4\%$), HOMA-IR ($3.2\pm1.2$ vs $3.1\pm2.1$) and microalbuminuria ($52.4\pm60.5$ vs $73.7\pm52$ mg/l) were comparable between groups. OGTT-derived measures of insulin sensitivity in PCOS and non PCOS women revealed equivalent values of early phase insulin secretion (insulinogenic index, $1.8\pm1.2$ vs $1.8\pm1.5$) and β-cell function (oral disposal index, $4.8\pm3.1$ vs $6.1\pm5.3$). Alternatively, the Matsuda index of insulin sensitivity was lower in PCOS than non-PCOS women ($2.8\pm0.8$ vs $3.7\pm2$, $P<0.05$). DXA-derived measures of fat body mass were similar between groups ($52.5\pm4.1$ vs $50.5\pm4.8\%$), while trunk-fat was increased in PCOS ($30.9\pm5.8$ vs $25\pm5.5\%$, $P<0.01$) and coexisted with an increase in leptin levels ($58.8\pm24.6$ vs $44.7\pm16.4$ mcg/l, $P<0.05$). Adiponectin levels were not different between the study groups ($14.7\pm11.2$ vs $10.4\pm5.8$ mcg/ml). Current data suggest that many indices of metabolic homeostasis are superimposed between PCOS obese and non-affected obese counterpart. However, dissimilarities in insulin sensitivity and fat accumulation emphasize the importance of adequate long-term management of PCOS women suffering from extreme obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1238

**Low dose 3-iodothyronamine increases acute lipolysis followed by protein catabolism in mouse**

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3-iodothyronamine ($T_1AM$) is a recently discovered fast-acting thyroid hormone derivative. To date, the physiological effects of endogenous$T_1AM$ remain elusive, although there is increasing interest in its physiological function and pharmaceutical potential due to the role it plays in lipid and glucose metabolism (1.2).The present study monitored the effect of weeklong, daily, low dose $T_1AM$ administration on weight and metabolism in spontaneously overweight mice. Mice treated with exogenous $T_1AM$ (10 mg/Kg/day) lost 8.2% of their initial body weight by day 9 and regained only 1.8% of initial weight in the following 2 weeks, indicating lasting effects of $T_1AM$ on weight maintenance. Real-time analysis of exhaled $^{13}CO_2$ by cavity ringdown spectroscopy (CRDS) detected increased lipolysis shortly after $T_1AM$ administration followed by a shift, later in the week, towards protein catabolism. Blood based metabolomics data obtained by nuclear magnetic resonance spectroscopy (NMR) were in agreement with the results from breath analysis, as indicated by the alterations found in key metabolites: valine, glycine, and 3-hydroxybutyrate. While this study begins to decode longer-term effects of $T_1AM$ on metabolism, additional studies with mildly feed restricted mice are currently underway to develop an appropriate dosing schedule for $T_1AM$ to be used as an effective weight loss and weight maintenance drug.
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Declaration of interest
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## P1239

**Lipid metabolism is impaired in obesity/diabetes model mice**

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A new genetic animal model of type 2 diabetes, the Tsumura Suzuki Obese Diabetes (TSOD) mouse, has been developed in 1999. The TSOD mouse

develops a moderate degree of obesity and diabetes. The aim of this study was to investigate the potential association of impairment of lipolysis and lipid metabolism on the development of obesity and diabetes in TSOD mice.
Methods
Three month old TSOD and the age-matched control (TSNO: Tsumura Suzuki Non-Obesity) male mice were obtained from The Institute for Animal Reproduction (Ibaraki, Japan). Serum glucose, total cholesterol (T-Cho), triglycerides (TG) and insulin were measured. Adipose tissues were removed and extracted lipids by acetone. Extracted lipids were analyzed by TLC and LC-MS. The expressions of mRNA of adipose tissue glycerol lipase (ATGL), hormone sensitive lipase (HSL), adipose phospholipase A2 (AdLPA2) and perilipin in epididymal adipose tissue were also measured by real-time PCR.
Results
Serum levels of glucose, TG and insulin were significantly elevated in TSOD mice than those of TSNO. Clear spot corresponding to diacylglycerol (DG) was observed in the sample from TSOD but not from TSNO by TLC. Ratio of total DG levels against total TG ones in TSOD mice were higher than those in TSNO by LC-MS. Expression of ATGL, HSL and perilipin was lower in TSOD than that in TSNO. However, mRNA expression of AdPLA2 in TAOD was significantly higher than that in TSNO.
Conclusion
Dysregulation of lipolysis and lipid metabolism in adipose tissue contribute in part to the development of obesity and diabetes in TSOD mice.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1240

**Comparing effects of lifestyle modification-induced weight loss on androgen levels, endothelial, sexual and urinary function, and quality of life in obese men with and without androgen deficiency**

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Introduction
Androgen deficiency (AD) is associated with endothelial and erectile dysfunction, lower urinary tract symptoms (LUTS) and reduced quality of life (QoL) in obesity. We aimed to compare effects of weight loss induced by lifestyle modification on endothelial and erectile function, LUTS and QoL in obese men with and without AD.
Methods
abdominally obese Asian (body mass index $\geq 30$ kg/m$^2$, waist circumference [WC] $\geqq$ 90 cm) men (mean age 43.1 years, range 30–61) with low libido were put on a weight loss program of caloric restriction (500 kilocalories/day below basal metabolic requirement) and moderate-intensity exercise (2000 kilocalories/week). Plasma sex-hormone binding globulin (SHBG) and total testosterone (TT), endothelial function (by Reactive Hyperaemia Index [RHI] using finger plethysmography on EndoPAT), International Index of Erectile Function 5-item (IIEF-5), Sexual Desire Inventory (SDI), International Prostate Symptom (IPSS) and 36-item Short Form Survey Instrument (SF-36) scores were measured at baseline and 12 weeks later. 34.2% (24/70) had AD, defined as TT $<$10.4 nmol/l.
Results (Table 1)
At baseline, men with AD had significantly lower SDI score, TT, SHBG and calculated free testosterone (FT), and higher IPSS score, but similar age, weight, WC, RHI, IIEF$-$5 and SF$-$36 scores. Similar weight losses were seen in men with (4.2$\pm$3.4%) and without (4.0$\pm$3.1%) AD. Men with AD had significantly greater increases in TT (30.2 vs9.3%), FT (27.5 vs 5.5%), and SDI score (37.1 vs 13.1%), and decrease in IPSS score (31.6 vs 22.9%). Improvements in WC, RHI, SHBG, IIEF-5 and SF-36 scores were similar. TT normalized in ten men (41.7%) with AD.
Conclusions
Weight loss induced by diet and exercise improves endothelial, sexual and urinary function, quality of life and sex hormones in obese men regardless of androgen status, with significantly greater improvements in testosterone, sexual desire and LUTS particularly in androgen-deficient men.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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**Table 1** Changes in anthropometry, sexual function, LUTS, sex hormones, sexual and endothelial function and LUTS after 12 weeks of lifestyle modifcation.

| | **Androgen deficient** ($n$=24) Mean $\pm$ s.d. | **Not androgen deficient** ($n$=46) Mean $\pm$ s.d. | **P value** |
|---|---|---|---|
| Baseline age (years) | 46.1$\pm$9.2 | 41.6$\pm$7.5 | 0.05 |
| Baseline BMI (kg/m$^2$) | 32.8$\pm$2.8 | 31.7$\pm$3.4 | 0.18 |
| Baseline weight (kg) | 98.6$\pm$9.7 | 94.7$\pm$10.8 | 0.14 |
| Baseline WC (cm) | 106.9$\pm$6.7 | 104.4$\pm$7.6 | 0.17 |
| Baseline TT (nmol/L) | 8.09$\pm$1.76 | 14.66$\pm$3.21 | $<$ 0.001 |
| Baseline SHBG (nmol/l) | 21.00$\pm$8.29 | 27.81$\pm$9.04 | 0.003 |
| Baseline FT (pmol/l) | 202$\pm$53 | 337$\pm$75 | $<$0.001 |
| Baseline IIEF-5 | 17.8$\pm$5.3 | 18.6$\pm$5.4 | 0.53 |
| Baseline SDI | 43.0$\pm$20.4 | 54.2$\pm$17.8 | 0.03 |
| Baseline IPSS | 6.8$\pm$4.8 | 4.1$\pm$3.2 | 0.01 |
| Baseline RHI | 1.77$\pm$0.42 | 1.96$\pm$0.63 | 0.13 |
| Baseline SF-36 (physical component) | 45.5$\pm$6.8 | 47.3$\pm$8.0 | 0.31 |
| Baseline SF-36 (mental component) | 48.8$\pm$7.3 | 49.2$\pm$8.2 | 0.84 |
| $\Delta$ weight (%) | $-$4.2$\pm$3.4 | $-$4.0$\pm$3.1 | 0.71 |
| $\Delta$ WC (%) | $-$2.7$\pm$2.2 | $-$3.7$\pm$2.8 | 0.07 |
| $\Delta$ TT (%) | 30.2$\pm$36.5 | 9.3$\pm$18.4 | 0.002 |
| $\Delta$ SHBG (%) | 9.9$\pm$16.4 | 10.1$\pm$13.8 | 0.95 |
| $\Delta$ FT (%) | 27.5$\pm$34.8 | 5.5$\pm$18.3 | 0.001 |
| $\Delta$ IIEF-5 (%) | 19.7$\pm$24.0 | 13.8$\pm$25.7 | 0.34 |
| $\Delta$ SDI (%) | 37.1$\pm$50.9 | 13.1$\pm$25.2 | 0.01 |
| $\Delta$ IPSS (%) | $-$31.6$\pm$30.4 | $-$22.9$\pm$57.2 | 0.04 |
| $\Delta$ RHI (%) | 25.8$\pm$30.1 | 23.3$\pm$33.1 | 0.75 |
| $\Delta$ SF-36 (physical component) (%) | 12.1$\pm$13.4 | 11.7$\pm$19.4 | 0.91 |
| $\Delta$ SF-36 (mental component) (%) | 11.3$\pm$10.1 | 9.3$\pm$2.4 | 0.58 |

Table 1

## P1241

**How safe is the use of herbal weight-loss products sold over the Internet?**

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Aim/background
The consumption of herbal weight-loss products sold over the Internet has rapidly increased, but the safety profile of these products has not been well known yet. Likewise, the declared ingredients in these products could be different than the marketed contents.
Material and Methods
Nine different herbal weight-loss products sold over the Internet were included. The content of each product were evaluated in the Laboratory of Forensic Medicine and the Scientific and Technological Research Laboratory of Inonu University.
Results
Even though analyzed weight-loss products were declared as pure herbal, three of them contain sibutramine, three contain caffeine, and three contain caffeine $+$temazepam. Sibutramine dosage in each capsule was found over 10 mg. We also measured toxic and trace element levels of nine herbal products and, we observed that these herbal products contain low amounts of Pb, Al, Ni and Ba.
Conclusions
Our results showed that herbal weight-loss products available without prescription and declared to be purely herbal may contain pharmaceutical substances like sibutramine or temazepam in high doses. Furthermore, they may also be contaminated with toxic metals. Without the suggestion or control of a physician, these products might cause various health problems that might be harmful. Strict legal rules and control mechanisms must be established to prevent their possible harmful effects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1242

### Obesity related comorbidities 2 years following bariatric surgery in a group of 215 German subjects

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Background
Bariatric surgery has shown to effectively reduce body weight and the prevalence of obesity associated comorbidities. However, especially data concerning diabetes is still conflicting. Also the benefit of surgical therapy varies strongly depending on individual patient characteristics.
Methods
Therefore we intended to investigate a heterogeneous group of obese patients ($n=215$) undergoing bariatric surgery in terms of related comorbidities and subgroup benefits.
Results
After one year mean reduction of body weight was $45\pm17$ kg (BMI $15.4\pm5.1$ $P<0.05$). High density cholesterol (HDL) increased on average by 8.0 $\pm9.2$ mg/dl ($P<0.05$), low density cholesterol (LDL)-Levels were reduced by $14.5\pm27.3$ mg/dl ($P<0.05$). The amount of patients who suffered from sleep apnoea reduced from 29.6 to 5.2% ($P<0.05$) after one and 5.3% after two years, those suffering from arthropathia from 83 to 20.7% ($P<0.05$) and 11.1% ($P<0.05$) respectively after one and two years. The prevalence of hypertension declined from 65.3 to 34.7% after 1 year ($P<0.05$) and 33, 3% after two years. If albuminuria was initially above 20 mg, available data demonstrated an average decrease of $126.9\pm214.3$ mg without reaching statistical significance.
Diabetes parameters showed a u-shaped curve with initial amelioration but deterioration of values from visit three on. However 85% of patients taking antidiabetics reduced the amount of oral antidiabetics and 100% the injected amount of insulin.
Subgroup analysis demonstrated a greater overall benefit regarding comorbidities for women or patients below 40 years (except for sleep apnoea). Reduction of HbA1c and loss of insulin was seen to a greater extent in patients with BMI $>50$ kg/m$^2$.
Conclusion
Bariatric surgery is an effective treatment of obesity which leads to a significantly lower BMI and has an immense impact on the improvement of comorbidities. Regarding the effect on diabetes close follow-up should be provided in order to optimise therapy after bariatric surgery.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1243

### Changes in gut hormone levels and glucose homeostasis in obese patients during the first year after laparoscopic sleeve gastrectomy

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Background
Changes in gut hormone levels after laparoscopic sleeve gastrectomy (LSG) has been proposed as a possible mechanism that explains the long-term weight loss and the improvement in glucose homeostasis after bariatric operations. In this prospective study we evaluated the changes in the fasting and postprandial levels of glucagon like peptide-1 (GLP-1), peptide YY (PYY), insulin and glucose during the first postoperative year after LSG.
Methods
Twelve morbid obese patients (three male, nine female, mean BMI $46.7\pm6.59$) were evaluated preoperatively and 6 weeks, 6 months and 12 months postoperatively. None of the patients had established diabetes preoperatively, however 85.7% of them (10/12) were insulin resistant. A standard 75 g oral glucose tolerance test (OGTT) was given after an overnight fast and blood samples were collected before and 30, 60, 90, 120 min after the OGTT for measurement of GLP-1, PYY, insulin and glucose.
Results
Body weight, body mass index (BMI) and waist circumference decreased markedly after LSG (all, $P<0.0001$). GLP-1 and PYY fasting levels did not change significantly postoperatively. GLP-1 and PYY postprandial responses as measured by Area Under the Curve (AUC) were significantly higher from the sixth postoperative week ($P<0.0001$). Fasting insulin levels were lower from 6 weeks postoperatively ($P<0.0001$), while insulin AUC decreased at 6 and 12 months postoperatively ($P=0.0093$). Finally, glucose AUC decreased at 6 and 12 months ($P<0.0001$) but glucose fasting levels decreased only at 12 months postoperatively ($P=0.0091$). Insulin sensitivity measured by Matsuda Index increased progressively postoperatively ($P<0.0001$) when the early insulin secretion measured by insulinogenic index did not change ($P=0.21$).
Conclusion
After LSG, GLP-1 and PYY postprandial responses in obese non- diabetic patients increased after an oral glucose tolerance test and was maintained for at least one year. Glucose and insulin levels decreased postoperatively showing a significant improvement in glucose homeostasis after LSG.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1244

### Changes of peripheral α-melanocortin stimulating hormone (α-MSH) in childhood craniopharyngioma patients in comparison with other forms of childhood obesity

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Objective
Relationships of blood circulating melanocortins to childhood obesity are not well established. We evaluated serum α-melanocyte stimulating hormone (α-MSH) in lean children and different study groups of childhood obesity.
Methods
We examined serum α-MSH in 52 otherwise healthy children with childhood obesity (Ob, mean age 11 years, 32 girls / 20 boys), 27 normal-weight children of same age, 7 additional obese patients with reduced melanocortin-4 receptor function (MC4Rmut), and 22 patients with craniopharyngioma (CP). Fasting serum α-MSH and leptin were measured by RIA. Serum α-MSH was also evaluated one hour after 500 kcal liquid meal (CP and Ob) and at the end of 1 year lifestyle intervention in 24 Ob patients.
Results
α-MSH levels were similar in obese vs. lean children but significantly lower in CP ($P<0.001$) and significantly higher ($P<0.05$) in MC4Rmut patients compared to Ob. 1 h after liquid meal, α-MSH increased in patients with SO but not with CP. After one year, α-MSH levels increased significantly in the successful weight reduction Ob subgroup despite unchanged cortisol levels. α-MSH changes correlated to weight status changes ($r=0.67$; $P=0.0003$) but not to changes of cortisol, insulin or insulin resistance index HOMA.
Conclusions
Persistently low α-MSH levels in CP patients are suspected to be due to pituitary or hypothalamic damage. High peripheral levels in MC4Rmut carriers indicate up-regulation of α-MSH. Changes of weight status are associated with changes of peripheral α-MSH. Synthetic α-MSH analogues might offer a promising therapeutic option for treatment of hypothalamic obesity in craniopharyngioma patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1245

**Measurement of serum high-molecular-weight (HMW) adiponectin level using recently-developed chemiluminescence enzyme immunoassay (CLEIA) at health check-up**

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Aim
A chemiluminescence enzyme immunoassay (CLEIA) for high-molecular-weight adiponectin (HMW-ADPN) was recently developed, and has been shown to be faster, more accurate and less expensive than conventional ELISA. In this study, we investigated the relationships between metabolic parameters and the HMW-ADPN level measured by CLEIA in a health check-up setting.
Methods
Informed consent was obtained from 1036 male and 416 female Japanese, 40 to 71 years of age, who participated in this study. Subjects receiving anti-diabetic medication or with suspected inflammation (serum CRP level $>$ 1 mg/dl) were excluded from the analyses.
Results
Median levels of serum HMW-ADPN measured by CLEIA were 2.5 μg/ml in males and 4.95 μg/ml in females. The correlation with the levels measured by ELISA was very good ($r=0.984$, $n=297$). The serum HMW-ADPN level in males with metabolic syndrome (MetS) ($2.13\pm1.14$, $n=173$) was significantly lower than that in those without MetS ($3.02\pm3.43$, $n=860$, $P < 0.0001$). HMW-ADPN correlated positively with the serum HDL-cholesterol level in both males and females ($r=0.41$ and 0.38, respectively, $P < 0.0001$ for both), and inversely correlated with the BMI, waist circumference and triglycerides ($r=-0.30$ to $-0.33$, $P < 0.0001$ for all). HMW-ADPN correlated also with insulin resistance index (HOMA-IR) and serum CRP levels in both males and females ($r=-0.33$ and $-0.28$ in males, and $r=-0.36$ and $-0.30$ in females, $P<0.0001$ for all). Stepwise multiple regression analysis revealed the serum HMW-ADPN level to correlate independently with serum HDL-cholesterol, sex, HOMA-IR and CRP ($F>20$, $P< 0.0001$, $R^2=0.392$).
Conclusions
These results suggest that measuring serum HMW-ADPN by CLEIA is useful, and that the serum HMW-ADPN level is closely correlated with parameters related to MetS and/or cardiovascular diseases.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P1246

**Anti-inflammatory effects of full-length and globular protein fragments of the new adipokine CTRP-3**

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Introduction
C1q/TNF-related protein-3 (CTRP-3) is a newly discovered adipokine with anti-inflammatory impact on monocytes by down-regulation of pro-inflammatory cytokines, e.g. interleukine 6 (IL6) and tumor necrosis factor alpha (TNF-alpha), in both adipocytes and monocytes. So far it is unclear which kind of interactions and molecular mechanisms are underlying the observed effects. Our aim is to characterize the differential effects of the globular and the collagenous domain of CTRP-3 and to compare these with the effects exerted by the full length protein.
Methods
DNA of recombinant full-length CTRP-3, its globular domain, and its collagen domain were transfected into and expressed in H5 insect cells. The secreted proteins were extracted from the cell culture supernatant and purified by affinity chromatography.
Costimulation experiments were performed in 3T3-L1 and monocyte-like THP-1 cells with pro-inflammatory stimuli lipopolysaccharide (LPS) and free fatty acids (FFA) plus CTRP-3 or its globular domain, respectively. Gene expression and secretion of pro-inflammatory cytokines were measured by real time RT-PCR and ELISA techniques. Protein levels of intracellular components of pro-inflammatory signaling were analysed by Western blot.
Results
Both full-length CTRP-3 and its globular domain were shown to decrease the amount of secreted IL6, MCP-1 and resistin after costimulation with LPS and FFA in differentiated 3T3-L1 cells. RT-PCR results confirmed down-regulation of these pro-inflammatory factors on mRNA level. Decrease of secreted IL6 in THP-1 cells upon costimulation with LPS and CTRP-3 globular domain could be shown as well.
Conclusion and outlook
The results suggest that the observed anti-inflammatory impact of CTRP-3 is mediated by its globular domain, whereas the collagen like domain is still to be tested on these effects. As a next step we plan to investigate the anti-inflammatory effects of CTRP-3 in an animal model of LPS-induced SIRS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1247

**Cholinergic and purinergic markers are increased in lymphocytes of patients with Metabolic Syndrome**

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Metabolic syndrome (MetS) is a condition characterized by abdominal obesity, insulin resistance, dyslipidaemia and hypertension, that is associated with cardiovascular disease and inflammatory metabolic disorders. This study aimed to ascertain the effect of selected parameters of purinergic and cholinergic systems in lymphocytes of MetS patients. The adenosine deaminase (ADA), dipeptidyl peptidase IV (DPP-IV) and acetylcholinesterase (AChE) activities, as well as γ-glutamyltransferase (GGT) and N-acetyl-b-glucosaminidase (NAG) activities in lymphocytes of patients with MetS ($n=38$) and controls ($n=41$) were evaluated. ADA and DPP-IV activities were markedly higher in lymphocytes of patients with MetS ($ADA=8.44\pm3.79$ U/l, $P < 0.05$; $DPP\text{-}IV=74.26\pm37.84$ U/l, $P < 0.0001$) when compared to the control group ($ADA=6.86\pm2.52$; $DPP\text{-}IV=46.02\pm20.93$). Experimental data also demonstrated that patients with MetS showed a significant increase on AChE activity when compared to the values of healthy subjects ($MetS=0.16\pm0.052$ vs $C=0.1062\pm0.028$ μmol AcSCh/h/ml; $P<0.0001$). However, no significant difference was found in NAG ($MetS=37.46\pm18.75$ vs $C=49.06\pm30.74$ μmol/l) and GGT ($MetS=5.568\pm3.09$ vs $C=5.22\pm2.99$ μmol/L) activities between the groups studied.
Our data suggest that the tissue distress and activation of the immune system observed in MetS patients affects enzymatic activities. The increase of DPP IV-activity observed in the MetS may be related to the interaction of ADA and DPP-IV at the T cells that results in co-stimulatory signs responsible for the activation of the T cell receptor. Also, the increase of AChE activity could be an inflammatory response caused by the insulin resistance state present by the MetS. These findings could most likely represent the sustained activation of lymphocytes in response to inflammatory stimuli of the MetS state.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1248

**Association between osteocalcin and insulin resistance in obese women**

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Introduction
Recent animal's studies suggest that osteocalcin have influence on beta cell proliferation, insulin secretion and insulin sensitivity. Osteoblasts appear to regulate energy expenditure by acting on adipocytes and pancreatic islet cells via osteocalcin, a 49-residue polypeptide. In turn, adipose tissue may also influence bone remodeling by regulating the activity of osteoblasts through adipokines, including leptin and adiponectin. Some of the complex mechanisms by which adipose tissue, brain and bone are able to regulate energy expenditure and glucose homeostasis are elucidated by recent studies.
Aim of the study
To investigate relationship between osteocalcin and insulin resistance in obese women with normal glucose tolerance.

Method
Fifty nine obese women (mean BMI = 35.2 ± 5.7 kg/m$^2$, mean age = 36.4 ± 10.2 years) participated in this study. We measured serum osteocalcin, fasting insulin level, fasting glucose level, HOMA-IR (Homeostasis model assessment of insulin resistance), AUC glucose (2 h OGTT), AUC insulin (2 h OGTT), adiponectin level and anthropometric parameters (body mass index, waist to hip ratio, total body fat). Statistics analysis was performed by SPSS 19. Pearson's correlation coefficients were calculated to evaluate the relationship between osteocalcin and other parameters.
Results
Serum osteocalcin was inversely correlated with fasting plasma insulin ($r = -0.379$, $P < 0.05$) and HOMA ir ($r = -0.349$, $P < 0.05$) and positively correlated with adiponectin level ($r = 0.603$, $P < 0.05$). There was no statistically significant correlation between serum osteocalcin and fasting plasma glucose, AUC glucose, AUC insulin and anthropometric values (body mass index, waist to hip ratio, total body fat).
Conclusion
Our results confirmed significant correlation between insulin and insulin sensitivity with osteocalcin as well as with adiponectin, which suggests possible novel role of bone via osteocalcin in regulation of glucose metabolism and insulin sensitivity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1249

**Serum leptin levels in patients with type 1 gaucher disease**

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Introduction
Leptin is an adipocyte-secreted hormone that plays a key part in energy homeostasis but also regulates reproductive, neuroendocrine, immune, and metabolic functions. Inflammatory cytokines may affect leptin secretion. Gaucher disease (GD) is autosomal recessive lysosomal storage disorder caused by the deficiency of enzyme glucocerebrosidase (GCD) with consequent massive acumulation of lipid-laden macrophages in various tissues. The pathology of Gaucher disease primarily results from the storage of sphingolipids in tissues throughout the mononuclear phagocyte system with subsequent macrophage activation and tissue inflammation with cytokine production.
Methods
We studied serum leptin concentration in a cohort of 15 patients with type 1 GD with and without enzyme replacement therapy (ERT) (9 females and 6 males, mean age 44.2+/-3.4 years,) and 20 age and BMI-matched healthy control subjects (13 females and 7 males, mean age 40.9+/−3.2 years).
Results
Serum leptin level is significantly higher in patients with GD type 1 as compared with healthy subjects (mean+/-SE, 21.0+/−5.8 vs 6.3+/-1.3 mcg/l respectively). There was no difference in serum leptin level between untreated ($n = 5$) and treated patients ($n = 10$) with type 1 GD. There was positive correlation between body mass index and leptin levels both, in GD patients irrespective of treatment status and healthy controls.
Conclusion
Our results confirm that serum leptin level is significantly higher in patients with type 1 GD in comparison with healthy subjects, and that this elevation is independent of body mass index. Also there was no effect of treatment status on serum leptin levels in GD patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1250

**Relationship between neck circumference and cardiometabolic parameters in non-obese brazilian adult population**

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Recent studies have suggested that upper body adiposity is associated with major cardiovascular risk (CVR). However most of them were conducted in obese patients. This study aimed to evaluate the relationship of neck circumference (NC) in a non-obese Brazilian adult population, considering that it would be a simple and time-saving measurement to identify patients with worse cardiometabolic profile. We evaluated 279 non-obese Brazilian adults [86 men (♂), 193 women (♀)], who had no known major medical conditions and were not receiving any medication living in Northeast of Brazil. Main anthropometric parameters evaluated were: BMI (kg/square meter): ♂25.0 ± 2.8/♀24.9 ± 2.9; NC (cm): ♂37.8 ± 2.0/♀33.0 ± 1.8; waist circumference (WC) (cm): ♂89.5 ± 8.0/♀85.6 ± 8.0; scapular circumference (SC): ♂95.1 ± 9.1/♀87.5 ± 5.2; systolic (SBP): ♂125.6 ± 14.8/♀118.0 ± 13.3, and diastolic blood pressure (mmHg) (DBP): ♂81.8 ± 8.3/♀78.7 ± 8.3; HDL (mg/dl): ♂43.5 ± 11.7/♀53.0 ± 12.6; triglycerides (mg/dl): ♂130.0 ± 76.8/♀107.0 ± 52.2; fasting glucose (mg/dl): ♂92.7 ± 10.7/♀91.4 ± 13.4; fasting insulin (uU/ml): ♂7.2 ± 4.0/♀9.0 ± 7.5; total cholesterol (mg/dl): ♂175.4 ± 37.4/♀182.7 ± 34.0; LDL (mg/dl): ♂104.6 ± 30.7/♀107.8 ± 30.2; HOMA-IR: ♂1.7 ± 1.0/♀2.0 ± 1.7. We found association (Pearson's correlation, $P < 0,05$) between NC and: WC ($r = 0.48$), AC ($r = 0.43$), SC ($r = 0.68$), BMI ($r = 0.35$), SBP ($r = 0.34$), DBP ($r = 0.27$), tryglicerides ($r = 0.24$), HDL ($r = -0.35$. These data suggest that NC could have a potential role in the clinical evaluation of cardiometabolic risk even in non-obese subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P1251

**A different method in obesity treatment; external gastric balloon**

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Obesity is an important and chronic disease. It is known that obesity closely related with many chronic disorders.Also it is known as a result of serious morbidity and mortality. For treatment of obesity, dietary, medicine, egzercise and surgery should be useful but they have also some adverse effects and surgery should be cause of mortality and severe morbidity.
Our method of EGB is completely non-invasive and we investigated the efficacy of obesity treatment by using 'External Gastric Balloon' which has no side effects, easy to use and cheap way to loose weight. It's produced by 'variteks company'. In this study we observed 91 patients, including 47 of study group and 44 of control group. In the study group patients used a corset for 2.51 ± 1.07 months with hypocaloric diet. And during this time average weight loss was 6.08 kg. On the other hand the control group was planned to take 1400–1600 kcal/day diet (∼1495.45 ± 256.95 kcal/day). Patients in the control group diet period was ∼6 months. Average weight loss was 6.06 kg. Patients in the study group weight loss with corset usage time between positive and statistically significant relationship at the level of 38.6% was observed ($P < 0.05$). A statistically significant relationship between duration of the diet in the control group patients and weight loss was not seen ($P > 0.05$).The higher weight loss can be seen with long corset using period.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1252

**Hyperuricemia and components of metabolic syndrome among patients of different age**

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Aim of research
To determine uric acid level in blood serum and incidents of hyperuricemia among women and men of different age and their relation with some components of metabolic syndrome.

Object of research
Women ($n=450$) and men ($n=120$), age of examined patients was from 20 to 89 years old. They were divided into the following groups: I group (BMI = 18.5–24.9), II group (BMI = 25.0–29.9), III group (BMI = 30.0–34.9), IV group (BMI > 35). Average age of examined patients was 60.4 ± 0.7 years.
Methods of research
Uric acid level of blood plasma was determined by uricase-peroxidase method. Statistic analysis was performed by Statistica 6.0.
Results
The level of uric acid increased with age in women and had a significant difference in women of 80–89 years ($r=0.18$, $P<0.05$). In men the maximal level of uric acid was in the group 60–69 years. Incidence of hyperuricemia among women was 17%, in men −30%. We determined that the highest level of triglyceride, cholesterol, systolic and diastolic pressure was among women and men with hyperuricemia. The higher level of uric acid was found among women in postmenopausal period with maximal body mass index (BMI > 35). In the I group level of uric acid was 277.52 ± 8.40 μmol/l; in II group – 286.81 ± 7.79 μmol/l; in III group – 291.81 ± 7.56 μmol/l, in IV group – 327.17 ± 12.17 μmol/l. Incidence of hyperuricemia among women in the I group was 10.2%, in II group – 15.9%; in III group – 21.2%, in IV group – 34.2%.
Conclusions
It was determined that the level of uric acid was increasing with age and the highest level of some components of metabolic syndrome (triglyceride, cholesterol, systolic and diastolic pressure) was among women and men with hyperuricemia. The higher level of uric acid was found among women in postmenopausal period with maximal body mass index (BMI > 35).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1253

**The comparison of obesity treatment with intragastric balloon and bihevioral-cognitive approach**

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Objectives
To compare obesity treatment with intragastric balloon (BIB – Bioenterics intragastric balloon) and behavioral-cognitive approach in order to determine the effectivness of these two methods in inducing weight loss, their impact on different features of metabolic syndrome and liver enzymes profile, and finally to evaluate patients attitudes and expectations regarding weight loss together with motivation and readiness to change their behaviour in order to lose weight.
Patients and methods
Subjects were required to be between 18 and 55 years old and to have a BMI between 30 and 45 to be eligible. Additional exclusion criteria, besides those required for BIB placement, were DM type 2, depression and binge-eating disorder. 60 subjects who had undergone the BIB procedure constituted the primary sample and 54 subjects who participated in weight loss cognitive-behavioral program constituted the paralel group. Anthropometric and biochemical parameters were assessed at baseline and after 6 months. Questionnaire assessment was used to evaluate patients attitudes and expectations regarding weight loss as well as their motivation and readiness to lose weight.
Results
The mean BMI during balloon treatment declined from 38.6 ± 3.9 to 32,84,39 kg/m$^2$ ($P<0.001$) with an excess weight loss (%EWL) of 44.6 ± 23.9 comparing with %EWL of 22.5 ± 11.5 in paralel group ($P<0.001$). A significant improvement in blood pressure, glycemia, trygliceride level and liver enzyme profile was seen in both treatment groups, but much more in subjects treated with BIB. Patients motivation and readiness to lose weight were high in both treatment groups, but subjects prior the balloon placement reported greater level of self-efficacy in weight control than those who decided to participate in cognitive-behavioral treatment.
Conclusion
Our results confirmed that intragastric balloon is useful method for promoting weight loss. Due to improvement of metabolic parameters and substantial benefit on liver function, obese people with metabolic syndrome appear to be the best candidates for BIB placement. Combining BIB with behavioral-cognitive approach might prove valuable for even greater weight loss.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1254

**Therapeutic efficacy of obesity treatment methods in teenagers**

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Aim
The aim of our study was to evaluate the effectiveness of the body mass (BM) decreasing program in pubertal children with alimentary obesity during the year of observation.
Materials and methods
Anthropometric changes in body mass index (BMI) and waist circumflex (WC) were analyzed every 1, 3, 6 and 12 months in 100 obese pubertal children (boys/girls = 50/50) with different treatment methods: the 1st group – only reducing diet (b/g = 30/30), the 2nd – combination with sport (12/11) and the 3rd – combined with metformin hydrochloride (9/8).
Results
There were no differences in initial BMI and WC between the 1st (30.3 ± 3.9 kg/m$^2$ and 92.1 ± 10.8 cm) and 2nd groups (29.8 ± 3.7 kg/m$^2$ and 89.6 ± 11.7 cm; $P>0.05$). The 3rd group's primary BMI and WC surpassed the same parameters in the 1st and 2nd groups (35.5 ± 7.4 kg/m$^2$ and 102.7 ± 15.8 cm), that was in keeping with abdominal obesity ($P=0.001$ and $P=0.007$; $P=0.01$ and $P=0.02$). There were no BMI changes in the 1st group during follow-up period ($P>0.05$). Gradual BMI decreasing was registered relating to initial ones starting from the 1st month of therapy (29.61 ± 3.29; 29.18 ± 3.53; 28.53 ± 3.45; 27.23 ± 3.63 kg/m$^2$, $P=0.003$). BMI reduction trend was observed every 3, 6 and 12 month ($P>0.05$) in the 3rd group. There were no differences between WC rates during the follow-up time regardless of treatment types. However WC decreasing tendency was noted in 3rd group (103.83 ± 17.65; 103.11 ± 17.13; 102.03 ± 16.52; 99.86 ± 15.97 cm).
Conclusions
Reducing nutrition combined with vigorous physical activity is enough for BM reducing in patient with uncomplicated obesity. Dietary intake combined with metformin hydrochloride should be recommended in children with abdominal and morbid adiposity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1255

**Insulin and C-peptide response after a test meal in obese patients before and 5 days after bariatric gastric by pass surgery**

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Different hypothesis exist about the mechanism of glycose control after bariatric surgery involving intestinal bypass. The aim of our study was to determine the insulin and C-peptide response after test meal (Fresubin drink a 200 ml; 200 kcal, 15% protein, 30% fat and 55% carbohydrate) before (day 0) and 5 days after gastric by pass surgery. Glycaemia (mmol/l; glucose oxidase), insulin (ECLIA, Roche Diagnostics, pmol/l) and C-peptide (ECLIA, Roche Diagnostics, pmol/l) were determined in 22 obese patients (age: 36.22 ± 12.66; BMI: 44.60 ± 4.31 kg/m$^2$) in two separate days in 0, 15, 30, 45, 60, 90 and 120 min. There were no significant difference between areas under the glucose curve (X ± S.D.; 645.562 ± 20.545 vs 621.600 ± 24.071 mmol/l per min; $P=0.304$) and under the C-peptide curve (293 074.125 ± 23 539.975 vs 267 750.375 ± 19 685.409 pmol/l per min; $P=0.317$) while there was significant lower area under the insulin curve in day 5 (38 263.075 ± 6079.509 vs 23 539.875 ± 2571.388 pmol/l per min; $P=0.032$). There was significant difference between basal glucose in 0 vs 5 day

($4.695 \pm 0.168$ vs $4.090 \pm 0.177$; $P = 0.0113$) while there were no significant difference between basal insulin ($100.514 \pm 24.191$ vs $61.229 \pm 7.166$; $P = 0.118$) and C-peptide ($1400.952 \pm 180.160$ vs $1074.571 \pm 96.986$; $P = 0.097$). There were no significant difference between peak levels in glucose ($6.330 \pm 0.218$ vs $6.165 \pm 0.236$; $P = 0.482$), insulin ($635.555 \pm 103.576$ vs $416.145 \pm 52.408$; $P = 0.066$) and C-peptide ($3300.900 \pm 279.453$ vs $3286.800 \pm 280.226$; $P = 0.960$) in day 0 and 5. In conclusion, insulin response after test meal is significantly decreased after gastric by pass surgery after 5 days without significant difference in glucose response, indicating early improvement in insulin sensitivity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1256

### The oxidative stress parameters within the context of metabolic syndrome evolution in men

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Introduction

The aim of study was to assess diversity of oxidative stress as a dynamic process throughout metabolic syndrome (MS) continuum ending with coronary heart disease (CHD) or diabetes mellitus (DM).

Methods

Main group (MG) of 154 men aged 25–60 years with abdominal obesity (waist circumference above 94 cm) was examined in comparison to 30 healthy men of similar age. In MG 28 men were diagnosed with CHD, 24 – with DM. Fasting glucose (FG) was assessed by hexokinase assay; fasting insulinemia (FI) – by ELISA; malonic dealdehyde (MDA), vitamins A (VA) and E (VE) in precipitated low-density lipoproteins (LDL) – by fluorometry; oxidative metabolism of polymorphonuclear leukocytes (PMN) – by spontaneous (spCLI), zimozan-induced chemoluminescence (indCLI), and stimulation indeces (SI); HOMA-IR index was calculated as FG (mM/l) $\times$ FI ($\mu$U/ml)/22.5.

Results

In MG (excluding DM patients) mean FG was similar to that in controls, whereas HOMA-IR index was higher ($3.54 \pm 0.12$ vs $2.22 \pm 0.24$; $P < 0.05$), reaching $6.08 \pm 0.52$ in DM subgroup. Mean MDA levels were higher in MG ($5.15 \pm 0.19$ vs $2.56 \pm 0.54$ nM/l per mg of LDL-protein; $P < 0.05$), being the highest in CHD ($5.72 \pm 0.26$) and somewhat less in DM patients ($4.78 \pm 0.67$). Mean VA levels were higher in MG than in controls only in patients without CHD and DM ($0.07 \pm 0.011$ vs $0.02 \pm 0.002$ mg/mg of LDL-protein; $P < 0.05$), however they fell down below controls to $0.01 \pm 0.001$ ($P < 0.05$) in CHD and DM patients; whereas VE did not differ between subgroups. Both spCLI (by 3.3 times) and indCLI (by 2.6 times) were higher in MG ($P < 0.05$), being the highest in patients without CHD and DM, whereas SI was the lowest in CHD ($2.18 \pm 0.13$) and DM ($2.14 \pm 0.15$) patients as compared to controls ($2.97 \pm 0.28$; $P < 0.05$).

Conclusion

The MS evolution towards CHD and DM is associated with cellular and humoral oxidative stress reactions with increase in MDA and PMN activation and concomitant deficiency of VA antioxidant potential and decreased PMN reserve reactivity.

Parameters of PMN oxidative metabolism (M $\pm$ S.E.M.).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1**

| Subgroup | N | spCLI | indCLI | SI |
|---|---|---|---|---|
| Controls | 30 | $0.015 \pm 0.002$ | $0.044 \pm 0.004$ | $2.97 \pm 0.28$ |
| Patients without CHD/DM | 102 | $0.049 \pm 0.005$ | $0.139 \pm 0.007$ | $2.85 \pm 0.25$ |
| Patients with CHD | 28 | $0.039 \pm 0.007$ | $0.085 \pm 0.004$ | $2.18 \pm 0.13$ |
| Patients with DM | 24 | $0.042 \pm 0.006$ | $0.090 \pm 0.010$ | $2.14 \pm 0.15$ |

## P1257

### Relationships between metabolic syndrome components and bone mineral density in ukrainian postmenopausal women

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Objective

Postmenopausal period in women is associated with the accelerated bone loss, contributing to the development of osteoporosis, fractures and decreasing of quality and duration of life. The metabolic syndrome (MS), which includes obesity, dyslipidemia, hyperglycemia, and hypertension, is a major public health problem also.

The purpose of our study was to reveal associations between tissue body composition, compounds of MS and BMD in postmenopausal women.

Design & method

The sample consisted of 90 postmenopausal 60–69 years old women (age: mean = 63.9; S.D. = 0.4); duration of menopause: mean = 14.1; S.D. = 0.9). MS was considered according to IDF (2005 years) criteria. Total body, lumbar spine, femoral neck, ulna radius bone mineral density (BMD), lean and fat mass distribution were measured by dual-energy X-ray absortiometry were compared for the cohorts with and without the MS. Other parameters including age, weight, height, the level of glucose, lipids were taken into account. Data were analyzed using Statistical Package 6.0 (Statsoft).

Results

Findings revealed that 55 (61.1%) of these postmenopausal women had MS. In patients with and without MS compared, fat mass was higher in the former group In patients with and without MS compared, BMD was lower in the former group at femoral neck ($0.77 \pm 0.02$; $0.89 \pm 0.03$, respectively; $F = 10.2$; $P = 0.002$), ulna radius ($0.57 \pm 0.02$; $0.64 \pm 0.02$, respectively; $F = 6.4$; $P = 0.01$).and at different body regions also. Lean mass comparing didn't show significant differences. Diabetes mellitus and hyperglycemia in subjects compared with age and body mass index matched non-diabetic subjects were associated with lower BMD. Serum concentration of triglycerides was a negative predictor of BMD. Increasing quantity of the MS components in women during the postmenopausal period had a negative correlation with BMD at every site.

Conclusion

Our findings suggest that body fat mass is not significant protective factor for BMD loss.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1258

### Allograft inflammatory factor 1 is a new human adipokine regulating adipose inflammation

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Introduction

Allograft inflammatory factor 1 (AIF-1) is a putative obesity gene which may influence the function of white adipose tissue (WAT).

Objective

To study the role of AIF-1 in human WAT.

Design and main outcome measures

mRNA expression and WAT secretion of AIF-1 was determined in subcutaneous and visceral WAT from non-obese subjects. Differentiated human adipocytes were treated with recombinant AIF-1 *in vitro*.

Results

AIF-1 was secreted in a time dependent fashion from WAT. The major source of AIF-1 was macrophages followed by lymphocytes; fat cells contributed only to a minimum extent. Expression of AIF-1 was similar in visceral and subcutaneous WAT and was markedly increased in obese subjects; the latter was normalized following weight reduction. AIF-1 stimulated the adipocyte production and secretion of monocyte chemoattractant protein 1 (MCP-1) without influencing other proteins involved in inflammation.

Conclusion

AIF-1 is a novel adipokine involved in the pathophysiology of obesity. It is produced mainly by macrophages within WAT and i. It's expression is increased in obesity as a secondary phenomenon. AIF-1 may play a paracrine role in the

inflammation of WAT through cross-talk between macrophages and fat cells, by enhancing adipocyte production and secretion of MCP-1.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1259

**Body composition and radiation exposure in the Adult Health Study**

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Background
Several studies have shown that medical radiation exposure in childhood might cause alteration in body composition, although all such studies were of a small scale and individual radiation doses were not estimated.
Objective
The aim of this cross-sectional study was to evaluate whether or not atomic-bomb (A-bomb) radiation exposure is associated with alteration of body composition using dual energy X-ray absorptiometry (DXA).
Methods
A total of 1729 participants in the Adult Health Study, comprising A-bomb survivors and their controls, were eligible for the study. Regional fat mass and lean mass were estimated by whole body DXA. Appendicular lean mass (ALM) /height$^2$(ALMH$^2$) and trunk (central) fat and limb (peripheral) fat ratio (trunk-to-limb fat-mass ratio) were calculated. We analyzed association between radiation dose and body composition using multivariate linear regression model.
Results
A-bomb radiation dose was associated with decreased levels of ALMH$^2$, after adjustment for smoking status and other conventional risk factors. Significant negative association between radiation dose and ALMH$^2$ was observed among A-bomb survivors exposed at young ages. Furthermore, there was significant positive association between radiation dose and trunk-to-limb fat ratio in young female survivors.
Conclusions
The observed effects on body composition may be useful information for elucidation of mechanisms behind late effects of radiation exposure on health outcomes including cardiovascular disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1260

**Usefulness of preoperativee exenatide in control of risk factors for bariatric surgery on type 2 diabetic patients with morbid obesity**

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Introduction
The aim of this study was to evaluate effects on glycemic control, body weight and cardiovascular risk factors (BP, lipids and Epworth sleepiness scale) in diabetic patients with morbid obesity treated with exenatide.
Material and methods
We studied consecutively 21 T2DM patients, all of them treated with metformin full dosage and insufficient metabolic control referred to our clinic for preoperative control of bariatric surgery. Twelve of these patients (treatment group) were treated in addition with conventional regimen exenatide (5 µg s.c./12 h, 30 days and 10 µg s.c./12 h later) for 6–12 months or until surgery. Not altered any other therapeutic regimen. All of them were attached to a specific program of diet and exercise and reviewed monthly by a specialist nurse practitioner. Mann–Whitney and Wilcoxon test were performed.
Results
The mean patient age was 38.8±10.8 years in treatment group and 44.9±12 years in control group, with a ratio female:male 2:1. The average time of diabetes mellitus in treatment group was 3.1±1.1 years an 3.05±1.5 years in control group. Two thirds of patients had a diagnosis of obstructive sleep apnea (OSA) and ambulatory CPAP used during nocturnal rest, without statistically significant differences between groups. At the end of the study, better results were obtained in the treatment group, with statistically significant differences in clinical parameters of control of OSA (Epworth sleepiness scale): 6.7±3.2 vs 12.1±1.5 $P<0.01$, and in glycemic control (HbA1c: 7.1±0.9 vs 8.6±0.9, $P<0.01$), but not in weight loss (BMI: 42.1±4.5 vs 44,8±6.6, $P=0.7$). The most frequent adverse event in treatment group was nausea mild to moderate. There were no losses to follow up due to this cause during the study period.
Conclusions
Adjunctive therapy with exenatide in morbidly obese T2DM-patients achieved a sustained improvement in glycemic control and OSA and persists after body weight stabilization.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1**

| | Group exenatide (basal) | Group control (basal) | P | Group exenatide (3 months) | Group control (3 months) | P | Group exenatide (1 year) | Group control (1 year) | P |
|---|---|---|---|---|---|---|---|---|---|
| BMI | 48.83 | 47.75 | NS | 42.79 | 43.6 | NS | 42.1 | 44.8 | NS |
| HbA1c | 10.1 | 9.1 | NS | 855 | 8.68 | NS | 7.1 | 8.6 | <0.01 |
| Epworth | 16.92 | 17.89 | NS | 11.16 | 14.33 | NS | 6.75 | 12.1 | <0.01 |
| TAS | 155 | 149 | NS | 137 | 147 | NS | 130 | 135 | NS |
| TAD | 96 | 89 | NS | 85 | 90 | NS | 79 | 91 | <0.05 |
| Cholesterol T. | 244 | 251 | NS | 185 | 212 | NS | 205 | 210 | NS |
| Triglycerides | 179 | 154 | NS | 90 | 114 | NS | 82 | 109 | NS |

## P1261

**Preclinical anti-obesity activity and safety assessment of a novel drug affecting CB1 receptor**

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Dietressa is a novel drug for the treatment of obesity containing ultra low doses of antibodies to CB1 receptor. A number of new drugs are now at different stages of development, and cannabinoid receptor type 1 is one of the most challenging targets for obesity treatment. However, there are some safety concerns over CB1 receptor antagonists, especially their psychological adverse effects.
The study included two parts: the first one was performed on 33 C57B1 male mice (baseline weight 12–14 g, 28 days) receiving a modified high-fat (45%) diet (MP Biomedicals, USA) in combination with distilled water (control, 0.2 ml/mice ig),

**Table 1** Body weight gain in mice on a high-fat diet, M±m

| | Body weight (g), week of the study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Control. $n=11$, | 12.2± 0.50 | 15.3± 1.00 | 16.0± 1.00 | 12.2± 0.50 | 18.2± 0.60 | 12.2± 0.50 | 20.2± 0.50 | 20.6± 0.60 | 21.5± 0.90 |
| Δ from baseline, % | | 25.4 | 25.4 | 31.3 | 49.3 | 41.8 | 65.7 | 68.7 | 76.1 |
| Dietressa, $n=11$ | 13.8± 1.10 | 13.5± 0.50 | 16.7± 0.40 | 19.8± 0.50 | 20.2± 0.80 | 20.9± 0.60 | 22.4± 0.90 | 23.6± 1.30 | 22.9± 1.10 |
| Δ from baseline, % | | - 2.6 | 21.1 * | 43.4** | 46.1 | 51.3 ** | 61.8 | 71.1 | 65.8 |
| Sibutramine, $n=11$ | 13.3± 0.60 | 16.2± 0.20 | 17.5± 0.30 | 19.6± 0.50 | 20.2± 0.40 | 20.2± 0.40 | 19.8± 0.50 | 21.6± 0.40 | 22.0± 0.50 |
| Δ from baseline, % | | 21.9 | 31.4** | 47.9 ** | 52.1* | 52.1** | 49.4 | 63.0 | 65.8 |

Differences from the control are significant at *$P<0.05$; **$P<0.01$.

sibutramine (10 mg/kg ig) or Dietressa (0.2 ml/mice ig) for 2 months. The second one was conducted on 20 white outbred laboratory male rats (230–250 g, 6–8 months) administered with distilled water (control, 2.5 ml/kg ig) or Dietressa at 2 (control, 2.5 ml/kg ig) for 5 days. Reinforcing potential was evaluated using the self-stimulation reaction (SSR) in Skinner box.
Starting from week 6 of administration Dietressa reduced body weight gain in mice at a high-fat diet. By the end of the study (week 8) body weight gain in the control group was 76.1%, and in Dietressa and sibutramine groups – 65.8%. Dietressa did not affect SSR frequency in rats. Discontinuation of the drug caused no withdrawal.
Preclinical studies of Dietressa revealed its ability to decrease body weight gain in experimental animals on a high fat diet without withdrawal symptoms.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P1262

### Green tea consumption, weight loss and resting energy expenditure

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Introduction
Green tea consumption has been reported to be associated with weight loss although the results of the studies are inconclusive and potential underlying mechanism is not yet clear. We aimed to evaluate the effect of green tea extract on weight loss, body composition and resting energy expenditure in obesity.
Methods
Twenty-nine obese patients (body mass index (BMI) $>30$ kg/m$^2$) were included in the study. Weight, BMI, waist circumference (WC), body composition and resting energy expenditure (REE) were recorded at baseline. Patients were randomly assigned to receive green tea extract (400 mg standardized green tea extract, 200 mg polyphenols and 100 mg green tela leaf powder) or placebo along with a standard hypocaloric diet. All measurements were repeated after 3 months.
Results
Fourteen patients received green tea exctract and 15 received placebo. Two groups were similar regarding all parameters. Both groups have lost weight significantly with a decrease in BMI, fat mass and WC. REE did not change in both groups. Addition of green tea extract did no increase REE, amount of weight loss or body composition.
Conclusions
Green tea extract does not have any effect on REE, weight loss or body composition in obese patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1263

### Waist and abdominal circumference in seven years old children: relationship between methods

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Introduction
Waist circumference serves as a key indicator of abdominal obesity and predictor of cardiovascular and metabolic risk even in children. Under the current WHO standard methodology, waist circumference is measured midway between the lowest rib and the iliac crest. However, comparison of current and earlier data is complicated as different waist circumference measurement methodologies have been used in the past. For instance, previous Czech studies commonly worked with abdominal circumference measured at the umbilicus level. Therefore, it is necessary to find a formula for comparison of both measurement methods (to enable comparison of waist circumference in Czech 7 years old children who participated in WHO Europe initiated COSI project with older Czech data from National Anthropological Surveys). In our new study we measured both waist circumference and abdominal circumference on a sample of the same age group, using the data to determine an index for recalculation of historic results.
Methods
At the moment, the sample includes 112 children (54 girls, 58 boys). Data were collected during morning classes. Measurements taken included: weight, height, waist circumference, abdominal circumference as specified above, and hip circumference. The relationship between abdominal circumference (AbdC) and waist circumference (WC) was evaluated using polynomial regression.
Results
Sample characteristics (mean $\pm$ S.D.):
Age $6.95 \pm 0.58$; AbdC $56.39 \pm 4.61$; WC $54.50 \pm 4.04$; BMI $15.53 \pm 1.55$; BMI $Z$-score $-0.10 \pm 0.89$.
Polynomial regression analysis of AbdC and WC data provided the following relationship:
Boys: WC $= 5.88661 + 0.868423 \times$ AbdC.
Girls WC $= 5.47176 + 0.866742 \times$ AbdC.
Conclusion
We suggest using the above equations to recalculate abdominal circumference to waist circumference for direct comparison of studies that use different measurement methodologies. We are planning to expand the sample to verify our preliminary results and increase the relevance of our conclusions.
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Declaration of interest
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## P1264

### Bariatric surgery in men: effects on gonadal hormones, body composition, glucose, lipid and bone metabolism

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Background
Obesity-related, isolated hypogonadotropic hypogonadism (IHH) occurs in more then 40% of morbidly obese men.
Hypothesis
IHH and prolonged persistence of low testosterone levels after bariatric procedures may reduce the beneficial effects of surgery.
Objective
To evaluate the impact of IHH on the results of bariatric surgery.
Patients and methods
observational study with measurement of gonadal hormone levels, assessment of body composition and glucose, lipid and bone metabolism during the first year after bariatric surgery in 13 hypogonadal (free testosterone $<225$ pmol/l) and 11 age-matched eugonadal morbidly obese men (free testosterone $>225$ pmol/l).
Results
serum free testosterone rose gradually after bariatric surgery in eugonadal as well as in hypogonadal men. The increase in free testosterone was directly related to the amount of weight loss. Gonadal hormone status prior to surgery did not affect the 1-year outcome of bariatric surgery, but the type of surgery did. Gastric bypass induced a greater loss of fat, but also caused a greater loss of muscle and bone than gastric banding.
Conclusion
Bariatric surgery raised serum T levels, but the improvement was less in hypogonadal than in eugonadal men. Obesity-related IHH did not reduce the efficacy of bariatric surgery.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1265

### Outcome of obese adults after laparoscopic adjustable gastric banding: a retrospective follow-up study

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Background
Laparoscopic adjustable gastric banding (LAGB) is now the most common surgical treatment for obesity. However, few data exist on the outcome of LAGB in our setting.
Objective
This study aimed to assess the outcome of obese adults after LAGB in terms of weight loss.
Methods
We conducted a retrospective review of records of all patients who underwent LAGB between January 2004 and December 2009 at St Luke's Medical Center. Patients were included in the analysis if they were at least 18 years old at the time of surgery and had one or more recorded weight measured during the follow-up period. Outcome was assessed using percentage excess weight loss (%EWL) at 1 year $\pm$6 months intervals. Analysis of variance was used to compare three or more groups of continuous variables while chi-square or Fischer's exact were used to analyse categorical variables. Logistic regression was done to determine independent factors that predict successful weight loss after gastric banding.
Results
A total of 97 patients met the inclusion criteria. The majority of the patients were Filipino (77%), and female (61%). Their ages ranged from 18 to 68 (mean 36.1 years $\pm$12.2). The mean BMI was 44.1 $\pm$ 0.1 kg/m$^2$ and mean excess weight was 61.4 $\pm$ 26.5 kg. Greatest %EWL (43.46%) and BMI reduction (21.55%) were attained at 2 years but was not sustained thereafter. The highest success rate (%EWL $>$ 50%) was also achieved at 2 years (48%). Follow up rate was high at 6 months (98%) and 1 year (68%), but, thereafter fell below the accepted value of 61%. Independent predictor of successful LAGB at 1 year was BMI $<$ 43.4 kg/m$^2$.
Conclusion
Obese adults achieved a substantial mean weight loss of 43.46% at 2 years after LAGB.
Outcome measures in obese adults after laparoscopic adjustable gastric banding over time of follow-up at St Luke's Medical Center, Philippines.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1**

| Outcome measure | Time after laparoscopic adjustable gastric banding <6 months | 1 year | 2 years | 3 years | 4 years |
|---|---|---|---|---|---|
| Percent excess weight loss | 18 | 31 | 44 | 28 | 32 |
| Percent BMI reduction | 9 | 15 | 22 | 13 | 18 |
| Rate of successful weight loss (%EWL > 50%) | 1.1 | 14 | 48 | 17 | 33 |
| Follow-up rate | 98 | 68 | 26 | 22 | 13 |

## P1266

### Proteomic analysis of adipose tissue in health and disease

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Adipose tissue is a highly active metabolic organ which, together with its role as an energy storage depot, produces a wide variety of bioactive molecules (i.e. adipokines) with important roles in the regulation of energy metabolism and homeostasis, immunity and inflammation. Alterations in lipid metabolism and/or in adipokine production associated to the excess of adipose tissue that defines obesity or to adipose tissue deficiency, as occurs in lipodystrophy, are linked to insulin resistance, which represents a major risk factor for the development of type II diabetes, dyslipidemia, hypertension and cardiovascular diseases. In order to identify potential biomarkers of adipose tissue (dys)function, we employed proteomic techniques for the molecular analysis of adipose tissue components (adipocytes and stromal-vascular fraction), adipose tissue depots (subcutaneous (SAT) vs visceral (VAT) adipose tissue), and adipose tissue-related pathologies (insulin resistance associated to obesity). These studies revealed that, in lean individuals, the stromal–vascular faction contributes to major protein differences between VAT and SAT; in particular, we identified several differentially expressed proteins (ezrin, lamin A/C) that could contribute to the distinct association of VAT and SAT to specific adipose tissue-related pathologies (metabolic syndrome and lipodystrophy respectively). Comparative analysis of paired samples of VAT and SAT from lean and obese individuals also unveiled that obesity evokes depot-specific changes in the proteomic profile of adipose tissue. Furthermore, our data support the view that development of insulin resistance linked to obesity involves changes in adipose tissue proteins related to the cytoskeleton, glucose metabolism, cellular iron metabolism and protein folding and stress response. Collectively, our proteomic studies support the relevance of specific biological processes for the maintenance of normal functioning of human adipose tissue and their dysregulation in relation to the development of insulin resistance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1267

### Is fat mass index superior to total fat mass percentage in predicting the metabolic syndrome?

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Aim
We aimed to compare the cut off values, defined to express the excess total body fat (TBF) percentange and increased fat mass index (FMI) as predictors of metabolic syndrome (MS).
Subjects and methods
A total of 162 obese (body mass index (BMI) $>$ 30 kg/m$^2$) subjects' TBF were measured by 'Tanita TBF 300 Body Composition Analyzer'. Patients were divided into two groups called normal obese (NO) and fatty obese (FO) according to the cut off values determined in De Lorenzo, NHANES and Switzerland models and cut off value determined for FMI (Table). Then we compared MS (National Cholesterol Education Program critera is used) prevalences in NO and FO groups.
Results
The number of FO patients according to the DeLorenzo, NHANES, Switzerland cut off values and FMI cut off values were respectively 142 (88%), 112 (69%), 119 (73%) and 88 (54%; Table). The prevalences of MS among FO patients were respectively 34% (*P*: 0.916), 35% (*P*: 0.858), 34.5% (*P*: 0.853) and 42% (*P*: 0.02; Table). According to these results, while cut off values for FMI were significantly associated with MS, the others were not. The percentage of MS among FO patients was highest (42%) and the percentage of the patients without MS among NO patients was highest (76%) according to the FMI cut off values.
Conclusion
Different cut of values used to define excess body fat according to gender and age by DeLorenzo, NHANES and Switzerland models seems not to predict the MS; but cut off values for FMI, significantly predicts MS. Contribution of other anthropometric measures such as sex, age, race and height to TBF, may increase its predictive value for MS development
Metabolic syndrome, total body fat, fat mass index

**Table 1** Comparison of MS prevelances between NO and FO patients according to the cut off values determined in De Lorenzo, NHANES and Switzerland models and cut off values determined for FMI.

| | DeLorenzo* | | NHANES† | | Switzerland‡ | | FMI§ | |
|---|---|---|---|---|---|---|---|---|
| | Normal obese | Fatty obese | Normal obese | Fatty obese | Normal obese | Fatty obese | Normal obese | Fatty obese |
| MS present | 13 (65%) | 94 (66%) | 34 (68%) | 73 (65%) | 29 (67%) | 78 (65.5%) | 56 (76%) | 51 (58%) |
| MS absent | 7 (35%) | 48 (34%) | 16 (32%) | 39 (35%) | 14 (33%) | 41 (34.5%) | 18 (24%) | 37 (42%) |

*$P$=0.916, †$P$=0.858, ‡$P$=0.853, §$P$=0.020. MS, metabolic syndrome; NO, normal obese; FO, fatty obese; FMI, fat mass index (total body fat (kg)/square of height (m$^2$)).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1268

**Relationships between hypovitaminosis D, low-grade chronic inflammation, and leptin-to-adiponectin ratio in PCOS: is this a matter of fatness?**

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Introduction
Several cross-sectional studies evidence the association between hypovitaminosis D, obesity, insulin resistance (IR), and polycystic ovary syndrome (PCOS) cohorts, although the role of obesity *per se* is still debatable. Increased leptin-to-adiponectin ratio (L/A) and low-grade chronic inflammation markers, both due to accumulation of dysfunctional adipocytes, have been recently reported in PCOS. Aim of our study was to investigate the relative roles of obesity, low-grade chronic inflammation and dysfunctional adiposity in hypovitaminosis D in PCOS according to BMI.
Methods
Eighty-eight women (age $24.3 \pm 4.3$ years; BMI range 18–47 kg/m$^2$; Ferriman–Gallwey score $\geq 8$) were consecutively recruited in the study from those referred for PCOS to our unit (42 lean and 46 overweight-obese (O/O) subjects). Testosterone, sex hormone-binding globulin, fasting plasma glucose and insulin, 25-hydroxy vitamin D (25OHD), C-reactive protein (CRP), interleukin (IL) 6, leptin and adiponectin were measured using commercially available kits. homeostasis model assessment of IR (HOMA-IR), free androgen index (FAI), and L/A were calculated.
Results
In O/O PCOS women FAI, HOMA-IR, 25OHD, CRP, IL6, L/A, and waist were significantly higher compared to lean PCOS ($P<0.001$). In subgroup analyses, the strengths of the associations between all the study variables were similar between lean and O/O PCOS, out of the association between 25OHD and CPR and IL6 that retained an inverse statistical significance in only O/O PCOS women.
Conclusion
While lower 25OHD and higher L/A were associated in both lean and O/O PCOS, lower 25OHD with higher CRP and IL6 were evidenced only in O/O PCOS. In other terms, in the complex relationships between hypovitaminosis D and PCOS, L/A might represent an early marker of the negative effect of adiposity on circulating 25OHD levels, while an increasing amount of dysfunctional adipocytes were likely required to evidence the association of low 25OHD levels with different markers of low-grade chronic inflammation, such as CRP and IL6.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1269

**Incidence of glucose and lipid metabolism disorders in overweight children and adolescents in Croatia**

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Prevalence of obesity in children and adolescents is reaching epidemic proportions. It is followed by the increase in incidence of type 2 diabetes (DM2) and dyslipidemia, and both disorders are risk factors of cardiovascular complications.
Aim
The aim was to define the prevalence of impaired glucose tolerance (IGT), DM2, hypercholesterolemia and hypertriglyceridemia in overweight children and adolescents in Croatia.
Subjects and methods
In 498 overweight children (266 girls, 232 boys) aged 1–19 years, with body mass index (BMI) >90%, a standard oral glucose tolerance test was performed (referent values according to American Diabetes Association) and cholesterol and triglyceride levels were determined (referent values according to Lipid Research Clinic Program Percentile Levels Defining, levels >95th percentile were considered increased).
Results
IGT was found in 52 children (10.4%) with no statistically significant difference according to age, sex or BMI. DM2 was found in two children (0.4%). Increased cholesterol levels were found in 86 children (17.3%), significantly more often in boys than in girls (21.6%: 13.5%; $P<0.001$). Increased triglyceride levels were found in 150 children (30.1%), also significantly more frequently in boys (35.8%: 25.2%; $P<0.01$). Increase of both cholesterol and triglyceride levels were found in 90 children (9.8%) with significantly higher BMI ($P<0.05$) and no difference according to age and sex.
Conclusion
Incidence of IGT in overweight children in our population (10.4%) is smaller than in USA (20–25%), and it resembles the incidence in Western Europe (6.3–11%). Incidence of DM2 was fortunately very small (0.4%).
Data of increased cholesterol and triglyceride levels in overweight children (17.3 and 30.1%) are close to those in literature.
Obesity prevention programs and interventional diet regimens in already overweight children are essential for reducing the risk of DM2 and cardiovascular complications.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1270

**Factors determining weight gain in adults and relation with glucose tolerance**

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Objective
Modifications in lifestyle and diet are major contributors for the high prevalence of obesity. Very few longitudinal studies have evaluated the changes in body weight over time. Dynamic changes in body weight are also an important indicator of risk for type 2 diabetes. The aim of this study is to assess factors associated with weight gain in a population of Spanish adults.
Methods
The study was undertaken in two population-based cohorts of Spain. The first phase of the 'Asturias Study' (1998–1999) included 1034 persons, of whom 701 were reassessed in 2004–2005. The first phase of the 'Pizarra Study' (1996–1998) included 1226 persons, of whom 783 were re-evaluated in 2002–2004. Both studies involved a questionnaire on socio-demographic variables and an oral glucose tolerance test (OGTT).
Results
During the follow-up, 32.3% of the participants lost weight, 34.5% gained fewer than 4 kg and 33.2% gained more than 4 kg. Weight gain was greater in persons younger than 50 years and in those with an initial body mass index below 30. Weight gain was associated with a greater incidence of diabetes and glucose dysregulation, whereas weight loss in persons with these disorders was associated with a normal OGTT 6 years later. Persons who ate watching TV, those who took less exercise and those who reported a higher daily calorie intake experienced greater weight gain.
Conclusions
The longitudinal changes in weight decisively affect the development of diabetes and glucose dysregulation. The weight is a dynamic phenomenon affected by several social customs.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1271

**Obesity prevention: main findings of the EPODE European network project**

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Introduction
EPODE is a coordinated, capacity-building methodology for communities to implement effective and sustainable strategies to prevent childhood obesity. Since 2008, the EPODE European Network (EEN), supported by the European Commission (DG SANCO), is aimed at facilitating the implementation of interventions using the EPODE methodology in other regions and countries.
Methodology
From 2008 to 2011, four committees – gathering universities and experts from different fields – have documented and conceptualized EPODE practices. This has been done through broad literature screening, 75 interviews and 20 committee meetings gathering more than 100 multidisciplinary contributions.
Results
Research results highlight: – The leading role of local authorities to initiate, lead, catalyse, federate and fund targeted and multisectoral actions, with the support of municipal services and local stakeholders;
– The importance of a tight collaboration between central and local coordination structures in order to develop relevant, concrete and tailored actions, addressing real life conditions;
– The interest of public-private partnerships to support actions on a long-term within the framework of a transparent governance and clear rights and obligations for all partners;
– The need of a multidisciplinary and significant evaluation (10–15% of total budget), adapted to the field and not only focusing on obesity prevalence but also on the process of actions.
Conclusion
Since the beginning of EEN, the use of EPODE methodology has been scaled up and is considered to be the largest global obesity prevention initiative at community level. EPODE is used in more than 8 countries, 500 cities and early evaluation suggests encouraging results.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1272

**Dietary habits and body weight in an unselected population of Italian high-school students**

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Aim
This study was aimed to estimate the nutritional status and the prevalence of weight excess in a wide unselected population of adolescent and young students from Emilia Romagna.
Material and methods
Three thousand and ninety-one high school students, aged 15–19 years were consecutively contacted. One thousand eight hundred sixty out of 3091 (60.2%) accepted to participate in an epidemiologic study, whose procedures included anthropometric measurements and a nutritional assessment, through a personal interview with a trained dietitian that employed the food-frequency questionnaire and multiple 24-h dietary recall. The percentage of males and females in the study population was 38.6 and 61.4% respectively.
Results
We found a prevalence of overweight or obesity (based on BMI values, standardized for age and sex) of 31.6% in the whole sample, with a higher rate in males than in females (38.7 vs 27.3% respectively). Underweight subjects were 5.9% of the whole sample, and 2.8 and 7.7%, of males and of females, respectively. Mean energy intake was 2409 and 1910 kcal/day for males and females respectively. The analysis of macronutrients showed an unbalanced diet, with excess of percent energy from lipids ($35.5 \pm 5.2\%$) and a deficit in percent energy from carbohydrates ($49.5 \pm 5.6\%$), when compared to the recommended daily intake (RDA). That was particularly evident for females, whose percent energy from lipids was slightly but significantly higher than in males (35.8 vs 35.1, respectively; $P = 0.006$). Moreover, among lipid and carbohydrate fraction a larger portion than recommended was represented by saturated fats ($31.6 \pm 5.2\%$) and oligosaccharides ($31.3 \pm 8.9\%$) respectively. As concerns micronutrients, we found a deficit of fibres (significantly more severe in males than in females) and of vitamin D and calcium whose consumption was about a half of the recommended quantity. In conclusion, this study shows that the quality of diet in the young Italian population has reached a critical state. In addition, it shows that the prevalence of overweight or obesity is higher than expected, similarly to published international trends.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1273

**Relationship between obesity and Leptin (G-2548A) and Leptin receptor (668A > G (Q223R)) gene polymorphisms in Turkish population**

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Obesity is due to the combined effects of genetic, environmental, lifestyle and the interactions of these factors. Leptin (LEP) is an adipocyte-derived hormone that acts to reduce food intake and increase energy expenditure by binding and activating its specific receptor in the hypothalamus. In humans, LEP and LEPR have been mapped to 7q31.3 and lp31, respectively. The LEP and LEPR genes have been investigated in the search for gene variants potentially related to the pathophysiology of obesity in diabetes and its associated complication. In this study, we were investigated LEP gene G-2548A and LEPR gene 668A > G (Q223R) polymorphisms and its association with obesity in Tokat district particular subjects. The study group comprised to 112 obese patients (81 female, 31 male, mean BMI 35.41 kg/m$^2$) ages from 18 to 82 (mean age; $50 \pm 14.25$ year), and 105 non-obese healthy subjects (62 female, 43 male, mean age $34.25 \pm 15.44$ year, and mean BMI 21.57 kg/m$^2$) were used the control group in this study. The obtained results showed that, there is no significant differences for allele and genotype distributions of LEP gene G-2548A and LEPR gene 668A > G (Q223R) polymorphisms between obese and control subjects ($P > 0.05$). In the comparison between BMI > 35 and BMI < 35 obese groups we were obtained significantly difference for GG genotype for LEP gene G-2548A polymorphisms ($P = 0.034$). This results shows that GG genotype for LEP gene G-25248A polymorphism maybe predisposed to morbid obese. There was a significant difference in LEP and LEPR double GG-GG (RR) genotype for frequency when comparing obese (BMI > 35) and control subjects ($P = 0.015$). According to these results, we thinking that, LEP gene G-2548A and LEPR gene 668A > G (Q223R) polymorphisms may be effective on the obesity in Turkish population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1274

**Atherosclerosis risk factors before and 90 days after bariatric surgery**

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Introduction
Morbid obesity is associated with systemic inflammation, possible impaired glucose toleration and changes in serum lipids. Association of these parameters is risk for atherosclerosis and cardiovascular diseases.
Methods
We have analyzed body weight, BMI, lipid profile, glucose, HbA1c and C reactive protein in 36 obese subjects, 8 males and 28 females, 37.9 ($\pm 18.6$)

years of age, before and three monts after bariatric surgery. There was performed laparoscopic restrictive-malapsorptive bariatric technique (Roux en Y gastric by pass). All of them were on 1200–1500 kcal balanced, medical nutritive therapy after surgery. Before surgery mean body weight was 130.4±44 kg, BMI 43 ±17.1 kg/m$^2$, blood glucose 5.13±3.8 mmol/l, HbA1c 5.78%, total s-cholesterol 5.23 mmol/, HDL-C 1.15 mmol/l, LDL-C 3.04 mmol/l, triglycerids 2.11 mmol/l, CRP 13.3 mg/l.

Results

Three monts after bariatric surgery body weight loss was 23±9 kg, 17.6% ($P<0.05$), BMI decreased 5.9 kg/m$^2$, 18% ($P<0.05$), glucose lowering was 1.5±1.4 mmol/l ($P<0.05$), HbA1c was lower 0.58, 12.3% ($P<0.05$). Changes in serum lipids was: s-cholesterol decreased 1.97 mmol/l, 37.6% ($P<0.01$), LDL-C 0.91 mmol/l, 29.1% ($P<0.05$), triglycerids 0.97 mmol/l, 42% ($P<0.01$). There was no significant changes in HDL cholesterol level and after 90 days HDL-C was 0.11 mmol/l lower, 9% ($P>0.05$). Decreasing in C reactive protein was 11 mmol/l, and that was most significant change of 82% ($P<0.001$).

Conclusion

Weight reduction due to bariatric restrictive-malabsorptive procedures and following medical nutritive therapy, improves lipid parameters, glycated hemoglobin, lowering systemic inflammation and risk for atherosclerosis and cardiovascular diseases in morbid obesity subjects.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1275

### Obesity prevalence in west black sea region of turkey, the Melen Study

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Aim

Obesity prevalence increase fastly and become important health problem all around the world. Aim of our study to determine obesity and abdominal obesity prevalence in west black sea region and to display the prevalence of chronic diseases especially diabetes mellitus (DM) in obese population.

Material and method

We evaluate 2222 (1418 female, 804 male, mean age 50) participant in Yigilca region. We took medical history and physical examination of all participant. Body mass indexes (BMI) was described as follows: BMI <18.5 as low body weight; 18.5–24.9 as normal body weight, 25–29.9 as overweight, ≥30 as obese and ≥40 as morbidly obese. According to waist measurement, >94 cm in male and > 90 cm in female accepted as abdominal obesity. Fasting blood glucose (FBG), electrolytes (Na, K), and lipid parameters were recorded.

Results

Mean BMI of participants were 30.6 in females and 27.5 in males. According to BMI obesity prevalence was 53.1% in female and 26.9% in male and mean obesity prevalence was 43.5% in generally. Abdominal obesity prevalence was 63% in female, 46% in male and generally was 57% in all participant. Obesity prevalences were increasing with age in both sex. Especially 3/4 (75%) of female and 1/3 (33%) of male were obese between 50 and 59 ages. Postmenopausal female had very high (64%) obesity prevalence but 43% in premenaposes. Coronary artery diseases, hypertension and especially DM were very high in obese population. While DM prevalence was 12.6% according to history, crude DM prevalence was increased 18.8% adding patients with FBG ≥126 mg/dl.

Conclusion

Obesity especially abdominal obesity and also DM prevalence were determined increased at exaggerated ratio in all section and ages of population especially postmenopausal females.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1276

### Association between metabolic syndrome and serum leptin level in postmenopausal women

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Menopausal status is associated with weight gain, increased central fat mass, abnormal lipid metabolism, insulin resistance, and susceptibility to metabolic syndrome (MetS). Leptin is synthesized and secreted by adipocytes. Serum leptin levels are highly correlated with fat mass. We determined the association between MetS and serum leptin levels in 153 postmenopausal women. The difference in serum leptin level between MetS and non-MetS groups showed a statistical significance after adjusting for body mass index (BMI; 19.9±9.5 vs 12.1±5.9 ng/ml, $P=0.013$). The indicator of abdominal obesity, waist-to-hip ratio (WHR) and visceral fat area (VFA), had a positive correlation with serum leptin level in non-obese subjects after adjusting for BMI ($P=0.017$, $P<0.001$ respectively). Of the components of MetS, abdominal obesity and the number of MetS components had a positive correlation with serum leptin level ($P<0.05$, $P<0.001$ respectively). In conclusion, our study showed that serum leptin was associated with MetS independent of BMI. The increase in prevalence in MetS in postmenopausal women may increase the risk of CVD; therefore, the prevention and treatment of MetS are important. Further researches are needed to investigate the additional direct and indirect mechanisms of action of leptin associated with MetS and to clarify the role of leptin on MetS.

Mean leptin levels in non-MetS and MetS groups. After adjustment for BMI, the difference of leptin between two groups showed a statistical significance ($P=0.013$). $P$ value was calculated by ANCOVA.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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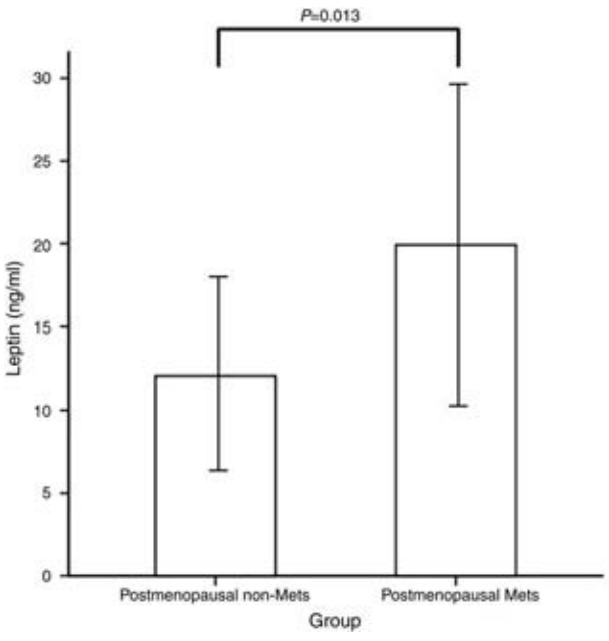
**Table 1** Coefficients of correlations between serum leptin and the components of MetS (A1), and partial coefficients of correlations after correction for BMI (A2).

| | A1 | A2 |
|---|---|---|
| WHR | 0.610* | 0.213* |
| Total triglyceride (mg/dl) | 0.270* | 0.163 |
| HDL cholesterol (mg/dl) | -0.254* | -0.111 |
| FPG (mg/dl) | 0.137 | 0.080 |
| SBP (mmHg) | 0.302* | 0.004 |
| DBP (mmHg) | 0.332* | 0.083 |

WHR, waist-to-hip ratio; HDL, high-density lipoprotein; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure. *$P<0.05$.



## P1277

### Effects of diet and physical activity-induced weight loss on insulin resistance in severely obese patients

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Introduction
Insulin resistance is quite common among obese individuals. Lifestyle interventions like diet interventions and regular physical activity are still important and safe first-line therapy.
The aim of this study was to assess the effects of diet and physical activity-induced weight loss on insulin resistance in extremely obese patients.
Methods
Forty-four patients (mean age 41.05±10.23 years) were consecutively recruited at Clinic of Endocrinology. They were on three weeks therapeutic fasting or semi-fasting diet in hospital conditions. At the beginning anthropometric measurements were performed. Subjects underwent an oral glucose tolerance test (OGTT) and insulin resistance/sensitivity was evaluated by the homeostasis model assessment (HOMA) and the oral glucose insulin sensitivity (OGIS). At home, patients underwent low calorie diet and dosed physical activity. After body weight reduction for at least 15%, all mentioned assessments were repeated. Statistics: Wilcoxon Signed Rank Test.
Results
None of the patients had significant adverse effects. The mean weight loss was 27 kg or 17% of the initial weight (149.48±25.52 kg vs 123.89±20.68 kg ($P<0.01$). This was followed by a decrease of body mass index (49.44±10.70 kg/m$^2$ vs 41.41±5.90 kg/m$^2$, $P<0.01$), waist circumference (140.90±17.17 vs 122.55±12.96 cm, $P<0.01$), glucose (4.71±0.80 vs 4.13±0.68 mmol/l, $P<0.01$), insulin (24.95±20.84 vs 16.80±10.06 mU/l, $P<0.05$), HOMA (5.56±4.55 vs 3.10±2.20, $P<0.05$) and OGIS (428.54±72.46 vs 490.92±80.56, $P<0.01$).
Conclusion
Based on aforementioned results, it can be concluded that 17% weight loss of initial weight in severely obese subjects, significantly increases insulin sensitivity and so substantially improve the pro-atherosclerotic milieu associated with obesity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1278

### Evaluation of depressive symptoms and quality of life in obese patients

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Objective
Obesity is a chronic disorder and a serious public health problem. Obesity which affects quality of life cause several physical and psychological problems. The aim of the current study was to evaluate depressive symptoms and quality of life in a group of obese patients.
Material and methods
A total of 203 subjects, 103 obese patients with no chronic disorders except diabetes mellitus and hypertension and 100 healthy subjects with normal weight were included the study. As subgroups, patients were assessed as obesity group and obesity with diabetes mellitus and/or hypertension. Data was collected through a face to face questionnaire and by body weight and height measurements. Obesity was defined as body mass index of >30 kg/m$^2$. All subjects were evaluated with the Beck depression inventory (BDI) and short form-36 (SF-36).
Results
In total, 26.1% of individuals had combined obesity, diabetes mellitus and hypertension; 24.6% had only obesity. The mean age was 39.8±10.1 years. The mean BMI was 28.4±6 (min: 19.0, max: 39.0). There was a statistically significant difference between the study groups in terms of age, sex, education, marital status, number of pregnancy, smoking story and socioeconomic status ($P<0.05$ for each). There was not a statistically significant correlation between obesity and BDI ($P>0.05$). SF-36 scores of the healthy subjects with normal weight were higher than the scores of the obese patients and also SF-36 scores of the pure obese patients were higher than the scores of the obese patients with diabetes and hypertension ($P<0.05$ for each).
Conclusion
In this study, it was demonstrated that obesity and the accompanying chronic disorders like diabetes mellitus, hypertension poorly affected to the quality of life. Preventive measures are necessary before the complications afflict to the patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1** The comparison of scale measurements between study groups.

| | Control | Obese | Obese and chronic disorders | *P* value |
|---|---|---|---|---|
| Beck depression Inventory | 6.5±5.7 6 (0–32) | 6.7±8.3 4.5 (0–42) | 8.2±6.3 7 (0–25) | 0.126 |
| Physical functioning | 27.8±2.7 28 (18–30) | 26.3±3.1 26.5 (19–30) | 23.9±3.6 24 (13–30) | <0.001 |
| Role limitations due to physical problems | 7.5±2.3 8 (4–28) | 6.2±1.9 7.5 (4–8) | 5.2±1.7 4 (4–8) | <0.001 |
| Social functioning | 9.6±1.7 10 (1–11) | 8.9±2.2 10 (3–11) | 8.5±2.1 9 (4–11) | <0.001 |
| Pain | 9.4±1.3 10 (5–11) | 7.6±2.4 8 (2–11) | 7.4±2.1 8 (3–11) | <0.001 |
| Mental health | 24.2±2.9 25 (13–28) | 21.6±5 23 (11–33) | 21.2±4.1 22 (12–28) | <0.001 |
| Role limitations due to emotional problems | 5.4±0.9 6 (3–6) | 4.5±1.4 4.5 (3–6) | 3.7±1.1 3 (3–6) | <0.001 |
| Vitality | 18.4±2.8 19 (9–22) | 15.1±4.3 16.5 (5–23) | 14.1±3.3 15 (9–22) | <0.001 |
| General health perceptions | 19.5±2.9 20 (11–24) | 16.7±3.8 17 (8–23) | 14.9±3.8 16 (8–23) | <0.001 |

## P1279

### Plantar heel pain and relation with different diseases in obese Turkish patients

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Plantar heel pain (PHP) is one of the most common musculoskeletal disorders of the foot, yet its etiology is poorly understood. Although obesity is the most common cause of PHP, there is little information available about the prevalence and associated factors in obese patient with PHP. The aim was to determine prevalence of various co-morbidities in obese patients with PHP and assess associations. A hundred and fourthy nine obese and overweight participants (29 males, 120 females and mean age 44 years) were matched by with and without PHP (34 with PHP and 115 without PHP). The two groups were then compared on various fatness, hormone and blood parameters. Diabetes mellitus (DM), coronary heart disease (CHD), chronic obstructive lung disease (COLD), asthma, smoking, varice, hemorrhoid, lumbago, constipation, osteoporosis, hormone replacement therapy (HRT), goiter, reflux were compared between the groups. Statistical analysis demonstrated that significantly greater increase in PHP prevalence with COLD ($P<0.001$), hemorrhoid ($P<0.01$), lumbago ($P<0.001$), constipation ($P<0.01$), reflux ($P<0.05$) prevalence but decreased prevalence of PHP with asthma ($P<0.05$) and varice ($P<0.01$) in patients. No statistically significant difference was identified between the PHP (+) and (−) groups for DM, CHD, smoking, osteoporosis, HRT and goiter in this study. This study showed that PHP is closely associated with obesity and the most important factor is duration of the obesity. The combination of obesity with different co-morbidities like as COLD, hemorrhoid, lumbago, constipation and reflux may be risk factors for PHP, but combination obesity with asthma or varice do not appear to play a role in PHP in this study.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1280

### Relationship between visceral fat and neck circumference in patients with metabolic syndrome

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The metabolic syndrome is a combination of multi-factorial risk factors, which affects more people because of increased incidence of obesity and diabetes. The

metabolic syndrome is one of the major health problems for the 21st century.
In this study, we compared neck circumferences measurements with some of the metabolic and anthropometric measurements in patients presenting with complains of weight, and aimed to show the correlation between visceral fat rates and neck measurements especially in patients with metabolic syndrome. 179 patients with complains of weight were included from 01.02.2009 to 01.02.2011, in Baskent University Umitkoy Polyclinic, diabetes and obesity clinic. This is a retrospective case–control study.
Research data were transferred to the statistic program SPSS version 16.0. The data control and analysis made with the same program. Pearson $\chi^2$ test used for the analysis of hypothesis, and $\chi^2$ test used for levels of significance of the data. Metabolic Sendrom was identified based on criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III).
Of the 179 patients, 77% ($n=137$) were women, 23% ($n=42$) were men (W: M ratio=3.26). The mean age was $37\pm13.15$. 40 of our patients (22.3%) were diagnosed the metabolic syndrome. The mean neck circumference was $35.15\pm3.48$ cm in those without metabolic syndrome, and $38.40\pm4.13$ cm in those with metabolic syndrome ($P=0.000$). Statistically significant positive correlation is demonstrated between neck circumference measurements and visceral fat rates in patients with metabolic syndrome ($r=0.71$, $P=0.01$; Table).
Our study revealed that, neck circumference measurement is a valuable and indicating statistically significant positive correlation measurement such as the waist circumference measurement which is one of the NCEP ATP III criteria, in metabolic syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1**

| Pearson correlation (*r*) | Neck circumference | Waist circumference | Hip circumference | Visceral fat | Abdominal fat | HDL | Triglyceride |
|---|---|---|---|---|---|---|---|
| Neck circumference | 1 | ,27 | ,27 | ,71** | ,40** | ,01 | ,14 |
| Waist circumference | | 1 | ,68** | ,56** | ,48** | −,12 | ,01 |
| Hip circumference | | | 1 | ,54** | ,41** | −,28 | ,08 |
| Visceral fat | | | | 1 | −,17 | −,07 | −,02 |
| Abdominal fat | | | | | 1 | −,20 | ,04 |
| HDL | | | | | | 1 | −,21 |
| Triglyceride | | | | | | | 1 |

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

## P1281

### Morbid child obesity-possible Rohhadnet syndrome: case report

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Introduction
ROHHADNET syndrome is a newly described condition characterized by rapid-onset obesity, pulmonary hypoventilation, hythalamic, autonomic dysregulation and, neural tumors. The severity of the clinical manifestations can rapidly lead to death by cardiorespiratory arrest.
Case report
We present the case of a 5.10 years old girl, who was admitted in the Endocrinology department for evaluation of a significant weight gain began 2.5 years ago.
Clinical examination releaved morbid obesity with facio-troncular distribution, height=123 cm (+2.5 SD Prader), weight =49.5 kg (+7.5 SD Prader), acrocyanosis, eyelid and, legs edema. The biochemical and hormonal profile releved increased natremia and transaminases, hypokalemia, increased urinary free cortizol with adequate suppression at Bricaire test, increased value for ACTH, TSH and prolactin. MRI exam at hypothalamic-pituitary and abdomen level showed a suggestive image for pituitary microadenoma.
Based on the clinical and laboratory investigations the possible diagnosis of ROHHADNET syndrome. The treatment followed correction of overweight and biochemical and hormonal changed parameters. At the age of 6 years and one month the patient died from renal failure and cardiopulmonary arrest.
Conclusions
Frequent associations of hypothalamicpituitary endocrine dysfunction creates real difficulty in diagnosis of ROHHADNET syndrome. This case illustrates the importance of complex endocrine evaluation and multidisciplinary approach to all forms of obesity with early onset and rapid evolution in order to establish the correct diagnosis and appropriate treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1282

### The correlation between metabolic syndrome and serum resistin, adiponectin, TNFα, leptin levels and abdominal adipose tissue thickness

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Advanced research to investigate possible methods of treatment in patients with metabolic syndrome is required. Adipocytokines are secreted by the adipose tissue. These polypeptides and their functions are also known last few years.
Adipocytokines are also anti-inflammatory and antiatherogenic, and they increase insulin sensitivity. As result of these skills, adipocytokines should be useful for diagnosis and treatment for metabolic syndrome. In this study we investigated the relationship between metabolic syndrome and adipocytokines levels.
In our study, 40 patients (22 males, 18 females) were divided into three groups according to the number of metabolic syndrome diagnostic criteria. Serum adiponectin, resistin, leptin, EGF, TNF-α levels and abdominal fat tissue thicknesses were compared.
At the end of this study there was no significant relationship between abdominal adipose tissue thickness, serum TNF-α, adiponectin, resistin levels and the number of diagnostic criteria for metabolic syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1283

### Study of insulin resistance in overweight and obese children by: M Hesham El Hefnawy and Ibrahim Emara

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Obesity is a major risk factor for chronic non communicable diseases and plays a central role in the insulin resistance or metabolic syndrome, which includes hyperinsulinemia, hypertension, hyperlipidemia, type 2 diabetes mellitus, and an increased risk of adult onset obesity and atherosclerotic cardiovascular disease (1). Insulin resistance is a state in which a given concentration of insulin produces a less than expected biological effect. The metabolic syndrome is a clustering of cardiovascular risk factors that arises from insulin resistance accompanying abnormal adipose deposition and function.
Aim of the work
The aim of this study was to the insulin resistance in overweight and obese children as insulin resistance resistance could be used an early marker of type 2 diabetes.
Subjects and method
overweight and obese children aged from 6 to 18 years (mean=11.94+3.51 years), were selected from the outpatient pediatric endocrinology clinic in National Institute of Diabetes & Endocrinology. Another 20 lean children were selected as a control group.
Results
The results of this study showed that All subjects were investigated for body mass index (BMI), study of HOMA-IR, B.P. measurements, serum cholesterol and triglycerides for study group were 33.09+6.07, 2.96+1.46, 152.16 +28.84 mg/dl respectively. There was a positive significant correlations between BMI and HOMA-IR, HOMA-IR and Cholesterol and between HOMA-IR and BMI.
Concussions
It could be concluded that we have to measure HOMA-IR in any overweight and obese for early detection of insulin resistance. It could be recommended to try to control overweight and obesity as obesity is the golden gate to insulin resistance and diabetes.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## Paediatric endocrinology

### P1284

**Insulin secretion and sensitivity in GH deficient children at the end of rhGH therapy and after 1 year of follow-up: influences of d3GH receptor polymorphism on metabolic parameters**

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Introduction
A rise in serum insulin levels during GH therapy is reported. Insulin resistance is a risk factor for type 2 diabetes, atherosclerosis, dyslipidemia, and hypertension. Few data describe insulin secretion and sensitivity after the end of GH therapy in children.
Methods
Aim of our study was to evaluate changes in insulin secretion and sensitivity in 24 children with isolated GHD at 3 times: i) during the last year of therapy (T0); ii) 6 months (T6) and iii) 12 months (T12) after stopped therapy. We measured glucose and insulin levels at fasting and after an oral glucose tolerance test (OGTT). Insulin resistance and sensitivity were estimated with classical indexes. Disposition index (DI) and insulinogenic index (INS) were also evaluated. The presence (Del) or absence (Ndel) of exon 3 deletion in the GH receptor was investigated (GHD: 4 Del, 16 Ndel). They were compared with 30 healthy puberty matched subjects.
Results
No subjects showed dysglycemia or hypertension. Fasting insulin, insulin and glucose levels at each point after OGTT were progressively lower at T6 and T12 compared to T0 ($P<0.001$). HOMA-IR and HOMA2 indexes were higher, and Matsuda and QUICKI indexes were lower at T0 respect to T6 and T12 ($P<0.01$). All the metabolic parameters were similar to controls at T12. INS and DI progressively decreased unexpectedly only in GHD Ndel arriving to values lower at T12 than NW ($P<0.05$). No alterations were found in GHD Del.
Conclusions
Insulin sensitivity decreased during rhGH therapy without the onset of dysglycemia and was associated with a compensatory insulin secretion and a reduction in DI only in GHD Ndel. Insulin and glucose secretion progressively restored after stopping treatment being similar to controls in the next 12 months. The presence or absence of exon 3 deletion, could affect some metabolic effects of rhGH in GHD children.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### P1285

**ACTH and cortisol are differently associated with metabolic syndrome components in a large cohort of Caucasian obese children and adolescents**

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Introduction
Higher cortisol levels and, in particular, hyperactivity of the HPA axis might play a role in the development of MS at least in adults. Data on the pediatric age are scanty.
Aim of our study was to evaluate ACTH and cortisol association with MS, its components, phenotypic parameters, family history of metabolic derangements in pediatric obesity.
Methods
Cross-sectional study. 271 Caucasian overweight or obese children and adolescents (age range: 1.8–18.0 years) were evaluated for: family history, detailed clinical examinations, fasting morning ACTH, cortisol, glucose, insulin, lipid profile and OGTT test; HOMA, ISI and QUICKI were calculated. We divided patients according to pediatric NCEP criteria for MS.
Results
62.4% of the subjects had MS (73.9% had hypertension, 58.4% HDL-cholesterol $<$10th percentile, 23.1% triglycerides $>$90th percentile, and 8.2% IFG, IGT or type 2 diabetes). Children and adolescents with MS presented higher ACTH ($P<0.0001$) and cortisol levels ($P<0.03$) than those without MS. Increasing numbers of features of MS were associated with higher ACTH, but not cortisol, also when adjusted for confounding factors ($P<0.001$). Subjects with IFG, IGT or type 2 diabetes, or altered HDL-cholesterol or triglycerides respect to the MS cut-offs had higher ACTH, but not cortisol levels ($P<0.01$). Subjects with hypertension had increased levels of both ACTH and cortisol levels ($P<0.02$) if the cut-off was 95th percentile, and only cortisol ($P<0.05$) if the cut-off was reduced to 90th percentile as suggested by MS criteria. ACTH and cortisol levels were associated with insulin resistance. Subjects with a family history of hypertension ($P<0.003$) had higher cortisol levels.
Conclusions
In obese pediatric subjects, MS is associated with higher ACTH and cortisol levels. The features of MS could be differently modulated by ACTH and cortisol. Cortisol secretion seems mainly involved in hypertension and its family history.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### P1286

**Subclinical hypothyroidism in obese children and metabolic implications**

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**Abstract**
Introduction
Aim of this study was to assess the prevalence of subclinical hypothyroidism in a paediatric population of overweight and obese children and its association with metabolic parameters and with metabolic syndrome (MS).
Subjects and methods
Clinical and metabolic evaluations in 600 overweight and obese children and adolescents (310 females, 290 males, 260 prepubertal, 240 pubertal, mean age: $10.7\pm3.1$ years) were performed. MS was defined according to paediatric NCEP-ATPIII criteria. TSH levels were evaluated both in quartiles and according to a fixed cut-off level (pathological value $>3.500$ μUl/ml). The presence of autoimmune thyroiditis was assessed in those subjects with abnormal TSH values.
Results
75 subjects (12.5%) had abnormal TSH levels and 10 of them (13.3%) showed antithyroid antibodies. Age decreased ($P<0.0001$) and the number of prepubertal children increased ($P<0.006$) with the rise of TSH levels. non-HDL cholesterol ($P<0.03$), triglycerides ($P<0.0001$), PNFI index ($P<0.0001$), fasting glucose ($P<0.04$), HOMA-IR ($P<0.04$), glucose peak ($P<0.03$) and glucose area ($P<0.01$) during OGTT increased with the rise of TSH levels. No differences in the prevalence of MS were found among the TSH quartiles and in subjects with altered TSH levels. A negative correlation between age and TSH levels was found ($\beta=-0.122$; $P<0.003$). Furthermore a positive correlation between TSH and non-HDL cholesterol ($\beta=0.095$; $P<0.02$), triglycerides ($\beta=0.232$; $P<0.0001$), PNFI ($\beta=0.185$; $P<0.0001$) and fasting glucose ($\beta=0.107$; $P<0.01$) was shown independently of confounding factors.
Conclusions
Subclinical hypothyroidism and autoimmune thyroiditis are frequent in overweight and obese children. The association between TSH levels and metabolic parameters suggests a role of the hypothalamus–pituitary–thyroid axis in the regulation of lipids and glucose metabolism. Conversely the absence of an association with the metabolic syndrome suggests that this axis may modulate specific metabolic alterations.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1287

**Addressing issues of diabetes patients community: Role of NGO's in resource poor settings**

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Adolescent diabetes social stigma in India. Such diabetics needs proper guidance/information/treatment-counselling outlets. This is burning issue in developing-nations like India. Hence we all need to unite and form a comprehensive diabetes care and counselling policy plan at ICE/ECE-meeting-2012. Treatment options must be suitable for developing-nations considering cost of Rx. Incorporating NGO's in such efforts is very effective.

Our project methodology

Our 15-year-old NGO started diabetes education-project in rural India from 2005. we started s education and surveillance project to analyze social and anthropological issues facing those affected by adolescent diabetes. Total 62 adolescents subjects enrolled by Feedback questionnaires to get their feedback on special needs, perceptions, social attitude on diagnosis of diabetes. Factors like community-inhibition, social-ostracism, economic-difficulties, marital discord, non-availability of treatment-guidance centres, lack of trained-staff analysed and draft policy is recommended to Govt-agencies.

Lessons learned

diabetes management must include care of nursing and psycho-social needs. Here role of NGO's is very effective in terms of cost-management, better impact and better-compliance of diabetics. Community mass intervention projects has proven useful in rural communities of resource poor-nations. ICE/ECE-Florence-congress-participants can collaborate with NGO-activists to address this issue. Uniform public health policy needed to implement and expand newer strategies to include broader range of diabetes care-issues.

Recommendations

Promoting dialogue between Government-health-services and NGO's accelerates diabetes education/awareness programs. NGO participation improves cost-efficacy of such initiatives in economically poor populations. This would reduce difficulties faced by young diabetics from Asian countries. It is essential that WHO, ICE/ECE form common guideline manual on this issue affecting developing-countries. We graphically present our NGO's project on diabetes patients education project in four phases to ICE/ECE-2012-congress-participants.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1288

**Influence of cranial and craniospinal irradiation in childhood on reproductive system of women and men cancer survivals**

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Introduction

Childhood cancer incidence increases all over the world, but it are optimistic that more than seventy percent of children with cancer survive due to radiation and chemotherapy. However cancer treatment may impair reproductive function. The aim of this study is to investigate level of pituitary hormones, testis/ovarian volume in women and men undergo cranial and craniospinal irradiation in childhood.

Methods

Participators were examined with ultrasound, hormonal analyze. 22 women and 12 men were included in this study.

Group 1 included 11 women and 11 men, after treatment for brain tumors in childhood. The median age of participators was 19.5 (16–25), the median age at original diagnosis was 11.4 (5–15). All patient received craniospinal irradiation 55 Gy and chemotherapy M-2000.

Group 2 included 11 women and 2 men after treatment for lymphoblastic leukemia. The median age of participators was 21.5 (16–30), age at original diagnosis – 7.7 (2.5–13). All patients received cranial irradiation 18 Gy, chemotherapy BFM-90.

Results

women after cranial irradiation had amenorrhea with depletion of follicles, high gonadotropin levels. Survivors after craniospinal irradiation had smaller ovarian volume per ovary than after cranial irradiation (average, 3,3 vs. 8.7 $cm^3$; $P < 0.001$), and higher LH (13.17 vs 4.5)and FSH (31.6 vs 5.65).

In men after craniospinal irradiation was detected lower testicular volume (7.03 vs 11.25), FSH level (13.4 vs 7.62). There were no significant differences in LH, testosterone, prolactin levels.

Conclusion

Women after craniospinal irradiation more likely had primary hypogonadism. Survivors with preserved menstrual cycles had sonographic and endocrine changes suggesting impairment of ovarian reserve. Men after cranspinal had lower testicular volume and FSH level with normal testosterone, LH that suggested impaired spermatogenesis. None of participates had low gonado-tropins. Thus craniospinal irradiation has a great impact on reproductive function in childhood cancer survivals.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This work was supported, however funding details unavailable.

## P1289

**Etiology of delay of puberty in adolescents with different neuroendocrine diseases**

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Aim

The work was initiated to compared extent of physical and sexual development retardation in adolescents with various neuroendocrine abnormalities.

Materials and methods

We examined 63 adolescents, 41 boys (59.4%) and 22 girls (34.9%) among them with various endocrine abnormalities. Mean age of boys and girls was 11.3 and 12 years, respectively. All patients underwent general clinical examination. Levels of STH, LH, FSH, prolactin, TSH, ACTH, cortisol were measured, biochemical and roentgenologic investigations, such as, CT, MRI of Turkish saddle, hand X-ray, clinical ultrasound of the thyroid and genitals as well as anthropometric measurements were performed. The patients' endocrine status was assessed with Tanner's score to determine puberty stage.

Results

By etiology of the abnormalities the patients were divided into four groups. The 1st group included 18 patients (28.6%) with the Turkish saddle neoplasms, 20 patients (31.7%) with empty Turkish saddle syndrome comprised the 2nd group; diabetes insipidus was diagnosed in 15 patients (23.8%) of the 3rd group, juvenile dyspiuitatarism being registered in 9 patients (14.3%) of the 4th group. Of 63 patients physical and sexual development retardation was observed in 26 (41.3%), growth retardation in 23 (36.5%), puberty arrest in 8 (12.7%), cryptorchidism and micropenis being found in two patients (3.2%) and 1 (1.6%) patient, respectively. As a whole, incidence of puberty retardation in 63 examinees exceeded isolated growth retardation by two times (44 vs 23). More than in 41.3% of cases growth retardation paralleled growth retardation suggesting panhypopituitarism in these patients. The isolated puberty arrest occurred less frequently, that is, in one patient (5.5%) of the 1st group, in two patients (10%) of the 2nd group, in three patients (20%) of the 3rd group and in one patient (11%) of the 4th.

Conclusions

i) Physical and sexual development retardation can be considered as a marker for high severity of neuroendocrine pathology. ii) Physical and sexual development retardation was observed in patients with the empty Turkish saddle syndrome (70%) and in those with the Turkish saddle neoplasms (60%).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1290

**Noonan syndrome: short stature and pubertal delay**

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Background
Noonan syndrome (NS) is a relatively common disease, clinically and genetically heterogeneous. It is characterized by facial dysmorphia, growth retardation, congenital heart disease, lymphatic dysplasia, among others. The diagnosis is clinical, according to van der Burgt criteria. In 61% of cases genetic mutations in the signaling pathway of RAS-MAPK are identified.
Clinical case
BVM, male, NS suspected. At 3 years old sent to Pediatric Endocrinology for macrogenitossomy. Personal history: polyhydramnios at 22 weeks of pregnancy, amniocentesis: karyotype 46XY; term birth, weight 4090 g (1.36 SDS), length 50 cm (0.09 SDS), Apgar score 9/10 and congenital heart disease (hypertrophic cardiomyopathy and pulmonary stenosis). Heart disease corrected at 2 years by pulmonary valvuloplasty. Family history: irrelevant. Physical examination (PE): weight 17.6 kg (0.62 SDS), height 103.2 cm (1.05 SDS), growth velocity (GV) 8.2 cm/year (0.1 SDS); dolichocephaly, ptosis, hypertelorism, antimongoloidal palpebral fissures, low-set ears and hair, extended filtrum, high-arched palate, short and broad neck, pterygium colli, shield chest and cubitus valgus, edema of lower limbs, scrotum and penis, without Godet's sign, Tanner: P1G1. Evaluation: target height 1.71 cm ($-0.82$ SDS). Bone age: 3 years. Laboratory: TSH 1.44 mIU/ml (RR: 0.35–5.50), IGF1 90 ng/ml (RR: 50–237), IGFBP3 4 μg/ml (RR: 0.2–6.6). Diagnosis of lymphedema. Referred to physical rehabilitation department for medical lymphatic drainage. At 15 years he returned to Endocrinology for delayed puberty. Presented progressive growth delay since 8 years, several hospitalizations for cellulitis and lymphangitis of lower limbs and genitals, and chylothorax. PE: weight 47.3 kg ($-1.45$ SDS), height 157.2 cm ($-1.94$ SDS), GV 3.5 cm/year ($-2.10$ SDS), lymphedema of lower limbs, scrotum and penis, Tanner P1G1. Bone age: 13 years. Laboratory: IGF1 70 ng/ml (RR: 143–996), IGFBP3 3.2 μg/ml (RR: 1.3–10), testosterone 0.1 ng/ml (RR: 3.5–13.5); LHRH test: FSH peak 11.3 IU/l, LH peak 7.1 IU/l. Testosterone therapy was initiated. Molecular study of PTPN11, SOS1, RAF1 and KRAS1 genes was negative; awaits further genetic study.
Conclusions
The case described points to clinical suspicion of Noonan syndrome and the need for regular endocrine evaluation. The GH–IGF1 and gonadal axis are the most affected. Early detection and treatment of endocrine abnormalities contributes to life quality improvement of these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1291

**The neuroendocrine complications of teenagers with diabetes insipidus of central genesis**

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Aim
to study features of growth and development in teenagers with Diabetes Insipidus of central genesis.
Material and methods
During 2009–2010 years we examined 37 teenagers with Diabetes Insipidus of central genesis. 19 boys (51.3%) and 18 girls (48.6%) among them. Mean age of boys and girls was 13.5 years.
All patients underwent general clinical examination. Levels of STH, LH, FSH, prolactin, TSH, free thyroxin, cortisol, etc. were measured, biochemical (blood and urine analysis, Zimnitskiy test of urine), and roentgenologic investigations, such as, CT, MRI of Turkish saddle, hand X-ray, clinical ultrasound of the thyroid and genitals as well as anthropometric measurements on the base of international growth-weight chart of Tanner-Whitehouse with evaluation of centile, growth insufficiency, SDS of growth and weight, growth velocity were performed. The patients' endocrine status was assessed with Tanner's score to determine puberty stage.
We have comparison of our data with healthy teenagers (10 boys and 10 girls).
Results
We founded, that average centile was 50. Besides that, our patients have very big growth and weight insufficiency. The average growth insufficiency in boys was on our data $8.3 \pm 0.7$ sm and $10.3 \pm 0.5$ sm in girls. The weight insufficiency in boys was $7.3 \pm 0.5$ kg and in girls $5.6 \pm 0.4$ kg. We founded that growth SDS $< -2$, and weight SDS $<$ -2 in boys and girls.
The evaluation of sexual development by Tanner's stages showed, that in 18 cases from 37 (48.6%) was founded delay of puberty, in this cases in 10 boys (55.5%) and in 8 (44.4%) girls. Three boys have criptorhism.
All of our patients have delay of skeletal development and bone age/passport age was 0.7 in boys and girls. The average bone age was small on 2.7 years from chronological (passport) age.
The hormonal investigation showed, that more of our patients have insufficiency of pituitary hormones: STH – in 32 patients (86.4%), LH – in 28 (75.6%), FSH – in 28 (75.6%), TSH – in 15 (40.5%) with functional hyperrolactinemiya.
Conclusions
The stage of delay of puberty and growth in teenagers with Diabetes Insipidus directly correlated with condition of compensation of electrolytes, which submitted insufficiency of our pathogenetic therapy and decompensation of patient.
The evaluation of sexual development by Tanner's stages showed, that in 18 cases from 37 (48.6%) was founded delay of puberty, in this cases in 10 boys (55.5%) and in 8 (44.4%) girls.
All of our patients have delay of skeletal development (100%).

## P1292

**Systemic administration of C-type natriuretic peptide rescues impaired endochondral bone growth in mice model of achondroplasia**

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Introduction
Recent studies have elucidated that C-type natriuretic peptide (CNP), a member of natriuretic peptide family, is a potent stimulator of endochondral bone growth; CNP and its membranous guanylyl cyclase receptor, GC-B are expressed in growth plate, and mice with targeted overexpression of CNP in growth plate exhibit prominent skeletal overgrowth, whereas CNP or GC-B knockout mice develop severely short stature phenotype owing to their impaired endochondral bone growth. We are trying to translate the strong stimulatory effect of CNP on bone growth into a medical treatment for patients with skeletal dysplasia, a group of genetic disorders characterized by disturbances in skeletal development. In the present study, we performed systemic administration of CNP to mice model of achondroplasia, the most common form of skeletal dysplasia.
Methods and results
We performed continuous i.v. administration of CNP to 3-week-old achondroplastic model mice for 4 weeks, and investigated the effects on their impaired skeletal growth. CNP stimulated the longitudinal growth of achondroplastic model mice dose-dependently, and the naso-anal length of achondroplastic mice treated with CNP at the dose of 1 μg/kg per min was almost comparable to that of wild-type mice treated with vehicle. CNP administration at the dose of 0.1 μg/kg per min considerably recovered the impaired growth of long bones in achondroplastic mice, and at the dose of 1 μg/kg per min, some bones grew rather longer than those in wild-type mice treated with vehicle. Histological examination revealed that narrowed growth plate observed in achondroplastic mice was recovered by administration of 1 μg/kg per min CNP to the extent of wild-type growth plate treated with vehicle.
Conclusion
We demonstrated that systemic administration of CNP is effective for the recovery of impaired skeletal growth in mice model of achondroplasia. Systemic administration of CNP is a potential therapeutic strategy for the treatment of skeletal dysplasia including achondroplasia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1293

**Exclusion of aldosterone signaling pathway genes as candidates for renal pseudohypoaldosteronism type 1 in 32 families**

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Background
Pseudohypoaldosteronism type 1 (PHA1) is a primary form of mineralocorticoid resistance presenting in the newborn with renal salt wasting, failure to thrive and dehydration. Inactivating mutations of the *NR3C2* gene, coding for the mineralocorticoid receptor (MR) are responsible for the vast majority of autosomal dominant and sporadic cases of renal PHA1. The underlying pathogenic mechanism involves both haploinsufficiency as well as a dominant negative mechanism. However, ~30% of kindreds with clinical signs of renal PHA1 do not present NR3C2 gene mutations or deletions.
Objective
To assess whether mutations in aldosterone signalling genes could be responsible for renal PHA1.
Design and methods
Five genes involved in the aldosterone-MR epithelial sodium reabsorption pathway (*SGK-1*, *NDRG2*, *GILZ*, *NEDD4-2*, *GPR48*) were sequenced in 32 patients with clinical signs and symptoms of renal PHA1 and without NR3C2 mutations.
Results
Different already described intronic and exonic single nucleotide polymorphisms (SNP) were found in the five genes sequenced. The frequency of these SNPs was similar to that previously observed in population studies. Three intronic variations were found in the sequence of *NEDD4-2* gene. No disease-causing gene mutation or new SNP were detected in the studied PHA1 patient group.
Conclusions
We present further evidence for locus heterogeneity in PHA1. Our data do not support that *SGK-1*, *NDRG2*, *NEDD4-2*, *GILZ* or *GPR48* gene variations are disease-causing factors in genetically unexplained renal PHA1. Further studies of genes encoding intracellular molecules involved in transduction of the signal from the mineralocorticoid receptor to ENaC are warranted.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1294

**Serum adiponectin levels in adolescents and young adults with GH deficiency**

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Introduction
Effect of GH deficiency on the endocrine function of adipose tissue (AT) is poorly understood. Data on serum adiponectin (ADIPO) concentrations in patients with GH deficiency (GHD) are scarse.
The aim of the study was the assessment of ADIPO serum concentrations in adolescents and young adults with GHD and evaluation of their correlations with the degree of GH/IGF1 axis function impairment as well as anthropometric and body composition parameters.
Subjects and methods
The study was performed in a group of total 122 subjects aged 16-25 years. Based on current peak serum GH concentrations in insulin tolerance test (ITT) patients were qualified for the following groups: i) severe GH deficiency – GHD (peak GH $<5.0$ ng/ml, $n=26$), ii) partial GH deficiency – PGHD (peak GH 5.0–110.0 ng/ml, $n=22$), iii) normal GH secretion – NGH (peak GH $>10.0$ ng/ml, $n=28$) and iv) healthy subjects – H ($n=46$). Following examinations were performed: i) anthropometric measurements, ii) analysis of body composition using BIA, iii) serum concentrations of ADIPO (Millipore, USA) and IGF1. Based on the analysis of body composition the total ADIPO content in the extracellular fluids per the unit of body fat mass (CADIPO/FM) was calculated.
Results
Mean serum concentrations of ADIPO did not differ significantly among the examined groups, however CADIPO/FM values were significantly lower ($P<0.05$) in GHD (8.6 mg/kg) compared with PGHD (30.1 mg/kg), NGH (27.9 mg/kg) and H (24.5 mg/kg) and well correlated with a degree of GH/IGF1 axis depletion ($r=0.54$; $P<0.0001$ for peak GH in ITT and $r=0.45$; $P<0.001$ for IGF1). A significant negative correlation between serum ADIPO and percentage of body fat (FM%) was found in GH sufficient subjects ($r=-0.30$; $P<0.01$), whereas it was positive in GHD and PGHD group ($r=0.37$; $P<0.05$)
Conclusion
Severe GHD impairs ADIPO secretion in the degree that correlates with GH–IGF1 depletion.
Declaration of interest
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## P1295

**Serum concentrations of chemerin, omentin and vaspin in girls with anorexia nervosa**

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Introduction
Production of chemerin (CHEM), omentin-1 (OMENT), and vaspin (VASP) in adipose tissue is determined by nutritional status. There is no data on CHEM, OMENT and VASP blood serum concentrations in patients with anorexia nervosa (AN).
Objectives
i) Assessment of CHEM, OMENT and VASP serum concentrations in girls with AN. ii) Analysis of correlations between the CHEM, OMENT, and VASP serum concentrations and body weight, BMI, serum insulin (INS) concentration in these patients.
Methods
Seventy-six girls: 46 with AN (mean age: $15.17\pm0.49$ years; mean BMI: $15.34\pm0.55$ kg/m$^2$; mean BMI–SDS: $-2.44\pm0.35$) and 30 healthy (H; mean age: $15.4\pm0.8$ years; mean BMI: $20.95\pm0.75$ kg/m$^2$; mean BMI–SDS: $0.131\pm0.41$). CHEM, OMENT and VASP serum concentrations were determined by ELISA method and the INS by IRMA.
Results
The CHEM and INS average serum concentrations were significantly lower in AN group than in H group ($130.24\pm2.15$ ng/ml and $2.35\pm0.09$ mIU/l vs $191.12\pm8.15$ ng/ml and $4.24\pm0.2$ mIU/l respectively, $P<0.00001$) but OMENT and VASP were higher ($46.19\pm0.92$ ng/ml and $0.52\pm0.01$ vs $34.09\pm0.96$ and $0.41\pm0.01$ ng/ml, respectively, $P<0.00001$). In the AN group the negative correlation was found between CHEM concentration and body weight ($r=-0.33$, $P<0.05$), and INS and BMI ($r=-0.31$, $P<0.05$). The positive correlation between serum CHEM and INS, and negative relationship between serum OMENT and VASP concentrations and body weight as well as BMI in all subjects was found ($P<0.00001$).
Conclusions
In anorexia nervosa CHEM concentrations are decreased in comparison with healthy but OMENT and VASP are increased.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1296

**Evaluation of circulating kisspeptin as a biomarker for differential diagnostic of central precocious puberty**

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Introduction
Kisspeptins and their cognate receptor GPR-54 (Kiss-1) were found to be regulators of the hypothalamo-pituitary–gonadal axis. It is under debate if the

onset of central precocious puberty (CPP) could be triggered by early increase of kisspeptin. We aimed to assess serum kisspeptin levels in girls in order to evaluate its potential as a reliable biochemical marker for differential diagnosis of CPP.
Materials and methods
The girls enrolled in our study were divided into three groups: central precocious puberty (CPP), premature telarche (T) and age-matched healthy controls (H). Hormonal serum levels (LH, FSH, estradiol) were measured by an automated immunochemiluminiscent assay. Kiss-1(112–121)amide was measured in sera, using EIA kit (Phoenix Pharmaceutical, Inc.), sensitivity 0.12 ng/ml, range 0–100 ng/ml, CV intra-assay 5–10%, CV interassay $<15\%$. Statistical analysis – SPSS version 16.0.
Results and discussions
Differential diagnosis between CPP and T was made by basal hormonal levels and after triptorelin test, ovarian ultrasound exam and bone age (BA) assessment. There were no differences between demographic characteristics in all three groups. No correlations were found between kisspeptin levels and age, body mass index, BA, LH, FSH (basal or stimulated), ovarian/uterus volume respectively, neither in the whole lot, nor in each group. A wide range of kisspeptin values in CPP girls (0.85–11.52 ng/ml) was obtained.
Surprinsingly, kisspeptin values were greater in healthy group than in CPP group ($P=0.04$) and than in T group (NS), showing a decreasing tendency with pubertal evolution.
Conclusions
Our results do not sustain circulating kisspeptin levels as useful biomarkers in differentiating between girls with idiopathic CPP and premature telarche.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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Key words: kisspeptins, central precocious puberty.

## P1297

**Two novel IGF1R gene heterozygous mutations in two unrelated children with pre and postnatal growth retardation, and microcephaly**

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Several IGF1R gene mutations have been described as a cause of growth retardation due to IGF1 insensitivity. The IGF1R gene was analyzed in two children suspected to have IGF1 insensitivity. Both were born small for gestational age (SGA) and showed no postnatal catch-up growth. Both patients presented microcephaly and developmental delay. A boy (P1) was born at 37 weeks, birth weight was 1900 g ($-2.98$ SDS) and body length 42 cm ($-4.7$ SDS). At 18 months chronological age (CA), height SDS was $-2.17$, weight SDS $-3.3$ and head circumference SDS $-4$. At 2.75 years CA, bone age (BA) was 1.5 years. He showed a mild dysmorphic phenotype. External genitalia and scrotal testes were normal. Basal serum GH (8 ng/ml), IGF1 ($+2.94$ SDS) and IGFBP3 ($+3.37$ SDS) were high for sex and age. A girl (P2) was born at 38 weeks, birth weight was 2650 g ($-1.4$ SDS), and body length 44 cm ($-3.3$ SDS). At 3.2 years CA, height SDS was $-2.95$, weight SDS $-2.73$ and head circumference SDS $-2.2$. At 2.6 years CA, BA was 1 years. She presented a Klippel Feil malformation. Basal serum GH (8.51 ng/ml) was high but serum IGF1 ($+0.42$ SDS) and IGFBP3 ($+0.61$ SDS) were normal for sex and age. No chromosome 15 anomalies were detected. Two novel heterozygous mutations, *de novo* R1256S (P1) and R1337C (P2) were detected in exon 21 of the IGF1R gene. The aminoacid substitutions were located at highly conserved aminoacid residues in the protein. These mutations were predicted to affect protein function using the sequence homology based SIFT tool and the structure-based PolyPhen approach. Given that phenotype, serum IGF1 and serum GH in IGF1R haploinsufficiency are quite variable, IGF1R molecular studies should be considered in children with an undiagnosed history of SGA without postnatal catch-up growth, and microcephaly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1298

**Pediatric and adolescent somatotrop adenomas**

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Introduction
Pituitary somatotrop adenomas are very rare in children and adolescents. They are supposed to be more invasive (invasion of cavernous sinuses or meninges $\pm$compression of adjacent neural structures) than adults'ones. We aimed to analyze their characteristics, and their complications.
Patients and subjects
are studied subjects whose clinical symptoms began before 20 years old. They all had biological, hormonal, ophthalmological, radiological explorations (TDM and or cerebral MRI) and histological study.
Results
From this retrospective study, we have collected 27 cases: 21 pures GHomas and 6 mixed adenomas (PRL/ GH). 20 were males and seven females. Mean age at diagnosis was $24.5\pm8.5$ years (12–55). Clinical symptoms were: 16 gigantisms $\pm$acromegaly, and 11 acromegalies without gigantism. 18 tumors invaded the cavernous sinuses and/or the meninges (66.6%), and 11 were giants (height $\geq$4 cm) or huge ($\geq$6 cm)$=40.7\%$. Mean plasma GH$=125.7\pm200$ ng/ml (7–840). Complications were: pituitary insufficiency ($\geq$1 deficit: $24=88.8\%$, $\geq$2 deficits:$13=48\%$), hydrocephaly$=7=25.9\%$, psychiatric troubles$=4=14.8\%$, epilepsy$=3$ (11%), blindness of one or both eyes: $5=18.5\%$, glucose disorders$=12=44.4\%$, dyslipidemia$=13=48\%$, systemic hypertension$=1$, dilated or hypertrophic cardiomyopathy$=6=22.2\%$, arterioneuropathy$=1$, bone problems$=5$. Association with a benign tumor was observed in 3 cases$=11\%$.
Conclusion
Children and adolescents somatotrop adenomas are diagnosed late. They prevail in males. They are often invasive, and giant or even huge which explains their numerous and severe cerebral complications. The resulting severe hypersomatotropism is responsible of metabolic, bone, and heart complications. On another side, a possible vicious circle between large tumors and severe hypersomatotropism is not excluded.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1299

**Wolfram syndrome: new cases and new mutations**

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Introduction
Wolfram syndrome (WS) is a genetic disorder that affects the quality of life of very young Patients. This condition is also referred to as DIDMOAD (diabetes insipidus-diabetes mellitus-optic atrophy-deafness).characterized by non immunogenic diabetes mellitus, and a progressive atrophy of the optic nerve, which occurs generally in the first decade of life.
In order to prevent and treat this condition, a better understanding of WS pathogenesis is needed through more detailed analysis of WFS1 gene product, i.e. wolframin protein.
Material and method
Seven new cases of WS were detected and WFS1 gene exon 8 was screened for mutations by PCR amplification followed by direct sequencing. Topology prediction of the protein was performed and the selected prediction was schematically drawn with the help of TOPO2 (Johns S.J., TOPO2, transmembrane protein display software (http://www.sacs.ucsf.edu/TOPO2/).
Results and conclusion
Five mutations were found to affect wolframin in these patients. From these mutations, W588X, A684G, and E752K are novel. E717K, which is a rare mutation in other populations, was observed in all WS patients in this study, and is suggested to be further investigated to be proposed as a WS marker in the Iranian population. Positions 684 and 752 of wolframin appear to be hot spots for mutations, as other missense mutations have been reported in these positions in other populations. W588X is a novel deletion that would lead toa truncated protein after the eighth transmembrane segment, and is related to the severity of symptoms observed in the patients who bears it.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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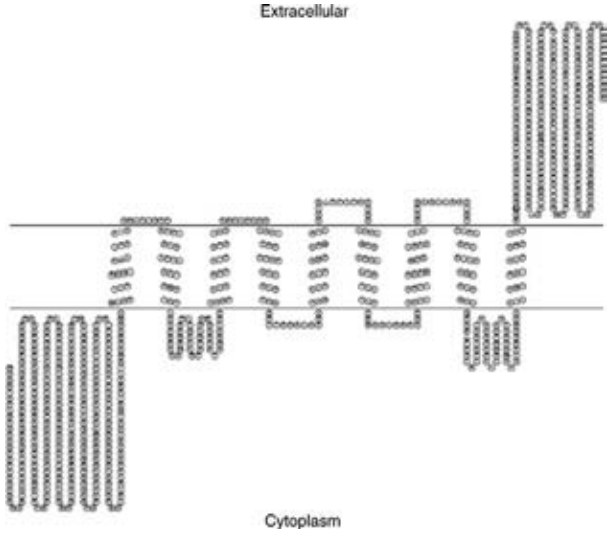
Keywords: Wolfram syndrome, wolframin, mutation, polymorphism.




## P1300

**Growth and response to rhGH treatment in patients with congenital combined pituitary hormone deficiency younger than 3 years of age**

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Congenital combined pituitary hormone deficiency (CCPHD) is a rare disease. Although most of them are growth hormone deficient, growth retardation is not always the presenting symptom.. There is no normative data in children younger than 3 years regarding dose and frequency with rhGH treatment. The objective was to evaluate the timing of growth retardation and the first and second year growth response to rhGH compared to the mathematical growth prediction model in a large cohort of patients with CCPHD younger than 3 years of age. Twenty seven children were included. The occurrence of growth retardation analyzed as time to event showed that median age was $0.89 \pm 0.74$ years with a mean height of $-3.55$ SDS ($n=19$). The remaining 8 grew well after a follow up of 2.29 years (range 0.42–3.64) despite low serum IGF1 in 6. rhGH treatment was started at a mean age of $1.76 \pm 1.0$ years with a mean dose of $0.23 \pm 0.05$ mg/kg per week($n=14$).
rhGH induced an increment in height SDS of $1.81 \pm 0.98$ and $0.88 \pm 0.81$ at the first and second year respectively. Distance of target height from height at onset of treatment and GH doses were correlated with gain in height SDS throughout the first year of treatment ($r=0.64$, $P$0.01 and $r=-0.66$, $P=0.01$ respectively).
Predicted growth velocity was similar to observed growth velocity at the first year $15.1 \pm 4.1$ vs $13.6 \pm 3.8$ ($P$=NS) and second year $11.02 \pm 4.1$ vs $10.09 \pm 2.3$ ($P$=NS) respectively.
In summary most of the patients with CCPHD presented growth retardation before the first year of life. However, a normal growth pattern could be seen in face of abnormal IGF1 levels. All patients grew according to the mathematical model of growth prediction.
The significant correlation between GH doses and gain in height SDS represent an additional tool for those patients urged to attain a better catch-up growth.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1301

**Hyperandrogenism and PCOS in adolescent girls with type 1 diabetes treated with intensive and continous insulin therapy**

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Women with T1DM experience high prevalence of hyperandrogenic disorders. Study aim was to examine whether they are present at adolescents and related to metabolic control and type of insulin therapy. Clinical characteristics, hormonal profile and ovarian volume were studied in 49 adolescent girls with T1DM. PCOS criteria were fulfilled in 11 (22.5%). Although girls with poor metabolic control had higher T ($P=0.02$) and 17-OH progesterone ($P=0.03$), frequency of PCOS did not correlate with T1DM duration and mean HbA1c and was independent of the type of insulin therapy. Girls with T1DM and PCOS were compared to 23 non-diabetic peers with PCOS (Table 1) and were found to have shorter cycle and lower ovarian volume and hirsutism score. This may be due to higher SHBG and lower FAI.
Conclusions
Although, irrespective of the type of insulin therapy, more than 20% of adolescents with T1DM experience PCOS, they show milder clinical and ultrasonographic symptoms of hyperandrogenism than their non-diabetic peer with PCOS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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**Table 1**

| | T1DM+PCOS girls ($n=11$) | Control PCOS girls ($n=23$) | P |
|---|---|---|---|
| Chronological age (years) | 16.5 (15.7–17.1) | 16.8 (15.5–17.3) | NS |
| Gynecological age (months) | 37.7±13.9 | 45.4±16.1 | NS |
| Cycle duration (days) | 31.0 (27.0–36.0) | 64.0 (35.2–104) | 0.004 |
| Hirsutism score | 2.0 (1.0–5.0) | 11.0 (3.0–13.0) | 0.004 |
| Volume of the right ovary (ml) | 5.4±1.6 | 7.3±2.2 | 0.03 |
| Volume of the left ovary (ml) | 4.2±1.8 | 7.1±3.2 | 0.01 |
| Testosterone (nmol/l) | 1.8±0.9 | 2.0±1.0 | NS |
| Androstenedione (nmol/l) | 10.5±4.9 | 10.1±4.5 | NS |
| DHEAS (μmol/l) | 5.4±2.0 | 7.1±2.4 | 0.05 |
| 17OH progesterone (nmol/l) | 4.2 (2.7–5.5) | 4.9 (3.6–6.7) | NS |
| SHBG (nmol/l) | 43.8±16.6 | 24.7±12.9 | 0.007 |
| FAI | 3.0 (2.6–4.3) | 8.5 (6.5–10.8) | 0.001 |

## P1302

**Diagnosis of glycemic abnormalities in thalassemic adolescents: continuous glucose monitoring versus glucose tolerance, and insulin: glucose parameters**

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We assessed glycemic status using oral glucose tolerance, 72-h continuous blood glucose concentrations by CGMS, calculate HOMA and QUICKI indices.
Population
15 adolescents with β thalassemia major.
Results
Oral glucose tolerance test (OGTT) In the 15 thalassemic adolescents (age=$19.75 \pm 3.08$ years) showed that four had impaired fasting blood glucose level (IFG)>5.6 mmol/l. One of them had diabetes (BG=16.2 mmol/l at 2-h, and two of them had impaired glucose tolerance IGT (>7.8<11.1 mmol/l). Maximum (postprandial) glucose levels recorded during CGM diagnosed IGT in six adolescents (>7.8<11.1 mmol/l) and diabetes (RBS>11.1 mmol/l) in two. HOMA and QUICKI revealed levels <2.6 ($1.6 \pm 0.8$) and >0.33 ($0.36 \pm 0.03$) respectively denoting non-insulin resistance state. There was a significant negative correlation between the β-cell function (B %) and the fasting and the 2-h blood glucose levels ($r=-0.6$, and $-0.48$, $P<0.01$)respectively. The average and maximum blood glucose levels during CGM were positively correlated with the FBS ($r=0.69$ and 0.6 respectively with $P<0.01$) and with the glucose level at 2 h after oral glucose intake ($r=0.87$and 0.86 respectively with $P<0.01$). The ferretin level was positively correlated with the fasting blood glucose (FBS), 2-h blood glucose levels in the OGTT, average, and the maximum blood glucose levels from the CGM ($r=0.69$, 0.43, 0.75, and 0.64 respectively, $P<0.01$) and negatively correlated with the β-cell function ($r=-0.41$, $P<0.01$).

Conclusion
CGM appears to be superior to standard OGTT for diagnosing glycemic abnormalities in TM. The glycemic abnormalities in our adolescents with TM is due to defective β-cell function with no evidence of insulin resistance state.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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Authors have nothing to disclose

## P1303

**Linear growth of children with congenital hypothyroidism detected by neonatal screening compared to normal children and their mid-parental height**

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We studied the growth data and bone maturation of 45 CH children (25 girls, 20 boys) with CH, diagnosed through the national screening program in Qatar, for 6 years or more to examine the effects of initial $T_4$ dosage (50 μg/day) with adjustment of $T_4$ dose to maintain serum $fT_4$ concentrations within the upper quartile of normal range and TSH < 4 mIU/ml. Birth weight, length and head circumference of patients (3.21 ± 0.43 kg, 50.5 ± 3.21 and 34.1 ± 1.5 cm respectively) did not differ than those for 10 560 normal newborns with normal thyroid function (3.19 ± 0.59 kg, 50.5 ± 2.2 cm and 34.2 ± 1.7). In CH children linear growth velocity during the first year (25.8 ± 2.8 cm/year) was similar to those for normal infants (25.5 ± 0.75 cm/year). During the first 6 years stature growth was normal in all children with CH. The mean HtSDS of CH children showed adjustment (± 0.5 S.D.) towards their mid-parental height SDS (MPHtSDS) only during the second year of life. Children mean HtSDS was higher by an average of 0.4 S.D. between the 2nd and 7th year of life. These data proved that effective screening and treatment completely assures normal linear growth in patients with CH. In conclusion, this treatment protocol maintains normal linear growth with HtSDS that slightly exceeds their MPHtSDS during infancy and childhood.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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Authors have nothing to disclose.

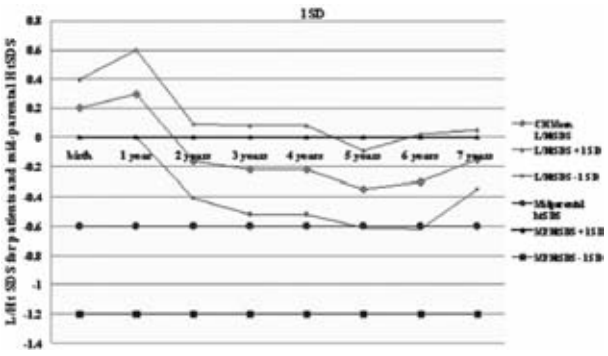


**Figure 1** LHtSDS ± 1S.D. of CH children vs midparental (MPHt) HtSDS ± 1S.D..

## P1304

**A novel splicing mutation of the POU1F1 gene in Japanese identical twins with mild combined pituitary hormone deficiency**

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Background
Mutations in POU1F1/PIT1 gene, a pituitary-specific transcription factor, affect the development and function of the anterior pituitary and lead to combined pituitary hormone deficiency (CPHD).
Objective
The clinical and genetic analysis of the twin patients presenting with mild form of CPHD and functional characterization of identified mutation.
Cases
Five-year-old identical twin brothers were referred to determine the etiology of their short stature in 1993. They were the first children of unrelated Japanese parents, born small for gestational age by caesarean section at term. Their heights were both less than −2.5 S.D. with delayed bone ages. Both parents were healthy, normal height father and rather small mother (−1.9 S.D.). Based on the clinical and endocrinological findings, they were diagnosed as having partial CPHD, with mild GH and PRL deficiencies and borderline TSH deficiency. It was decided that medical therapy, including GH and levothyroxine administration, was not necessary. Their growth chart showed similar growth curves throughout the entire period of growth, and indicated lack of pubertal growth spurt. Their adult heights were −1.8S.D. and −1.9S.D..
Results
A novel heterozygous splice site mutation (Ex2 + 1G > T; c.214 + 1G > T) was detected. This mutation was also present in their undiagnosed mother, but not in any of the controls. In vitro splicing studies suggested this mutation to result in an in-frame skipping of exon2. Heterologous expression studies of the mutated POU1F1 protein showed only modest reductions in its transactivation activities in HEK293T cells, while acting as a dominant-negative inhibitor of the endogenous activities of POU1F1 in pituitary GH3 cells.
Conclusions
This is the first report of a mutation at the exon2 donor splice site of POU1F1. The addition of this mutation to the growing list of pathological POU1F1 mutations may provide deeper insights into clinical heterogeneity and better understanding of the structure-function relationships of POU1F1.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1305

**Analysis of selected FOXP3 gene polymorphisms in children and adolescents with Graves' disease and Hashimoto's thyroiditis**

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Introduction
FOXP3 is a critical determinant of T regulatory cells (Tregs) development and function. Treg cells play a crucial role in modulating potentially self-reactive clones, and dysfunction of this cell type contributes to autoimmune disease such as Graves' disease (GD) and Hashimoto's thyroiditis (HT). The aim of our study was to estimate the association of three polymorphism of FOXP3 gene with the predisposition to GD and HT in Polish population.
Description of methods
The study was performed in the group consisting of 98 patients with GD (mean age, 17.3 ± 6), 39 patients with HT (mean age, 18 ± 4.5) sequentially recruited from the endocrinology outpatient clinic and 158 healthy volunteers (mean age, 16.3 ± 3). DNA was extracted from the peripheral blood leukocytes using a classical salting out method. The three SNPs rs3761549 (−2383C/T), rs3761548 (−3279G/T) and rs3761547 (−3499T/C) in the FOXP3 gene were genotyped by TaqMan SNP genotyping assay using the real-time PCR method. The levels of thyroid hormones, TSH and anti-thyroid autoantibody were determined using chemiluminescence method.
Results and conclusion
In our study the frequencies of −3279TT (rs3761548) genotype was less frequent in female patients with HT in comparison to healthy female (1 vs 18%, $P = 0.033$). There were no differences in the distribution of other analyzed polymorphisms of FOXP3 gene between the studied group. This result may suggests that −3279G/T polymorphism in FOXP3 gene could have a protective role in predisposition to HT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1306

**Characterization of a population of patients with 46,XX disorders of sex development followed at a Pediatric Center of Argentina**

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Disorders of sex development (DSD) are those congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical. The main aim of this study is to characterize a cohort of 46,XX DSD patients followed at the Garrahan Pediatric Hospital, Buenos Aires, Argentina. Medical records of all patients followed at the Endocrinology Department because of DSD between January 1, 2000 and January 1, 2011 in whom laboratory tests were requested were reviewed. We analyzed the records of 156 patients with the 46,XX karyotype. In 133 patients (85.3%) the final diagnosis was congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency. This diagnosis was established on the basis of consistent hormonal measurements (in all cases basal serum 17OH-progesterone was higher than 100 ng/ml). Diagnosis was confirmed by genotyping of the CYP21A2 gene in 90% of the cases. Diagnoses of the remaining 23 patients were the following: ovotesticular DSD in 5 patients (3.2%), testicular DSD in 6 (3.8%), aromatase (CYP19) deficiency in 7 (4.5%), oxidoreductase deficiency (POR) in 2 (1.3%), CAH due to 11-hydroxylase deficiency in 2 (1.3%), and CAH due to 3βhydroxysteroid dehydrogenase in 1 patient (0.6%). In patients with testicular and ovotesticular DSD the presence of the SRY gene was investigated. SRY was positive in two patients, one with testicular DSD and the other with ovotesticular DSD. As it has been previously described, CAH is the most common diagnosis in patients with 46,XX DSD. Testicular and ovotesticular DSD was the second most frequent diagnosis. Further studies should be performed to better characterize the ovotesticular and testicular 46,XX DSD population. Aromatase deficiency is believed to be a rare disease, but in our experience, it was as frequent as ovotesticular DSD.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1307

**Randomized multicenter trial on patients with childhood craniopharyngioma (KRANIOPHARYNGEOM 2007): update after 49 months of recruitment**

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Despite high survival rates (92%) in patients with childhood craniopharyngioma (CP), quality of life (QoL) is frequently impaired due to sequelae such as severe obesity resulting from hypothalamic involvement of CP. Based on the results of the multicenter prospective study KRANIOPHARYNGEOM 2000 radical surgery is no appropriate treatment strategy in patients with hypothalamic involvement. Furthermore, tumour progression/relapses are frequent early events in CP patients. The analysis of event-free survival-rates (EFS) in 117 prospectively evaluated patients with CP showed a high rate of early events in terms of tumour progression after incomplete resection (EFS: $0.31 \pm 0.07$) and relapses after complete resection (EFS: $0.63 \pm 0.09$) during the first 3 years of follow-up.

Accordingly, in KRANIOPHARYNGEOM 2007 QoL, and survival rates in CP pts (>5 years at diagnosis) are analyzed after randomization of the time point of irradiation (XRT) after incomplete resection (immediate XRT vs XRT at progression of residual tumour). Up to now (November 2011) 93 pts with CP were recruited (53 pts in the randomization arm; 40 pts in the surveillance arm; 4 pts in the process of review of imaging). 15 of 53 pts were randomized. 34 pts could not be randomized due to parental decision (13 pts), late schedule (12 pts) and due to decision of the physician (5 pts).

In conclusion, KRANIOPHARYNGEOM 2007 represents the first randomized trial in CP and the first study in pediatric neurooncology analyzing QoL as an endpoint. Aim of the study is to analyze the appropriate time point of XRT in order to improve QoL in patients with hypothalamic involvement. The recruiting compliance is high. However, the randomization compliance has to be improved in order to reach cohort sizes necessary for reliable statistical analysis and to answer the questions assessed by the randomized trial KRANIOPHARYNGEOM 2007.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1308

**Effect of calcitriol on bone metabolism in adolescents with type 1 diabetes**

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Vitamin D supplementation in childhood improves the achievement of peak bone mass. We investigated the effect of calcitriol on bone turnover in recent-onset type 1 diabetes (T1D). Moreover, the association between osteocalcin (OC) and metabolic control was examined.

We conducted a *post-hoc* analysis of a double-blind, placebo-controlled study of calcitriol supplementation to preserve β-cell function. Twenty-seven recent-onset T1D, mean age 25.6 years (range 10–35) were randomized to calcitriol (0.25 μg/die) or placebo (1:1) and followed-up for 1 year. Changes in bone formation (OC) and resorption (β-CTx) markers, and differences between placebo and calcitriol-treated group were evaluated.

At T1D diagnosis, OC levels were lower in females than in males ($P<0.01$). No significant correlations were found in relation to HbA1c, insulin requirement and C-peptide. At 1 year follow-up, OC levels were increased (11%) in the placebo group while dropped by 25% in the calcitriol group, but their levels were not significantly different compared to diagnosis. By stratifying patients according to age, we found that at 1 year follow-up as compared to diagnosis, calcitriol-treated patients $\geq 18$ years of age ($n=6$) showed significant 61% drop of OC ($P=0.04$) and a 67% reduction in β-CTx ($P=0.09$). In the same subgroup, OC tended to be lower ($P=0.08$) and β-CTx were significantly reduced ($P=0.03$) compared to placebo. Differences were not significant in patients >18 years of age ($n=13$). In the placebo group, OC levels were inversely related to C-peptide ($r=-0.79$; $P<0.01$) and tended to be positively related to insulin requirement ($r=0.59$; $P=0.07$). Baseline OC levels were inversely related to C-peptide changes from baseline ($r=-0.68$; $P=0.03$).

Our preliminary data suggest that calcitriol decreases bone remodeling in T1D adolescents and may preserve bone mass. After 1 year of insulin treatment in the placebo group, OC was negatively associated with C-peptide, thus questioning the role of OC as stimulator for β-cells.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This work was supported, however funding details unavailable.

## P1309

**Vitamin D levels in a paediatric population of normal weight and obese subjects**

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Introduction

Vitamin D plays an important role on musculoskeletal composition, but new evidences highlight others possible pleiotropic effects on many tissues and also metabolic functions. Vitamin D insufficiency should be associated with all-cause mortality, in particular with cardiovascular disease and metabolic syndrome. International studies suggested that 25(OH)D level sufficiency should be established at 30 ng/ml, insufficiency between 30 and 20 ng/ml and deficiency lower than 20 ng/ml.

Methods

To evaluate vitamin D status, we studied vitamin D levels in a population of normal weight (NW) and obese (OB) children: 113 were NW children, 105 M and 8 F, 46 prepubertal and 67 pubertal children and 444 were OB, 219 M and 225 F, 299 prepubertal and 145 pubertal children.

Results
Only 28.3% of NW children showed normal levels of vitamin D, 49.6% showed vitamin D insufficiency while 22.1% showed a clear vitamin D deficiency. Among vitamin D deficient children, 8.8% demonstrated vitamin D levels lower than 14.5 ng/ml. Obese children showed 18.9% of subjects with normal levels of vitamin D, 36.7% of subjects with vitamin D insufficiency and 44.4% of subjects a status of vitamin D deficiency. Among these, 23.2% showed vitamin D levels lower than 14.5 ng/ml. Mean vitamin D levels in NW children ($27.3 \pm 1.2$ ng/ml) resulted higher than in OB children ($21.8 \pm 0.6$ ng/ml). No differences have been found between prepubertal and pubertal children in terms of vitamin D levels.
Conclusions
Our pediatric population demonstrates a low percentage of vitamin D levels sufficiency. In particular obese children show only 19% of subjects with normal levels while almost half of this population show a clear insufficiency. Further studies are needed to support these results and to evaluate the possible metabolic consequences.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1310

**A particular phenotype in a case of Down-Turner syndrome**

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Introduction
Double aneuploidy involving both sex and autosomal chromosomes (chr) is very rare, Down–Turner syndrome being the most frequent. Fourty-seven cases of Down–Turner mosaicism have been reported, only nine with a karyotype containing Y chr (phenotype: seven male, two ambiguous genitals).
Case report
We describe a girl affected with Down-Turner syndrome. The cytogenetic analysis on peripheral lymphocytes, performed 2 months after birth because of mild facial dysmorphisms, showed a karyotype 45,X/47,XY,+21 (84/16%). Analysis of cultured skin fibroblasts confirmed the karyotype, with similar proportion of mosaicism (79/21%). The girl came to our attention at the age of 15.4 years. She presented few, if any characteristics of Down syndrome apart from primary autoimmune hypothyroidism. The phenotype of Turner syndrome was mild too: wide thorax with a large internipple distance, short neck, no signs of puberty with female external genitals, no mental retardation. Hormonal assessment showed hypergonadotropic hypogonadism (FSH 93.2 U/l, LH 37.7 U/l, estradiol 5 pg/ml, AMH $<$ 0.1 ng/ml, testosterone 0.24 ng/mL). MRI abdominal imaging showed streak dysgenetic gonads with an infantile uterus. Because of the presence of Y chr, along with streak non-functioning gonads, a prophylactic gonadectomy was performed and estrogenic replacement was started. Histological examination revealed a mixed gonadal dysgenesis, with the presence of both ovarian and testis tissue. Cytogenetic analysis performed with conventional techniques confirmed the karyotype 45,X/47,XY,+21 (77/23%) in gonadal fibroblasts. FISH analysis with SRY probe showed the presence of a hybridization signal on the Y chr.
Conclusions
We report the first case of Down–Turner syndrome presenting a Y chr with a female phenotype. To confirm the mosaicism as causative event, ongoing genetic studies will be carried out to exclude the presence of chimerism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1311

**Dysfunction of adrenal steroidgenesis in preterm infants with late-onset circulatory collapse**

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Objective
To investigate if the event of late-onset circulatory collapse (LCC) implicated in dysfunction of adrenal steroidgenesis due to impairment of adrenal remodeling in preterm infants.
Methods
Eleven in the patient group (case) with clinical signs compatible with LCC diagnosis (hypotension, oliguria, and hyponatremia) and 11 in the control group (control) matched them for gestational age without such signs. Steroids in serum were separated and purified by HPLC. Then, all samples were analyzed using ELISA or a gas chromatography-mass spectrometry.
Results
Serum levels of sulfoconjugated steroids (pregnenolone sulfate (Preg S), 17-hydroxypregnenolone sulfate (17-OH-Preg S) and dehydroepiandrosterone sulfate (DHEA S)) in case were significantly higher than those in control. However, there was no significant difference of unconjugated steroid levels (Preg, 17-OH-Preg and DHEA) between case and control. To investigate 3β-hydroxysteroid dehydrogenase (3β-HSD) activity, serum concentration ratio (17-OH-progesterone (17-OH-P)/(17-OH-Preg + 17-OH-Preg S)) was measured in case and control. The ratio was significantly decreased in Cace compared with control. Preg-$>$P-$>$-$>$cortisol is a major pathway for cortisol synthesis. 3β-HSD catalyzes step Preg-$>$P. Serum concentration of Preg in case (8.7 ng/ml (3.1–14.1), median and quartiles) was ten-times higher than control (0.8 ng/ml (0.7–6.1). However, there were no significant differences of serum P and cortisol levels in case (P: 1.1 ng/ml (1.0–1.5); cortisol: 7.8 μg/dl (3.1–11.3)) and in control (0.5 ng/ml (0.1–1.7); 2.5 μg/dl (1.4–5.5)).
Conclusion
Cause of the event of LCC is at least in part dysfunction of steroidgenesis due to impaired adrenal remodeling.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector

## P1312

**Thyroid abnormalities in a 13 year old girl with an androgen secreting juvenile granulosa cell tumor of the ovary: insight into the thyroid-androgen axis in girls**

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Introduction
Granulosa cell tumors account for ~ 2–3% of all ovarian malignancies. There are two types: adult granulosa cell tumor (AGCT) and juvenile granulosa cell tumor (JGCT). JGCTs are rare and constitute 5% of all granulosa tumors. Androgen production by a JGCT is even rare and may produce virilization in patients. Thyroid abnormalities in female patients with androgen-secreting JGCT has not been documented before.
Objective:
We describe a unique case of a 14 year pubertal Caucasian girl presenting with hoarseness, hirsutism, irregular menstrual cycles and a large multi-nodular goiter with three large complex solid-cystic nodules measuring $>$ 2 cm.
Results
Thyroid function was normal and thyroid antibodies were negative. Thyroid nodule FNAC showed benign follicular changes. Testosterone level was 488 ng/dl and androstenedione levels were elevated. Karyotype, α fetoprotein and beta HCG were normal. LH was normal but FSH and estradiol levels were suppressed. Computed tomography revealed a dense, cystic solid, heterogeneous 14 cm left ovarian mass. Unilateral oophorectomy was performed. Histopathology showed intermediate grade juvenile granulosa cell tumor positive for inhibin, calretinin and negative for thyroglobulin, TTF1, PAX8 and aromatase.

Testosterone (13 ng/dl), inhibin and androstenedione levels dramatically decreased immediately after surgery; LH and FSH normalized, with return of normal menstrual cycles. TSH steadily increased as $FT_4$ dropped over the next few months. Thyroid US showed reduction of thyroid size and nodule size by 30%. Patient was treated with L-thyroxine for hypothyroid symptoms and was stable 6 months after surgery.

Conclusion

This is the first pediatric case in the literature of ovarian androgen secreting JGCT associated with multiple large thyroid nodules. The effect of testosterone withdrawal leading to a reduced thyroid size, low FT4 with elevated TSH, has never been described before. We feel that thyroid function needs to be checked in female patients with high testosterone levels, especially after normalization of testosterone. Larger studies need to be done to look at this relationship in detail.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1313

**Clinical practice guideline on linear growth measurement of children**

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Introduction

Growth is an important and sensitive indicator of child health. Abnormal growth is a common consequence of many conditions, therefore its identification acts as a useful warning of possible pathology. Effective growth monitoring requires precise linear growth measurements; however, measurements are often inaccurate and unreliable. Measurement error influences the interpretation of growth patterns resulting in failure to identify underlying pathology or apparent growth divergence in normally growing children.

Purpose

The purpose of this project was to develop a clinical practice guideline to assist professionals in applying evidence-based knowledge in measuring children using standardized instruments and techniques to facilitate accurate and reliable growth assessments.

Methods

Systematic methods were used to identify evidence to answer focused clinical questions about linear growth measurement. A multidisciplinary team of health professionals critically appraised and synthesized the evidence to develop explicit clinical practice recommendations using an evidence-based practice rating scheme. The guideline was prospectively evaluated through internal reviews, external expert reviews, and a pilot study.

Results

Data analyses were used to improve guideline clarity, applicability, and feasibility, while demonstrating validity and intraexaminer and interexaminer reliability ($P<0.0001$). The guideline provides clinical practice recommendations for measurement techniques, measurement instruments, use of less expensive instruments, calibration of instruments, diurnal height variation, measuring height versus length, replicate measurements, and special considerations. Each recommendation is linked with scientific rationale and supporting references. Tools are available to facilitate guideline implementation.

Conclusions

The clinical practice guideline on linear growth measurement is based on the best available evidence and was developed using rigorous methods. Health professionals and parents need accurate and reliable growth information. Guideline use will improve timely recognition, diagnosis, and treatment of growth disorders, while reducing unnecessary and costly evaluations in normally growing children. Widespread dissemination and adoption of the guideline can significantly impact child health around the globe.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1314

**The presence of thyroid nodules in our morbid obese patients and its relation with metabolic parameters**

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Objective

Insulin resistance may contribute to the development of nodular thyroid disease. Changes in thyroid morphology have been reported in obese patients. In this study, we examined the frequency of nodular thyroid disease in morbid obese patients ($BMI>40\ kg/m^2$) and investigated the metabolic parameters which may play a role in thyroid nodule formation.

Methods

Three hundred and six morbid obese patients (296 women, 10 men) (age 46.2 $\pm$11) without known thyroid disease were included in the study. Thyroid ultrasonography was performed, HOMA-IR, HOMA-% B and HOMA-% S was measured in all patients.

Results

Age, waist circumference, HOMA-% B, vitamin-D3, LDL-cholesterol, TSH was significantly correlated with thyroid volume after regression analysis ($P<0.05$) (adjusted $R^2$ value is 0987). A 153 (50%) patients had nodules which was greater then normal population. Presence of nodules were significantly correlated with the age, weight, waist circumference, and HDL-cholesterol level of free $T_4$ after logistic regression analysis ($P<0.05$). Nodule volume was significantly associated with age, HOMA-% B, HOMA-IR, vitamin B12, and fasting glucose after regression analysis ($P<0.05$) (adjusted $R^2$ value 0689). There were no significant differences in the incidence of nodules according to presence of insulin resistance or presence of diabetes in these patients. There was no correlation with metabolic syndrome criteria and nodule presence.

Conclusion

Unlike low frequency of thyroid nodule in obese people in recent studies, we found higher frequency of thyroid nodule in our morbid obese patients. In obese patients, metabolic parameters may play an important role in thyroid volume, nodule formation and nodule volume.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1315

**Mutations of the SRD5A2 gene in patients with 5 $\alpha$-steroid reductase deficiency**

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Introduction

5 $\alpha$-steroid reductase deficiency (5$\alpha$ SRD) is a rare autosomal recessive disorder.

Aim

To identify mutations in the SRD5A2 gene in patients with 5$\alpha$ SRD.

Methods

Patients registered in the endocrine clinic of our hospital and new patients with diagnosis of 5$\alpha$ RD were eligible to be included in this study. The study was approved by AIIMS ethics committee, written informed consent was obtained from patients (parents in case of children). All patients had detailed history, physical examination, chromosomal analysis and hormonal studies done. Electrochemiluminiscence method was used for the estimation of LH, FSH and T. DHT was done after Celite chromatography by (RIA)(kit based method (Immunotech,Prague)). DNA was isolated from the blood samples,quantified and subjected to PCR amplification using specific primers for all five exons of the SRD5A2 gene.

Results

There were 32 patients (from 28 families) with 5$\alpha$ RD.The age of the patients ranged from 2 to 28 years (13.01$\pm$7.4) and the mean age at which medical attention was sort was 5.6 years. Molecular genetic studies were performed in 13 cases from 11 families. Five patient had homozygous mutation of R246Q and nine patients had mutation of V89L on exon 5,one patient had mutation of A52T on exon 1. Besides an intronic insertion of C at g.31805880-5881 was present in all samples.

Conclusion
Mutation of V89L and R246Q (two cases reported earlier) on exon 5 and a new mutation of A52T has been found in our patients and needs further work for significance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1316

**Lipid storage myopathy in a child with idiopathic short stature**

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Introduction
Lipid storage myopathy (LSM) is characterized by increased lipid droplets in muscle fibers. Primary carnitine deficiency is the most frequent cause of LSM, clinical presentation ranging from asymptomatic to progressive muscle weakness or cardiomyopathy, carnitine supplementation being effective with remission of symptoms.
Case report
In february 2007 R.A. born in 1996 presented progressive muscle weakness with elevated muscular enzymes (LDH = 1155 μI/ml $n$ = 125–234, CPK = 1082 μI/ml N = 25–195). Starting from a muscular biopsy, which suggested polymyositis, a glucocorticoid trial was initiated with partial amelioration followed by clinical relapse. Development of cushingoid syndrome and growth retardation (height 123 cm, −3 s.d.; weight 22 kg, −2.5 s.d.; normal parental heights) determined endocrinological evaluation which revealed normal IGF I (172 ng/ml $n$ = 111–551), and basal GH (3.3 μI/ml) with response at stimulation (stimulated GH 125 μI/ml) and delayed bone age of 8,5 years. Functional pituitary evaluation was normal.
Reevaluation by muscular biopsy revealed presence of increased lipid droplets in type 1 muscle fibers suggesting LSM. Carnitine supplementation was started in december 2008 and continued without pause (1 g/day) with progressive clinical improvement and normalization of muscular enzymes.
Absence of GH deficiency justified expectative but the stagnant height after 6 months was an argument for hGH therapy, which was started in October 2008 followed by a satisfactory growth rate.
Reevaluation in December 2011 revealed Tanner pubertal stage IV, growth velocity of 0.7 cm/month, actual height of 147 cm (−2s.d.), delayed bone age of 13 years.
Conclusion
We report a patient with idiopathic short stature and LSM who responded well to hGH therapy and carnitine supplementation. To our knowledge, children with LSM do not usually associate short stature or GH deficiency. Impact of hGH therapy on body composition and muscle structure in LSM cases needs to be further evaluated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1317

**Care of patients with childhood onset growth hormone deficiency before and after the transition period**

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Objectives
To investigate how CO-GHD patients are taken care of in paediatrics, during the transition period and in long-term adult follow-up.
Methods
Retrospective cohort study of CO-GHD patients, transferred in our adult department between 1994 and 2011. Paediatric charts were available for all patients. For adult follow-up, parameters of metabolic, bone and cardiovascular status were recorded at several hospitalisations: the first one (V0) and at 1 (V1), 3 (V3) and 5 (V5) years after transition. Three groups of patients were studied: treated (G1) and untreated (G2) persistent GHD, and resolutive GHD (G3).
Results
We present a cohort of 113 patients with median age at transition of 19.5 years. Aetiologies of GHD were acquired in 54%, congenital in 34% and idiopathic in 12% of cases. GHD was complete in 72% of patients. Other pituitary deficits were often associated in congenital (70%) or acquired GHD (78%), idiopathic GHD were generally isolated (64%). In childhood, GH was started at a mean dose of 34.6 μg/kg/day. Mean difference between final and target heights was −0.4s.d.. At transition, 14% of patients had a normal GH axis. 48 patients completed V1, among whom 30 were under GH (mean dose 0.62 mg/day). At V3 and V5, 32 and 22 patients were studied respectively (18 and 5 under GH). Preliminary data show that, over time, mean IGF1 drops in G1, in parallel with GH doses, but always remains higher than in G2, in which it is stable. Mean BMI shows overweight in both G1 and G2: it remains stable in G1, but tends to rise in G2. Surprisingly, mean spine BMD tends to increase in G2 and drop in G1.
Conclusion
This is the largest cohort of CO-GHD patients followed until adulthood. It allows us to evaluate our medical practices, in times when adult GH supplementation remains controversial.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1318

**Psychiatric symptoms in Addison's crisis in childhood: the complexity of diagnosis**

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Introduction
Since Addison disease is relatively rare and difficult to recognize in childhood delayed diagnosis is common. Patients with adrenal insufficiency generally show hypotension, hypoglycaemia, hyponatriemia, but can also manifest mild symptoms like chronic fatigue, nausea, vomiting, weight loss, recurring abdominal pain and psychiatric symptoms. In this regard mild disturbances in mood, motivation and behavior represent the main clinical features showing a prevalence between 64 and 84%.
Case report
The patient was admitted at age of 12 years old with severe physical impairment; in the past 3 years he had a marked decrease in height and weight growth velocity with values below 3rd centile and BMI 12. He presented hyponatremia, food refusal, vomiting and psychiatric symptoms like emotional lability, depressed mood and fear of dying. He alternated these symptoms with episodes of euphoria with rapid cycles, strong opposition temper tantrums. During hospitalization the patient was fed by parenteral nutrition and then by enteral nutrition by Nasogastric Tube with poor weight recover and costant hyponatriemia. In this period psychiatric symptoms had been slightly improving with antipsychotic therapy. After several weeks an endocrinological assessment was requested, with subsequent diagnosis of autoimmune adrenal insufficiency (serum cortisol 48 nmol/l, ACTH 3330 ng/l and antiadrenal antibodies positivity).
Replacement therapy with corticosteroids, led to correction of electrolyte disturbances and a dramatic improvement of the eating disorder, with fast weight recovery (BMI 16 two months later). After a temporary exacerbation of psychiatric symptoms a gradual improvement was observed with a complete remission. Despite a good compliance to therapy, the delay in growth velocity persisted. After 1 year GH deficiency was diagnosed, likely related to autoimmune pituitary disease.
Conclusions
Symptoms in Addison's disease can be heterogeneous and difficult to recognize in childhood; a careful evaluation is therefore necessary. Furthermore psychiatric symptoms can be prevalent, leading to incorrect or delayed diagnosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1319

**Insulin like growth factor-I (IGF-I) levels and metabolic parameters in a population of obese children and adolescents**

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Introduction
The risk association between the insulin like growth factor-1 (IGF1) and cardiovascular risk is inconclusive in adults and under-explored in the pediatric population. We aimed to investigate the associations between serum concentrations of IGF1 and cardiovascular risk factors in obese children and adolescents.
Methods
Cross-sectional study. Clinical and metabolic evaluations including an oral glucose tolerance test (OGTT) were performed at fasting in 594 overweight an obese children and adolescents (295 males and 299 females). IGF1 levels were measured by Immulite and IGF-SDS for each age and gender subgroup was calculated and, then, divided into quartiles.
Results
Two hundred and thirty-nine subjects were in Tanner 1, 206 in Tanner 2 or 3, 149 in Tanner 4 or 5 stages, respectively. Subjects in the lowest quartile of IGF1 SDS were older ($P<0.01$) and with higher BMI ($P<0.03$) respect to the highest quartile. After correction for age, gender and pubertal stages, subjects in the highest quartile presented higher insulin levels at fasting ($P<0.01$), post-OGTT ($P<0.03$) and higher HOMA-index ($P<0.01$). No significance was detected for glucose, lipids or pressure with exception of higher triglycerides in the lowest quartiles of IGF-SDS in the crude ($P<0.03$) but not in the corrected models. Continuous IGF-I levels maintained the same associations observed for IGF SDS. Acanthosis index did not correlate with IGF-I levels.
Conclusions
IGF-I levels were directly associated with insulin levels and insulin resistance in obese children and adolescent irrespective of gender and puberty. The association with other cardiovascular risk factor observed in adults could be modulated by age.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1320

**The median age of the onset of puberty in Uzbek boys of Tashkent city**

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Background
The age of the onset of puberty is much lower today than in the past century, due to the stability of the socio-economic conditions, improved quality of life and overall health of the population.
Materials and methods
We examined healthy 818 boys aged from 7 to 18 years, the study was conducted between 2009 and 2011.
Boys were examined in three urban districts of Tashkent city (i.e. Mirzo-Ulugbek, Shakhantaur, Yunus-Abad). Testicular volume was evaluated with a Prader orchidometer. The age of the onset of puberty was defined as the age at attainment of testicular volume of 4 ml or greater.
Results
The earliest age of the onset of puberty was 9.0 years (3.3%). All healthy boys had testicular volume greater than 4 ml by 14 years. The data suggest for healthy Uzbek boys is defined upper reference value of the age of the onset of puberty. Thereby the diagnosis of delayed puberty may be established at 14 years, which generally coincides with the international guidelines for the diagnosis of delayed sexual development. The median age of the onset of puberty for Uzbek boys of Tashkent city was 11.9 (95% CI 11.1–12.7), this data agree with Great Britain (11.9) and Byelorussia (11.9), but different from USA (11.5), Netherlands (11.5).
Conclusion
1. The earliest age of the onset of puberty in healthy Uzbek boys of Tashkent city was 9.0 years and 14 years ultimate one.
2. The median age of the onset of puberty for Uzbek boys of Tashkent city was 11.9 years.
3. We have defined criteria for the diagnosis of delayed puberty at 14 years.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1321

**Thyroid function in a group of type 1 diabetes children without clinical evidence of thyroid diseases**

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Background
Type 1 diabetes mellitus is frequently associated with endocrine autoimmune diseases especially thyroid disorders. These could have a negative impact on glycemic control even if there is no clinical evidence of thyroid dysfunction.
The aim of the study was to evaluate thyroid function in a group of children with type 1 diabetes mellitus (T1DM) and no clinical evidence of thyroid diseases and to determine its impact on glycemic control.
Material and methods
We collected demographic data from 72 children with T1DM and no clinical evidence of thyroid disorders, physical examination data, and laboratory data: glycated hemoglobin, thyroid peroxidase antibodies (TPOAb), TSH. Subclinical hypothyroidism has been defined as TSH above 4.5 μU/ml without clinical evidence of hypothyroidism.
Results
Thirty-eight (52.7%) patients were boys; mean age $10.89 \pm 4.26$ years, mean span of type 1 DM $3.41 \pm 2.56$ years. TPOAb were present in 23.6% of patients. Mean glycated hemoglobin was higher in patients with thyroid autoimmunity (9.7% $\pm 2.05$ vs $8.6\% \pm 2.11$ ($P=0.05$)). TSH was significantly higher in patients with thyroid autoimmunity (3.39 vs 2.15 μU/ml; $P<0.05$). A number of 8 patients of 72 (11%) had TSH over 4.5 μU/ml (range 4.5–5.76 μU/ml) and only one child had TSH under 0.5 μU/ml. Subclinical hypothyroidism was observed in 29.4% (5/17) among patients with thyroid autoimmunity compared with only 5.4% (3/55) in patients without thyroid autoimmunity ($P<0.001$).
Conclusions
Thyroid autoimmunity was found in 23.6% children with T1DM and no clinical evidence of thyroid disorders. Patients with thyroid autoimmunity had significantly higher glycated hemoglobin and higher TSH versus patients negative for TPOAb. The most frequent subclinical thyroid dysfunction found out was hypothyroidism. Subclinical hypothyroidism was much more prevalent in patients with thyroid autoimmunity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1322

**The EURO-WABB project**

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Introduction
EURO-WABB is a European research project within the field of rare diabetes diseases. The general objective is to support efficient diagnosis, treatment and research for the overlapping rare genetic diseases Wolfram, Alstrom and Bardet-Biedl (WABB) syndromes.

Methods
The project is supported by the EU DG-SANCO by the collaboration of 8 Associated Partners (AP) and 15 Collaborating Partners. University of Birmingham work as the Project Leader (TB) and coordinating centre. A web-platform was launched in 2011 (www.euro-wabb.org) and a virtual registry will be soon available.
Results
Key achievements of the project are yet on the ground and include: i) coordination: Project Management and Scientific Advisory Committee established; ii) dissemination: awareness raising through conference attendance and development of multi-language website; iii) evaluation: regular conference calls with AP to monitor project progress; iv) core datasets: core and extended dataset agreed in September 2011; 44 + 370 data fields respectively; phenotyping information standardised used ICD-10 and ESPE classification coding systems; v) genetics: mutation database completed for ALMS1, WFS1, SLC19A2 and EIF2AK3; vi) Virtual Registry and Information Environment: prototype core dataset registry developed for testing in November 2011. First recruitment of patients started in August 2011 and local Ethical Committee approval was obtained in UK, Italy and Poland. In the period from 20th June–2nd January 2012, 2814 visits from 73 countries accessed the website (2295 from EU). Actually, 22 people have formally registered and include a mix of patients, families, clinicians and other interested parties.
Conclusion
EURO-WABB is a new reality in the world of rare diseases. By the virtual registry and the website it will be possible in the next future to address the main topics of this project. Next milestones will include the publication of consensus management guidelines, diagnostic criteria, health professional education and patient information material to support the registry.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1323

**Kearns-Sayre syndrome: clinical and molecular diagnosis of the disease and treatment with recombinant GH (rGH) complicated by a severe cardiac conduction deficit and cardiomyopathy**

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Introduction
Kearns-Sayre syndrome (KSS) is a rare mitochondrial cytopathy. The diagnosis of KSS is made on the classical triad of symptoms: onset of the disease $<20$ years of age, progressive external ophthalmoplegia (PEO) and pigmentary retinopathy (PR). KSS is manifested also by other systemic abnormalities: cardiac conduction defects, different neurological abnormalities and several endocrine disorders. A variety of deletions and/or duplications in mtDNA, affecting genes encoding respiratory chain proteins, are found in most cases. The mutated mtDNA coexists with normal molecules (heteroplasmy) and the proportion of mutated to normal mtDNA correlates with the severity of clinical symptoms.
Case report
The adopted boy was born small for gestational age at term. From the 2nd year of life chronic PEO was observed. Additionally the PR was observed. From early childhood he presented short stature. In the age of 11 years, in the EMG the myogenic pattern was revealed and KSS was diagnosed. MRI of the head showed hypoplasia of pituitary gland. Long-range PCR analysis disclosed a 7663 bp long deletion in mtDNA in 6340–14 003 nucleotide region. In the age of 12 years GH deficiency was recognized and the rGH therapy was started. In the age of 15 years complete atrioventicular block was diagnosed. The patient was applied with pacemaker. During next 6 months progressive insufficiency of left ventricle was observed, the echo sound showed the features of dilated cardiomyopathy. The rGH treatment was finished with final height 163 cm.
Conclusions
The effect of rGH therapy in our patient was satisfactory even a big mtDNA deletion and severe cardiac disturbances occurred. Cardiac disturbances were a part of the syndrome, not related to rGH therapy. Based on the literature and the examined patient we state that growth of KSS patients, spontaneous or promoted with rGH, is unpredictable.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1324

**Newborn screening for congenital hypothyreoidism(CH) in Georgia**

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Background and aim
Revelation of the incidence of CH is of great value at the background of iodine deficiency (ID) existing in Georgia. Screening on CH in our company with consequent monitoring of the newborns with neonatal hyperthyreotropinemia (NHT) started in September 2010.
Materials and methods
During the first year period 70 731 newborns from all over Georgia underwent TSH (mU/l) determination from blood spots, taken at 24–72 h after birth and venous blood $FT_4$ (ng/dl) and TSH at two weeks after birth using DELFIA kits were measured. Infants with NHT(cut off level 20 mU/l) were consulted by pediatrician, endocrinologist and their blood thyreoglobulin (TG) level(ng/dl) and thyroid volume ($cm^3$), using ultrasound investigation were examined.
Results
Transitory NHT had 280 infants (1:253 live births), mediana 46.5(range 28.7–210), boys 151, girls 129.23 infants had CH (1:3075 live births), girls -boys ratio-2,8/;ten with subclinical CH (Gr.1) and 13 -overt CH (Gr.2). Most of the babies were clinically euthyroid except 8.3% having prolonged jaundice, 5%-constipation, 3.3%-sleepiness and 0.33% macroglossia. Most of the mothers with high NHT did not receive iodide supplementation during pregnancy. No one received high doses of iodine or anti-thyroid drugs. Heredity for thyroid pathology ranged 34–41%. No case of goiter and thyroid agenesis was detected. Gr.1 thyroid volume median- 1.3 was 2.6 times greater than of Gr.2- 0.5 ($P<0.001$) and their neonatal and 2-weeks TSH levels were lower compared to GR.2 $P<$O.O5. Two infants of Gr.2 with pronounced thyroid hypoplasia (volume 0.2 $cm^3$) had low TG level-2.5–3.6. The rest had moderately elevated TG levels with median of Gr.2- 168.5, that was significantly higher compared to Gr.1- 72.1, $P<0.01$.
Conclusion
Thus, CH in Georgia develops without goiter and with marked hypoplasia in severe cases that is possibly conditioned by ID and hereditary predisposition to thyroid pathology.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1325

**GH deficiency in the transitional age: retesting and reassessment of ninety personal cases**

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Transition is defined as the period of the life of a subject ranging from the conclusion of puberty to full adulthood (20–24 y. o.).
The management of patients with GH deficiency in this period of life is related to: assessment deficit etiology: idiopathic or organic; dose of the drug; gain of the full somatic development; maturation of the sexual-reproductive; cardio-metabolic risk assessment; educational aims.
Purpose of the study
In our DH service, since 1999, 1800 children was referred for growth failure. 638 patients was enrolled for GH treatment The average age for the start of therapy was 11.3 years and mean age at discontinuation of therapy was 16.3 years. The mean duration of therapy was 5 years. Currently 250 children with GH are on GH therapy and are monitored with a six months follow-up. All patients have stopped GH treatment after final height attainment (transition phase), we are re-evaluating them for residual GH supply, performing stimulation tests. 90 patients (54 males and 38 females) performed GHRH + arginine re-testing to assess GH secretory capacity after 6 $\pm$ months of discontinuation of therapy.
Materials and methods
Patients undergoing stimulation test with GHRH + arginine: GH was measured at the time 0 '30' 60 '90' and 120 '. We also have evaluated basal value of IGF1. Fully auxological and clinical examination evaluation of body composition, was performed at the time of retesting.
Results
The results obtained so far show that many patients with idiopathic GH deficiency recover secretion of the hormone. Rarely, patients with organic (multiple congenital onset pan-hypopituitarism) or acquired deficits (radio-and chemo-treated tumors) recovering residual GH secretion.
Conclusions
It's essential to identify patients whose GH deficiency persists in the transition phase until to adulthood. In these patients the treatment with GH needs to be

evaluated balancing benefits vs the overall systemic costs and adverse effect. Complications (survival, quality of life, cardiometabolic outcome), that could result from untreated GH deficit, should be correctly predicted.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1326

**Building a Brazilian national network for pediatric endocrinology: the RUTE SIG experience**

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Providing good information, exchanging expertise and stimulating clinical discussions are usually main goals when structuring academic services. Nowadays, especially in large countries with social/economic differences between regions, such as Brazil, telemedicine and telehealth are resources with main strategic importance in a country's Public Health System. Since its creation, RUTE network (Rede Universitária de Telemedicina, supported by brazilian Ministry of Science and Technology in association with the Ministry of Health) is enforcing the creation of SIG (Special Interest Groups) with the purpose of integrating and enhancing collaboration between universities and educational health institutions with graduation and/or post graduation medical programs, including medical residences, in a great (and ever expanding) array of subjects and themes. Starting in august, 2011, the SIG of Pediatric Endocrinology was structured to integrate services from all over the country, in monthly videoconferences, following a program previously discussed. These conferences are designed to stimulate the full participation of top specialists and also residents and medical students. Presently, six institutions are enlisted (UFRN – Universidade Federal do Rio Grande do Norte; UNICAMP – Universidade de Campinas; UFBA – Universidade Federal da Bahia; UnB- Universidade de Brasília; Santa Casa (Porto Alegre); USP – Universidade de São Paulo; UFPR – Universidade Federal do Paraná e FAMERP – Faculdade de Medicina de Rio Preto). After each one of the first four videoconferences, conclusions were summarized in order to subsidize national politics in critical pediatric endocrinology issues, like national protocols of growth hormone and insulin analogues dispensation by federal and state governments. The main effort is to expand the group of institutions, which, even in this very initial moment, includes institutions from four of five great regions of the country. In a such a large and diverse country as Brazil, this activity must be enforced, since its advantages and potential usefulness had already been shown.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1327

**Peculiarities of autoimmune thyroiditis in children**

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Aim
Evaluate anamnesis, clinical and laboratory parameters in children with autoimmune thyroiditis (AT).
Methods
We analyzed retrospectively 211 children in the endocrinological department of University hospital (Minsk) over 2002–2011 years. The number of boys (B) 42 (19.9%), of girls (G) -169 (80.1%) (stage on Tanner1- 15 (35.7%) B, 58 (24.3%) G; stage2-3 – 19 (70.3%) B, 65 (58.6%) G; stage 4-5 – 8 (29.7%) B, 46 (41.4%) G). Ultrasound of thyroid gland, levels of TSH, free $T_4$, Anti-TPO were analyzed. The results were processed using the Statistic 6.1.
Results
Positive family history on the endocrinopathies were in 87 (41.2%) patients (thyroid diseases – 61 (28.9%), DM2 – 22 (10.4%), DM1 – 2 (0.95%), other endocrinopathies – 2 (0.95%) cases). The average volume of the thyroid was $138.4 \pm 40.4\%$ – B, $153.4 \pm 64.8\%$ – G from the age norms ($P = 0.1$). 97 (45.9%) patients – subclinical hypothyroidism, 74 (35.1%) – euthyroid, 40 (19%) – primary hypothyroidism.
TSH level in prepubertal B – $18.7 \pm 8.36$ (0.23–3.4 μIU/l), G – $12 \pm 2.76$ ($P = 0.5$); early pubertal B – $10.5 \pm 6.8$, G – $10.4 \pm 1.9$ ($P = 0.8$); late pubertal B – $3.3 \pm 0.5$, G – $12.36 \pm 5.14$ ($P = 0.5$).
Free T4 level in prepubertal B – $15.46 \pm 1.01$ (10–23.2 pmol/l), G $-14.67 \pm 0.55$ ($P = 0.2$); early pubertal B $-17.48 \pm 1.46$, G $-14.26 \pm 0.38$ ($P = 0.3$); late pubertal B – $19.57 \pm 0.7$, G $-15.7 \pm 0.77$ ($P = 0.1$).
Anti-TPO level in prepubertal B – $331.2 \pm 78.6$ ($< 30$ U/ml), G – 581, $7 \pm 100.8$ ($P = 0.04$); early pubertal B – $613.5 \pm 140.5$, G – $710.4 \pm 100.5$ ($P = 0.3$); late pubertal B – $584 \pm 293$, G – $376 \pm 53.2$ ($P = 0.6$).
There was positive correlation between Anti-TPO level and thyroid volume in all cases ($r = 0.18$ $P = 0.007$).
Conclusions
AT was manifested with the development of subclinical hypothyroidism and goiter. The maximum volume of the thyroid and level of Anti-TPO was in early puberty. The most pronounced decrease of thyroid function was in prepubertal boys, in girls reliable differences of TSH level with the development of puberty was not observed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1328

**Short stature due to late-onset congenital adrenal hyperplasia**

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Introduction
Precocious pubarche is defined as pubic hair onset before age eight in girls and age nine in boys. In 5–20% of cases the cause is late-onset congenital adrenal hyperplasia (LO-CAH), which is due mainly to non-classic 21-hydroxylase deficiency. If not promptly diagnosed, it can lead to accelerated bone maturation, short final height, and in adulthood to severe acne, hirsutism and infertility.
Case report
Adolescent male, 15 years-old, had a first Pediatric Endocrinology consultation in May 2011 due to short stature. No complications reported during pregnancy and neonatal period. Pubarche occurred at age 7. No information about age of onset of testes and penis development. No relevant family history. Family estimated target height 168 cm (10th–25th percentiles). On physical examination with weight 69 kg (90th percentil), height 159 cm (3rd–10th percentiles) and Tanner stage 5 for genital and pubic hair development. Stature growth chart with few records in the last years but crossing four percentiles curves from age 12 to 15. Laboratory assays showed an increase of baseline 17-hydroxyprogesterone (7.57 ng/ml) and Δ4-androstenedione (4.71 ng/ml; *n*: 0.60–3.10). Measurements of free testosterone, dehydroepiandrosterone sulfate, gonadotropins, IGF1, IGFBP-3 and thyroid function were normal for age group. LO-CAH diagnosis was confirmed by ACTH stimulation test (17-hydroxyprogesterone increased to 27.7 ng/ml 60 min after). Genetic testing detected the mutation p.Val281Leu in homozygosity in gene CYP21A2. Radiography of non-dominant hand and wrist revealed a bone age of 17 years. Testicular ultrasound showed no adrenal rest tumors.
Conclusions
In this clinical case, earlier onset of pubarche was not identified and LO-CAH was not promptly diagnosed, leading to accelerated bone maturation and subsequent committed final height. Early diagnosis of LO-CAH is fundamental to control signs and symptoms related to androgen excess.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1329

**Actual preparation of the growth hormone treatment for small-for-gestational-age children**

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In 1997, Japanese Child Life Specialist (CLS) was born by earning license in the United States, and then the concept of 'preparation' has spread in Japan. Needs for 'preparation' has been established as the channel to enhance child abilities with an increase in Hospital Specialists (HPS) got licenses in Britain. However, CLS and HPS do not exist in all facilities, so nurses had individually provided explanation of examinations and procedures through pictures or picture-story-shows to these children previously in Japan.

In our facility, nurses has provided the 'preparation' of the GH treatment (i.e. admitting into the hospital, having examinations and procedures, and receiving growth hormone injections) using picture-story-shows and/or stuffed toys for hospitalized small-for-gestational-age (SGA) children and their families, since 2008 when the growth hormone treatment cost became reimbursed for SGA by the national medical insurance. Actual practices will be reported.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1330

**47XYY syndrome is one of the cause of tall in children**

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Symptom of tall is manifestation of many endocrine and genetic diseases. Their early detection is necessary for timely therapy, for improvement of disease state and prognosis for a disease. 47 XYY syndrome is one of frequent occurrence diseases that are accompanied with primary tall. Materials and methods. Patient L., 12 years old, was under our observation. Chief complaint – tall height. Intracranial hypertension, delay in speech development and gross motor delay were registered on the first year of life.

Results

Children's weight – 97.3 kg, height – 195 sm. Rate of growth for current year made up 12 sm. Physical growth and development – high and disharmonious, body mass index – 25.5 kg/m$^2$. Sexual formula of Tanner: P3, Ax1, gonads in marsupium 12 ml. Predict final height of bone age (14 years old 6 months) constituted 204 sm. This data exceed significant index of genetic height (188.5 sm). Indices of biochemical blood assay, glucose loading had normal values. Levels of thyroid-stimulating hormone (TSH), free thyroxin, follicle-stimulating hormone, interstitial cell-stimulating hormone, chondrotropic hormone, prolactin and of cortisol were within the established norms. Values of insulin-like growth factor-1 (761.0 ng/ml, *n* 249 – 642 ng/ml) and of testosterone (18.9 ng/ml, *n* 2.8 – 8.0 ng/ml) had high normative indices. In the process of karyotyping chromosome pathology (karyotype 47 XYY) was established in child. Consultation of neurologist: asthenia, pseudoneurotic tics.

Conclusions

i) It was mad the exclusion: 47 XYY syndrome. ii) Treatment: testosterone enanthate at doses 500 mg, 2 weeks, in the course 6 months. iii) At this moment present patient do not require treatment (bone age > 13.5 years old, treatment is prescribed at the age of 13–13.5 years old, after effect of exogenous androgens on precipitation of closing of bone growth plate is insignificant). It is necessary supervision of endocrinologist, control of rate of height, of rate of sexual maturation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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# Pituitary – Basic

## P1331

**A recessive mutation resulting in a disabling amino acid substitution (T194R) in the LHX3 homeodomain causes combined pituitary hormone deficiency**

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LHX3, a LIM-homeodomain transcription factor, plays a critical role in pituitary and nervous system development. Mutations in the LHX3 gene are associated with combined pituitary hormone deficiency disease (CPHD). Two female siblings with neonatal complications were diagnosed with CPHD resulting from a novel, recessive mutation in LHX3. The index patient presented with classical symptoms of CPHD, featuring deficiencies of GH, LH, FSH, prolactin, TSH and later onset ACTH deficiency. Other complications included a hypoplastic anterior pituitary, respiratory distress, hearing impairment, hypoglycaemia and limited neck rotation. Upon sequencing of the LHX3 gene, it was found that the patients harboured a homozygous C→G transversion mutation in the fourth coding exon of the LHX3 gene, resulting in a change in amino acid 194 from threonine to arginine (T194R) in the DNA-binding homeodomain. Computer modeling predicted that the homeodomain structure resulting from the T194R mutation would be altered. Consistent with this prediction, biochemical analyses demonstrated that the T194 protein did not bind tested LHX3 DNA recognition sites nor did it activate the α glycoprotein subunit and prolactin target genes. This study describes a novel mutation altering a critical residue of the LHX3 protein and thus expands our understanding of the phenotypic features, molecular mechanism, and developmental course associated with mutations in the LHX3 gene.

Declaration of interest

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## P1332

**Evidence of the involvement of gherline and obestatin in the regulation of GH secretion during pubertal development in boys**

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The oxyntic mucosa of digestive tract secretes two peptide hormones, ghrelin and obestatin, which are derived from the same gene transcript and play an important role in food intake, metabolic responses and growth and reproduction. Ghrelin and obestatin bind GH secretagogue receptor GHSR1α and G-protein coupled receptor GPR39 respectively, in the pituitary. A limited data in the literature demonstrate that levels of ghrelin peak during first 2 years of life and thereafter decline until the end of puberty, whereas the sensitivity of GHSR increases during puberty since hexareline, a synthetic GHSR analogue, induces greater GH responses in pubertal boys compared to prepubertal boys. Though a role of obestatin in augmentation of GH secretion *in vitro* has recently been observed, the involvement of obestatin in GH secretion in vivo especially at puberty is 1. A possible association of ghrelin and obestatin with increase in GH secretion during puberty was aimed at. Blood samples were collected from 10 to 20 years old 407 boys and plasma concentrations of T, GH, ghrelin and obestatin were determined using specific ELISA. Data were analyzed using Student's *t* test, ANOVA and Pearson correlation. The concentrations of T remarkably increased during mid puberty. Ghrelin showed 1st peak at 14 year, followed by a peak of GH at 15 years and a 2nd peak of ghrelin at 17 years. Obestatin exhibited an abrupt increase at early puberty, gradually augmented during mid puberty and peaked at 17 years. T, ghrelin and obestatin were positively correlated with GH secretion at early and mid puberty. GH, T, ghrelin and obestatin were positively correlated with linear growth velocity at early and mid puberty. The present study demonstrates that the secretion of ghrelin and obestatin increases during puberty and may play a role in increase in GH secretion at puberty.

Declaration of interest
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## P1333

**T3 and T4 rapidly regulate the TSH, FSH and LH $\alpha$ subunit gene expression at posttranscriptional level**

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Several studies demonstrated that the thyroid hormones (TH) downregulate the transcriptional rate of beta and alpha TSH subunits. Recent data showed that $T_3$ also acts posttranscriptionally reducing the polyadenylation and translational rate of the beta TSH mRNA, when acutely administered to thyroidectomized (Tx) rats. This study aimed to investigate whether similar effects occur for the alpha subunit synthesis after acute administration of $T_3$ or $T_4$, which would also influence the synthesis of LH and FSH. For this, euthyroid (sham-operated, SO) and Tx rats were treated with physiological or supraphysiological doses of $T_3$, $T_4$ or saline, and killed by decapitation, 30 min thereafter. Some Tx rats were also subjected to $T_3$ treatment with supraphysiological dose for 5 days. The pituitaries were removed for evaluation of the fraction of alpha subunit mRNA associated with ribosomes, the total mRNA content and its degree of polyadenylation and the total protein content by RT/qPCR, RACE-PAT assay and western blotting, respectively. It was shown in Tx group an increased alpha mRNA content, without alterations on the polyadenylation degree, and reduced protein content compared with SO group. $T_3$ administration for 30 min or 5 days reduced the content of mRNA associated with ribosomes, and increased the total protein content; $T_4$ treatment for 30 min decreased the total mRNA content, and increased the poly(A) tail length and the total protein content in pituitary. Thus, both $T_3$ and $T_4$ rapidly regulate the posttranscriptional steps of alpha subunit synthesis modifying the content, stability and translational rate of its mRNA, as well as its secretion, which might affect TSH, FSH and LH economy.
Declaration of interest
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## P1334

**Development of novel AIP (Aryl hydrocarbon receptor-interacting protein) gene study models using the fruitfly and the zebrafish**

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Introduction
Mutations in AIP have been associated with the familial isolated pituitary adenoma (FIPA) syndrome, an autosomal dominant disease with incomplete penetrance characterised by pituitary adenomas in a familial setting. However, the mechanisms by which AIP inactivation promotes pituitary tumorigenesis are unknown. Studies of AIP in model organisms, such as the fruitfly, harbouring the AIP orthologue CG1847, as well as the zebrafish, are theoretically promising models in discovering possible functions of the AIP gene and understanding pituitary tumorigenesis.
Aim
To establish AIP study models in fruitfly and zebrafish.
Methods
We used RT-PCR and *in-situ* hybridization (ISH) to study the expression of AIP orthologues in fruitfly and zebrafish, and we have generated transgenic AIP-knockdown fruitfly strains by RNA interference using GAL4/UAS-system.
Results
FlyBase RNA ISH showed that CG1847 gene is expressed in fruitfly from early stages (stage I). We generated several fruitfly crosses, using two different GAL4 drivers (actin and elav, expressed in all cells or all neurons respectively) and two different CG1847 RNAi lines (same RNAi construct on ChrII and ChrIII respectively). When actin-GAL4 flies were crossed with CG1847-R2 RNAi line no viable adult offspring were observed suggesting that complete AIP-knockdown is lethal. When using the nervous system-specific elav-GAL4driver, we were able to confirm CG1847 knockdown in the viable offsprings. Wing-targeted knockdown of AIP resulted in specific wing abnormalities. In zebrafish we were able to prove AIP expression from very early stages of development with a wide-spread expression profile.
Conclusions
We have shown that equivalents of mammalian AIP are present and well conserved in the fruitfly and the zebrafish. We have demonstrated that complete AIP deficiency is lethal in the fruitfly, similar to results in the AIP-KO mice. We suggest that these model organisms are useful to identify AIP protein interactions and to explore possible mechanisms of tumour suppression.
Declaration of interest
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## P1335

**Multiple enhancers regulate expression of the human LHX3 gene in the developing pituitary**

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LHX3 is a LIM homeodomain transcription factor necessary for proper development of the pituitary and central nervous system. Patients with mutations in coding regions of the LHX3 gene have complex syndromes including combined pituitary hormone deficiency and nervous system defects resulting in symptoms such as dwarfism, thyroid insufficiency, infertility, and developmental delay. Although previous studies from our group and others have identified promoter and intronic elements of LHX3 that are important for basal gene expression *in vitro*, the mechanisms by which the LHX3 gene is regulated *in vivo* were not known. Using transgenic mouse models and bioinformatic approaches, we have mapped conserved enhancer and repressor regions that direct tissue-specific expression to the pituitary gland and spinal cord in a pattern consistent with endogenous expression. Several transferable cis elements can individually guide nervous system expression; however, a 180 bp minimal enhancer is alone sufficient to confer specific expression in the developing pituitary. Within this sequence, tandem binding sites recognized by the ISL1 LIM homedomain protein are essential for enhancer activity in the pituitary and spine and a PITX1 bicoid class homeodomain element is required for spatial patterning in the developing pituitary. This study establishes ISL1 as a novel transcriptional regulator of LHX3 and describes a mechanism for regulation by PITX1. Ongoing research focuses on lineage tracing experiments using an enhancer-directed Cre recombinase mouse model. This approach will identify what differentiated cells and their progeny originated from cells with enhancer-directed expression. Characterization of novel genetic defects will facilitate patient treatment and enable genetic counseling. Sources of Research Support: NIH HD42024 to SJR.
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## P1336

**Role of a preformed signalosome in GnRH-stimulated FAK and paxillin phosphorylation by ERK at focal adhesions: possible role in gonadotropes migration**

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Introduction
We have recently described a preformed signalosome associated with the GnRH receptor (GnRHR), which undergoes dynamic rearrangement upon stimulation with GnRH. This signaling complex included c-Src, focal adhesion kinase (FAK), paxillin, vinculin, tubulin, caveolin-1, protein kinase C (PKC) δ, PKCε, PKCα, Ras, kinase suppressor of Ras-1 (KSR), MAPK kinase (MEK) 1/2, ERK1/2 and

the GnRHR. To sort out the role of the signalosomes associated with the GnRHR we investigated the activation of ERK1/2 and its substrates in the complex. First, caveolin-1, FAK, vinculin, and paxillin were phosphorylated on Tyr residues by the GnRH-activated c-Src. Then, GnRH activated ERK1/2 in the complex in a c-Src-dependent manner, and the activated ERK1/2 subsequently phosphorylated FAK and paxillin.
Methods
LβT2 gonadotrope cells were transfected with GnRHR-mCherry and paxillin-GFP, or GnRHR-mCherry and ERK-GFP, stimulated with GnRH and visualized in live imaging.
Results
Addition of GnRH to LβT2 gonadotrope cells transfected with GnRHR-mCherry and ERK-GFP resulted in bleb formation and ERK accumulation in the blebs. Furthermore, the cells seem to be migrating after GnRH stimulation. Also, addition of GnRH to LβT2 gonadotrope cells transfected with GnRHR-mCherry and paxillin-GFP resulted in enrichment of paxillin in focal adhesions in the newly formed blebs.
Conclusion
We therefore propose that the role of the signalosome is to sequester a cytosolic pool of activated ERK1/2 to phosphorylate FAK and paxillin at focal adhesions apparently to mediate gonadotropes migration.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1337

### T3 acts posttranscriptionally reducing the poly(a) tail length and the translational rate of alpha and beta TSH mRNA in TαT1 cells

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Recent data have shown that, in parallel to its transcriptional action down-regulating TSH synthesis, triiodothyronine ($T_3$) also acts on posttranscriptional steps of beta TSH synthesis, reducing the polyadenylation and translational rate of the beta TSH mRNA, when acutely administered to thyroidectomized rats. This study aimed to investigate whether these effects also occur in TalphaT1 cells, and if they are dependent on gene transcription, which would characterize novel actions of $T_3$ on its own regulatory axis. Cells were seeded on matrigel-coated plates, plated in DMEM high glucose and firstly divided in two groups: control (C) containing 10% FBS and hypothyroid (H) – cells plated with 10% FBS depleted of TH by treatment with AG1X-8 resin for 48 h. After this, 5,6-dichloro-1-β-D-ribobenzimidazole (DRB-50 mM) was administered to inhibit the gene transcription for 2 h, followed by the $T_3$-treatment in the doses of $10^{-8}$M or $10^{-10}$M for 30 min or $10^{-7}$M for 4 h. The total RNA and the fraction correspondent to the polisomes were extracted for investigation of mRNA content by RT/qPCR and polyadenylation degree by RACE-PAT assay. The results obtained showed that the $T_3$ rapidly reduces the total content of both subunits of TSH mRNA, and that this reduction is lost by DRB treatment only for alpha TSH mRNA subunit. Moreover it was shown that the $T_3$ ($10^{-10}$M) specifically decreased the poly(A) tail length of beta TSH mRNA, and diminished the content of mRNA of both subunits associated with ribosome, which indicates that the translational rate of TSH synthesis was reduced. These data led us to conclude that the $T_3$ down-regulates TSH synthesis in TalphaT1 cells acting in several posttranscriptional steps of gene expression.
Declaration of interest
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## P1338

### Estradiol induces the rapid trafficking of membrane-associated ERα isoforms in pituitary cells

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In the pituitary, estrogens regulate several cell functions via the activation of membrane-associated estrogen receptors (mERs). We have previously reported that both 17β-estradiol ($E_2$) and membrane-impermeable E2-BSA induce rapid apoptotic actions in anterior pituitary (AP) cells, lactotropes and somatotropes through the activation of mERs.
In the present study, we investigated the involvement of mERα in the apoptotic action of E2 in lactotropes. We also studied the expression of mERα variants in AP cells during the estrous cycle and the regulation of this receptor expression by E2.
Cultured anterior pituitary cells from ovariectomized (OVX) Wistar rats were incubated with E2-BSA ($10^{-9}$ M) in the presence of an ERα selective antagonist, MPP ($10^{-7}$ M), for 120 min. Apoptotic lactotropes were identified by TUNEL and immunocytochemistry for prolactin. The expression of mERα isoforms was studied in AP cells from rats at proestrus (P) or diestrus I (D) by surface biotinylation and Western Blot. The expression of mERα was also studied in AP cells from OVX rats incubated with E2 ($10^{-8}$ M) for 0–120 min.
E2-BSA increased the percentage of TUNEL-positive lactotropes ($P<0.001$, $\chi^2$) but failed to induce apoptosis in lactotropes coincubated with MPP. Three mERα isoforms of 66, 39 and 20–24 kDa were detected in AP cells. mERα66 expression was higher at $P$ vs D ($P<0.05$), whereas mERα20-24 was lower ($P<0.05$). E2 increased the expression of mERα66 and mERα39 ($P<0.05$, ANOVA) but not of mERα20-24 at 30 min. Intracellular ERα isoforms remained unchanged during the estrous cycle or after E2 incubation.
Our results suggest that E2 induces the rapid trafficking of mERα isoforms to the plasma membrane of AP cells. At proestrus, the high circulating E2 levels could participate in the transient expression of mERα66, which may be involved in the apoptotic action of E2 in the pituitary at this stage of the estrous cycle.
Declaration of interest
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## P1339

### Pasireotide (SOM230) prevents sulfonylurea-induced hypoglycemia in fasted male rats

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Introduction
Severe hypoglycemia induced by sulfonylurea overdose is a result of insulin hypersecretion. Pasireotide (SOM230) is a multireceptor-targeted somatostatin analogue with high affinity for somatostatin receptor subtypes $sstr_{1,2,3}$ and especially $sstr_5$, which are expressed on rat and human β cells; pasireotide has been shown to inhibit insulin secretion in both species. The aim of these studies was to investigate the effect of pasireotide on glyburide (glibenclamide)-induced hypoglycemia in fasted male Lewis rats.
Methods
Adult male Lewis rats ($n=6$ per group) were fasted for 24 h and received glyburide 30 mg/kg by oral gavage alone (group 1) or in combination with pasireotide 10 or 30 μg/kg by s.c. injection (groups 2 and 3, respectively). Blood samples were taken by sublingual bleeding 1 h before treatment and 1, 3 and 6 h after treatment. Glucose levels were measured using Accu-check.
Results
Overall, 24 h food restriction significantly reduced plasma glucose levels from $8.0\pm0.4$ mmol/l on day $-1$ to $6.4\pm0.3$, $6.1\pm0.4$ and $6.1\pm0.2$ mmol/l at 1, 3 and 6 h, respectively, on day 1. Following glyburide application in group 1, plasma glucose levels were further reduced to $5.5\pm0.2$, $4.2\pm0.2$ and $3.5\pm0.4$ mmol/l at 1, 3 and 6 h after application. Co-application of glyburide with pasireotide 10 μg/kg in group 2 resulted in glucose plasma levels of $7.0\pm0.4$, $6.2\pm0.4$ and $6.5\pm0.5$ mmol/l at 1, 3 and 6 h after application. In group 3, co-application of glyburide with pasireotide 30 μg/kg resulted in plasma glucose values that were close to those seen in non-fasted animals (ranging between 7.0 and 7.6 mmol/l) 1–6 h after the application.
Conclusion
In rats with glyburide-induced hypoglycemia, low-dose pasireotide increased plasma glucose levels to those seen in fasted rats, and high-dose pasireotide resulted in plasma glucose values similar to those seen in non-fasted rats. These preclinical results suggest that pasireotide may have a role in managing patients with severe hypoglycemia induced by excessive insulin levels, such as those due to glyburide overdose.
Declaration of interest
I fully declare a conflict of interest. Details below.
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## P1340

**mTOR inhibitor Torin1 induces antiproliferative effects in MtT/E cell line and human pituitary tumors**

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As for many tumor types, it has been shown that the Akt pathway is overexpressed and activated in human pituitary tumors. Thus, pituitary tumors may be sensitive to the anti-proliferative effects of mTOR inhibitors. However, part of non-functioning pituitary tumors are rapamycin-resistant. Torin1, second-generation ATP-competitive mTOR kinase inhibitor, suppresses both mTORC1 and mTORC2 complexes. To evaluate the *in vitro* effects of the mTOR inhibitor Torin1 on pituitary cells, a rat non-secreting pituitary tumor cell line, MtT/E, and human non-functioning pituitary adenoma (NFPA) cells were used.
Treatment of MtT/E cells with Torin1 induced a significant dose- and time-dependent decrease of cell viability and cell number. Incubation of cells from four NFPAs with Torin1 significantly reduced the number of viable cells by 25–45%. The anti-proliferative effects of Torin1 on pituitary tumor cells were found to be mediated by G0/G1 cell cycle arrest associated with cyclin D1 and cyclin D3 suppression, apoptosis reflected by increased fraction of cleaved caspase and subG1 events, and autophagy tested with autophagy markers, LC3 and p62. Torin1 increased p53 levels and p53 inhibitor, pifithrin-alpha, blocked the effects of Torin1 on both cleaved caspase and p62 expression. Expression of phosphorylated-p70S6K and phosphorylated-Akt was significantly reduced by Torin1. Interestingly, the protein expression of the negative regulator of PI3K, the PTEN phosphatase, was significantly decreased by Torin1 in MtT/E cells.
Our results show that Torin1 potently inhibits pituitary cell proliferation suggesting that mTOR inhibitors may be a promising antiproliferative therapy for pituitary adenomas. This therapeutic manipulation may have beneficial effects particularly for patients harboring invasive pituitary tumors unresponsive to current treatments.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1341

**Effect of estrogen and FGF-2 interaction on lactotroph cell proliferation mediated by membrane-initiated signaling**

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Estradiol ($E_2$) may directly interact with growth factors stimulating cell proliferation and differentiation. Previously, we have demonstrated that $E_2$ through membrane estrogen receptor α (mERα) modulates the lactotroph cell population which exhibit noticeable changes in the pituitary gland. The aim of this study was to analyze the mERα contribution on lactotroph cell proliferation in response to $E_2$ and FGF-2 interaction evaluating the pathway involved in this effect. Pituitary cell cultures from Wistar female rats were treated with 10 nM of $E_2$, $E_2$ membrane-impermeable conjugated ($E_2$-BSA), PPT (ERα agonist), DPN (ERβ antagonist) alone or combined with FGF-2 (0.6 nM) for 30 min or 4 h. The lactotroph cell number was quantified by BrdU/PRL and PKCα and ε; phosphorylated (p) and total Akt or ERK1/2, and β-actin protein expression by western blot. In addition, MEK (PD98059) and ER (ICI 182780) inhibitors were used. The subcellular translocation of PKCα and ε was visualized by confocal microscopy. FGF receptors were identified by immuno-electron-microscopy. Statistical analysis: ANOVA-Tukey. In serum free condition, $E_2$, $E_2$-BSA, PPT, DPN or FGF-2 alone did not modify the lactotroph cell number, whereas $E_2$/FGF-2, $E_2$-BSA/FGF-2 or PPT/FGF-2 co-incubation significantly increased the mitogenic activity of these cells after 30 min or 4 h, being this effect blocked by ICI 182780 or PD98059 pre-treatment. The subcellular distribution of PKCα and pAkt did not exhibit any significant variation after treatments. However, the stimulation for 30 min with the combined treatments described above induced a remarkable increase of pERK1/2 and PKCε translocation to lactotroph plasma membrane that was accompanied with a significant increase of PKCε expression after 4 h. These findings show a cooperative effect of $E_2$ and FGF-2 on lactotroph proliferation triggered by signaling initiated at the plasma membrane with the contribution of mER α and the activation of PKCε/ERK1/2. This regulatory effect could participate in the homeostasis of pituitary cell populations.

Declaration of interest
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## P1342

**Importance of topoisomerase IIα and Ki-67 as markers of invasiveness and predictors of recurrence in pituitary tumours**

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Introduction
During clinical observation the behaviour of pituitary tumours is frequently unpredictable. The work was aimed at evaluating Ki-67 and topoisomerase II alpha indices as markers of tumour invasiveness and prognostic factors for postoperative course of disease.
Material and methods
The Ki-67 and topoisomerase IIα (Topo IIα) indices were determined by immunochemistry in specimens excised from neurosurgically removed pituitary tumours. In 48 examined patients (29 females and 19 males, mean age $46.5 \pm 18.9$ years) who underwent pituitary tumour surgery, micro- and macroadenoma occurred in 13 and 35 cases, respectively.
Results
The Ki-67 index, unlike the Topo IIα index, was significantly related to tumour size category (median Ki-67 value 0.1 vs 1.8 in micro- and macroadenoma, respectively, $P<0.005$) while both indices were correlated with tumour size (for Ki-67: $r=0.4$, $P<0.005$; for Topo IIα $r=0.3$, $P<0.05$). Local tumour invasiveness was significantly related to both Ki-67 and Topo IIα indices. Postsurgery tumour recurrence (confirmed by MRI) was significantly related to Topo IIα ($P<0.01$) and not related to Ki-67 index ($P>0.05$). No correlation between Ki-67 and Topo IIα indices, nor between either index and patient age was found.
Conclusion
Ki-67 and topoisomerase IIα expression, as determined in pituitary tumour specimens, is related to the tumour size and invasiveness. Topoisomerase IIα appears to be a more valuable predictor of further disease course than Ki-67 (specifically for local recurrence) and may be helpful in planning postoperative therapy.

Declaration of interest
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## P1343

**Aryl hydrocarbon receptor interacting protein in somatotroph adenomas: a molecular target for somatostatin analogues?**

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The aryl hydrocarbon receptor interacting protein (AIP) gene is abundantly expressed in normal somatotrophs. Somatotropinomas in patients with germline AIP mutations (AIPmut) are typically more aggressive than non-AIPmut adenomas and associated with higher GH/IGF1 secretion. AIP downregulation may also occur in sporadic somatotropinomas, especially in invasive tumours. We wished to evaluate the impact of somatostatin analogues (SSA) pre-treatment on AIP expression in sporadic somatotropinomas.
Patients and methods
46 sporadic somatotropinomas have been operated on in 44 Italian patients, including 20 cases treated by SSA pre-operatively. Pre-operative clinical, radiological and hormonal data were collected. AIP sequencing was performed in most cases and AIP expression was determined by immunohistochemistry (AIP–IHC). $\chi^2$ and non-parametric tests were used for statistical analysis.

Results
A single AIP mutation was identified and 17 macroadenomas displayed a faint or negative AIP staining (36.9%). Low AIP–IHC was significantly associated with the presence of suprasellar extension ($P=0.003$), cavernous sinus invasion ($P=0.024$), higher MIB1 proliferation indexes ($P=0.026$), with a trend in higher pre-operative GH levels ($P=0.066$) and no significant difference in PRL/IGF1 levels. Pre-operative SSA treatment was associated with a significantly higher AIP expression, only three pre-treated adenomas displaying low AIP–IHC (15 vs 53.6% in untreated cases, $P=0.007$): all were male GH/PRL-secreting adenomas, poorly responsive to SSA, including the AIPmut case. Introducing SSA pre-treatment as a covariant in statistical analysis, low AIP was confirmed to be associated with tumour characteristics but not with GH secretion. Significant correlations between tumour characteristics and AIP–IHC were also confirmed in untreated tumours taken as a subgroup, but not in treated tumours.
Conclusion
While confirming that AIP downregulation is associated with tumour aggressiveness in sporadic somatropinomas, regardless of AIP mutations, these data also suggest a role for AIP in SSA signalling in these tumors. This could contribute to the frequent pharmacological resistance of AIPmut somatotropinomas.
Declaration of interest
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## P1344

**Estradiol regulates the expression of c-FLIP in anterior pituitary cells**

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Our previous results demonstrate that estrogens increase the expression of death receptors TNFRI and Fas in the anterior pituitary and sensitize anterior pituitary cells to pro-apoptotic factors. c-FLIP is a protein that interacts with procaspases 8 and 10 modulating death receptor activity. In normal tissues, two isoforms of c-FLIP have been identified, the short isoform (c-FLIPs) and the long one (c-FLIPL). c-FLIPs has an anti-apoptotic action whereas c-FLIPL has either anti-apoptotic, pro-apoptotic or proliferative actions depending on the tissue and its level of expression. To investigate whether c-FLIP is involved in the apoptosis of anterior pituitary cells, we evaluated the action of estradiol on c-FLIP expression in anterior pituitary cells from ovariectomized (OVX) Wistar rats and in the somatolactotrope cell line GH3. Both c-FLIPL and c-FLIPs, identified by Western blot, were detected in the anterior pituitary gland and GH3 cells. In cultured anterior pituitary cell from OVX rats, 17β-estradiol ($E_2$, 10-9M) increased the expression of c-FLIPL (control: $1.50\pm0.42$, $E_2$: $1.91\pm0.59$, $P<0.05$, *t*-test) but did not modify the expression of c-FLIPs (control: $0.30\pm0.05$; $E_2$: $0.37\pm0.08$). Also, the administration of $E_2$ (ip, 20 mg/100 g body weight) to OVX female rats increased the expression of c-FLIPL in the anterior pituitary (OVX: $5.7\pm0.40$, OVX+$E_2$: $6.8\pm0.41$, $P<0.05$, t-test) but did not modify the expression of c-FLIPs (OVX: $1.08\pm0.10$; OVX+$E_2$: $1.18\pm0.05$). The effect of $E_2$ on c-FLIPL expression was not observed in GH3 cells (control: $1.1\pm0.29$; $E_2$: $0.9\pm0.13$)
These results indicate that estrogens regulate the long isoform expression of cFLIP in the anterior pituitary suggesting that this action could be involved in the pro-apoptotic effect of estrogens in this gland.
Declaration of interest
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## P1345

**Increased interleukin 22 circulating levels in patients with tumors involving the hypothalamic-pituitary region**

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Introduction
Cytokines have been suggested to be involved in modulating anti-tumor responses and promoting tumor growth. Some studies have indicated that cytokines could also be involved in the pathogenesis of pituitary tumors. Th17 cells, characterized by expression of several cytokines as interleukin 22 (IL 22), might have a role in tumor immune-surveillance. IL-22 is a cytokine involved in the modulation of tissue responses during inflammation and, through activation of Stat3-signaling cascades, in the proliferative and anti-apoptotic pathways. Moreover, IL 22 expression requires the ligand-dependent transcription factor aryl hydrocarbon receptor. The aim of our study was to evaluate serum IL 22 levels in patients with tumors involving hypothalamic-pituitary region.
Design
Serum IL 22 levels were measured by a quantitative enzyme immunoassay technique in 38 consecutive patients (27 with secreting or non functioning pituitary macroadenomas; six with craniopharyngiomas; five with meningiomas or disgerminomas; 26 M, mean age $50.3\pm17.7$ years) and in 37 healthy subjects (C: 23 M, mean age $44.9\pm11.72$ years). Other diseases, causing increased IL 22 levels, were excluded in all cases.
Results
Serum Il 22 levels were significantly higher in patients overall than in C ($28.6\pm13.8$ vs $7.2\pm5.7$ pg/ml, $P<0.001$). No differences were observed between patients with pituitary macroadenomas and those with other brain tumors. No correlation was found between serum IL 22 levels and age, gender and positive family history of autoimmune disease. Multivariate analysis did not demonstrate significant association of increased IL 22 levels with hormone hypersecretion (in pituitary adenomas), hypopituitarism or concomitant hormone replacement.
Conclusion
Patients with pituitary tumors show increased serum IL 22 levels, suggesting its hypothetical role in tumor proliferation. Nevertheless, further studies need to elucidate the involvement of this intriguing cytokine in pituitary tumorigenesis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1346

**Expression of Ras related proteins in ES cell-derived AVP secreting cells**

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Rab proteins (Rab GTPase) have been implicated in the trafficking and exocytosis of secretary vesicle and play a central role in the secretion of hormones or neurotransmitters. However, the involvement of Rabs and Rab effectors in the regulation of AVP secretion is still unknown. Embryonic stem (ES) cells are pluripotent cells that differentiate into all cell types. Recently, it has been reported that when dissociated ES cells are quickly reaggregeted in low cell-adhesion culture wells and cultured as floating aggregates in growth fator free chemically defined medium (SFEBq/gfCDM culture), ES cells differentiate into rostral hypothalamic progenitor cells including vasopressin (AVP) secreting cells. The aim of this study is to examine the involvement of Rab proteins in SFEBq/gfCDM cultured ES-cell aggregates (SgESa). To examine the expression of Rabs in SgESa, whole cell lysates from SgESa cultured for day 28 were subjected into SDS-PAGE and immunoblotting was performed. We confirmed that AVP was expressed in the SgESa and found that Rab3a, Rab27a, and Rabphilin3a were expressed in the SgESa. Next, AVP release upon high potassium and osmolality stimulation in SgESa on day 28 was analyzed. AVP concentrations in conditioned media were higher in high potassium- or osmolality- stimulated SgESa (incubated with high K+ artificial cerebrospinal fluid (aCSF) or high mannitol aCSF respectively) compared to control (incubated with aCSF). These results confirmed that ES cell-derived AVP secreting cells (ES–AVP cells) were successfully induced. Immunocytochemical analysis of ES–AVP cells using confocal laser scanning microscopy showed that rabphilin3a was mainly distributed in the subplasma membrane region, and partially colocalized with copeptin (precursor of AVP). After the high potassium and osmotic stimulation, the distribution of copeptin colocalized with rabphilin3a seemed to be enriched in plasma membrane region. Our results revealed that Rab proteins and Rab effectors may be involved in AVP release in ES-AVP cells.
Declaration of interest
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## P1347

### Inhibition of methylation stimulates *POMC* expression and ACTH secretion in human pituitary ACTH-secreting tumors

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DNA methylation has recently been shown to play a role in postnatal as well as developmental gene regulation. Transfer and removal of methyl groups from CpG islands in the promoter region of specific genes has been associated with gene silencing or activation, respectively. *POMC* is known to present several methylation sites and demethylation of these sites has been shown to be linked to *POMC* expression by neuroendocrine tumors outside the pituitary.

Aim

Of the present study was to evaluate whether inhibition of methylation by DNA-metyltransferases (DNMTs) modulates *POMC* expression and ACTH secretion by pituitary corticotroph tumors.

Methods

Thirteen human pituitary ACTH-secreting tumors were collected at surgery and established in culture. Cells were incubated with 1 μM Aza-2′-deoxycytidine (AZA), an inhibitor of DNMTs, with or without 10 nM CRH. After 24 and 48 h, medium was collected for measurement of ACTH by IRMA and RNA extracted for evaluation of *POMC* expression by real-time PCR. Data was normalized to control wells (DMEM and BSA alone) and analyzed by Wilcoxon's test.

Results

Incubation with AZA for 24 h increased *POMC* expression and ACTH secretion compared to control wells; lesser effects were observed after 48 h incubation. AZA did not appear to exert an additional stimulatory effect on *POMC* and ACTH stimulated by CRH.

Conclusions

The present study shows that inhibition of methylation increases *POMC* gene expression and ACTH secretion in human pituitary ACTH-secreting tumors. This can be taken to indicate that methylation plays a role in the regulation of gene expression in tumoral corticotrophs and paves the way to further study on epigenetic changes in Cushing's disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1**

| **ACTH** (% control) | **24 h** | **48 h** |
|---|---|---|
| Control | 100.0±3.2 | 100.0±2.9 |
| 10 nM CRH | 690.4±227.5* | 525.9±154.4* |
| 1 μM AZA | 164.9±32.8* | 101.3±6.5 |
| AZA+CRH | 691.7±214.4* | 523.0±151.9* |
| *POMC vs βGLUC* | | |
| Control | 1.00±020 | 1.00±0.17 |
| 10 nM CRH | 1.43±0.15* | 2.12±0.43* |
| 1 μM AZA | 1.46±0.13* | 1.43±0.26* |
| AZA+CRH | 1.90.22* | 2.55±0.49* |

*$P<0.05$ vs control.

## P1348

### Effect of valproate on inositol 1,4,5 triphosphate and protein kinase C pathways activity in anterior pituitary cells of female rat

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Introduction

VPA, antiepileptic drug, may cause a number of side effects including reproductive deficits in humans. Previously we found that VPA (1 μM) may suppress GnRH-stimulated release of LH from rat anterior pituitary cells *in vitro* without affecting its basal secretion (*Neuroendocrinol Lett* 2011 **32** 101–106). However mechanism of VPA suppressive activity is still unknown. Since pharmacological effects of VPA require GnRH-R activation, the mechanism of its action might occur via modulation of $IP_3$/PKC pathway activity.

Aim

The aim of this study was to evaluate: firstly – an effect of VPA on phorbol ester - PKC activator (TPA) -induced LH release, secondly – an effect of VPA treatment on $IP_3$ synthesis in the primary culture of female rat anterior pituitary cells.

Material and methods

Dispersed cells ($5\times10^5$/ml) were incubated for 3 h with TPA (100 nM), VPA (10 nM–10 μM) or both (TPA+VPA). As controls, incubations with GnRH (100 nM) as well as GnRH+VPA (1–10 μM) were prepared. In the second experiment cells were preincubated (37°C) for 24 h with 1 μCi myo-[$^3$ H]-inositol, then for 30 min with 10 mM LiCl and finally for 3 h in the presence of GnRH (100 nM), VPA (100 nM, 1 μM) or both (100 nM GnRH+100 nM or 1 μM VPA). Medium rLH concentration were estimated by RIA method, cellular $IP_3$ concentrations were estimated by ion-exchange chromatography analysis.

Results

VPA administration did not affect TPA-induced LH release from anterior pituitary cells at any VPA dose tested. VPA treatment resulted in a dose independent significant increase of $IP_3$ cellular concentrations. Moreover, VPA-induced $IP_3$ synthesis reached the same level as was detected after GnRH stimulation.

Conclusions

Obtained data suggest that the inhibitory effect of VPA on GnRH-stimulated LH release is not exerted via down-regulation of $IP_3$/PKC pathway activity.

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Declaration of interest

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## P1349

### The role of familial pituitary adenoma gene, aryl hydrocarbon receptor-interacting protein, in the proliferative and invasive activity of a malignant pancreatic cell line

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Background

Heterozygote mutations in AIP predispose carriers to young-onset pituitary adenomas, most often somatotroph or lactotroph adenomas. No other tumour type has been consistently detected in AIP mutation positive families, despite the fact that AIP is ubiquitously expressed. Current clinical and experimental data suggest that AIP is a tumour suppressor gene.

Aims

To investigate the tumour suppressor role of AIP in a non-pituitary malignant cell line by exploring whether silencing of AIP would affect the invasive and proliferative properties of a highly invasive human pancreatic cancer cell line BxPC3.

Methods

Functional assays (invasion, MTS and colony formation) were carried out after silencing of AIP in BxPC3 using siRNA targeting human AIP. Invasion assays were performed using BioCoat-Matrigel Invasion Chambers. MTS assays were used to measure the metabolic activity at 24, 48, 72, 96 and 120 h after transfection. Colony formation assays were also performed to detect changes in proliferation rates of these cells.

Results

Silencing endogenous AIP in BxPC3 cells significantly increased the number of invading cells compared to non-targeting siRNA controls ($P<0.04$). MTS assays showed AIP silencing caused an increase in metabolic activity compared to non-targeting siRNA controls at 72, 96 and 120 h ($P<0.01$ for all). Furthermore, knockdown of AIP led to an increase in the number of colonies compared to siRNA controls ($P<0.01$).

Conclusion

These results indicate that lack of AIP increased the proliferative and invasive behaviour of this non-pituitary malignant cell line, suggesting an inhibitory role in cell invasion and proliferation and supporting the function of AIP as a tumour suppressor. These data suggest that the tumour suppressor effect of AIP is not limited to pituitary cells and can counteract aggressive behaviour of a cancer cell line. This raises questions as to why AIP mutations are solely associated with pituitary adenomas and no other tumour types.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1350

### Combined intra- and inter-individual variability in macroprolactinaemia

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Objective
Macroprolactinaemia is the phenomenon of forming immunoglobulins (Igs) against prolactin (PRL). The Ig–PRL complex has an increased half-life resulting in elevated levels of immunoreactivity in most PRL immunoassays without having any significance. Identifying macroprolactinaemia-induced hyperprolactinaemia is essential in order to prevent unnecessary testing and treatment. This study is to establish the combined intra- and inter-individual variability in macroprolactinaemia and to verify if multiple measurements to diagnose macroprolactinaemia are justified.
Method
Macroprolactinaemia was determined by size-exclusion chromatography (SEC) combined with the AutoDelfia PRL immunoassay. The percentages of monomeric PRL (%PRL), dimeric PRL (%BIG–PRL) and Ig–PRL complex (%BIG–BIG–PRL) were calculated. Abnormal macroprolactinaemia was diagnosed if the measured %PRL $>$ reference %PRL. From a group of 298 patients suspected for macroprolactinaemia and subjected to SEC combined with immunological testing, 17 patients, who had undergone multiple testing, were selected; 15 patients had been tested twice and two patients three times. The combined intra- and inter-individual variability was calculated based on inter-individual coefficient of variance (CV) of the relative intra-individual change (RIC) according to the model of running means between two time points: $RIC = (abs(A-B))/((A+B)/2) \times 100\%$ in which A is the percentage of the respective PRL form at one time point and B that of the subsequent time point.
Results
CVs of %PRL, %BIG–PRL and %BIG–BIG–PRL were 21, 32 and 27% respectively. The period between the time points was not standardized, varied significantly and was $16 \pm 19$ months (mean $\pm$ s.d.; $n = 19$). The presence of absence of abnormal macroprolactinaemia proved to be unchanged in subsequent samples; 12 patients suffered from abnormal macroprolactinaemia of which nine patients had normoprolactinaemia after correction.
Conclusion
The combined intra- and inter-individual variability's of the PRL forms were $\leq 33\%$ and the diagnosis of macroprolactinaemia was consistent during the time period evaluated. Therefore, no indications were found that justify multiple measurements to diagnose macroprolactinaemia.
Declaration of interest
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## P1351

### Reduced expression of glucocorticoid stimulated genes in large and dedifferentiated corticotroph adenomas

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Introduction
E-cadherins are found in epithelial tissue. Reduced expression in somatotroph pituitary adenomas has been demonstrated to correlate with dedifferentiated phenotype; reduced tumour size, increased invasiveness, and reduced somatostatin analog response. Recently, correlations between corticotroph tumour dedifferentiation and both E-cadherin immunostaining and reduced E-cadherin gene (*CDH1*) mRNA was demonstrated.
Glucocorticoids induces transcription of some genes, and inhibit others. Corticotroph adenomas are characterised by a relative resistance to the normal inhibitory effect of glucocorticoids on proopiomelanocortin (*POMC*) gene expression and ACTH secretion.
Our aims were to explore if glucocorticoid resistance in corticotrophs was associated with both positively and negatively regulated genes, and if the resistance correlated with level of tumour dedifferentiation.
Methods/design
Twenty patients with verified Cushing's disease or Nelson's syndrome, operated at Rikshospitalet, Oslo, were included. RT-qPCR of *CDH1*, growth arrest-specific 5 (*GAS5*), *POMC*, the reference gene *GAPDH* and the normally glucocorticoid stimulated genes *GILZ* and thioredoxin-interacting protein (*TXNIP*), as well as immunohistochemical analysis of E-cadherin, were performed. Correlations between expression of the *POMC*, *GILZ* and *TXNIP* genes in different stages of corticotroph adenomas, defined clinically (microadenomas, macroadenomas or Nelson tumours), by *CDH1* gene expression, and by E-cadherin immunoreactivity (nuclear, intermediate or membranous), were evaluated. Expression of the glucocorticoid regulated genes, *GAS5* expression and preoperative 24-h urinary cortisol excretion were investigated.
Results
*TXNIP* and *GILZ* expressions were positively correlated to *CDH1* expression, and were highest in microadenomas and in tumours with high membranous E-cadherin reactivity. In contrast, *POMC* expression was not significantly different between the groups. No correlations to *GAS5* expression or urinary cortisol were found.
Conclusions
The expression of the glucocorticoid responsive genes *POMC*, *GILZ* and *TXNIP* in corticotroph adenomas shows a remarkable variation. The pattern and mechanism of glucocorticoid resistance in corticotroph adenomas seem dynamic, and covariate with loss of epithelial phenotype associated with corticotroph tumour dedifferentiation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1352

### Effects of the combined treatment with AMPK activator and somatostatin-14 on hormone secretion and cell proliferation in cultured GH-secreting pituitary tumor cells

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AMP-activated protein kinase (AMPK) activation decreased GH release and intracellular GH storage in rat pituitary cell cultures and reduced cell proliferation in rat pituitary adenomatous cells (GH3) cultures.
We investigated the effects of the AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) as compared to somatostatin-14 on cell viability (MTT assay after 48-h) and GH secretion (over 24 h) in 16 human GH-secreting pituitary adenomas *in vitro*.
AICAR (0.4 mM) reduced cell viability in four adenomas out of fourteen (mean decrease vs control: $16 \pm 9\%$). Four adenomas were responsive to SS-14 (100 nM; mean decrease vs control: $15 \pm 6\%$). One adenoma was responsive to both somatostatin and AICAR. The effects of cotreatment with SS-14 and AICAR was investigated in eight adenomas. In two adenomas, the effects of AICAR + SS-14 did not exceed the effect of AICAR. In three adenomas which were not responsive to either AICAR or SS-14, the cotreatment was able to reduce cell viability by $27 \pm 5\%$ vs control. Three adenomas were not responsive to any treatment. As to the effects on GH secretion, nine adenomas out of fourteen were responsive to AICAR (mean decrease $35 \pm 20\%$). Ten adenomas were responsive to SS-14 (mean decrease $40 \pm 15\%$). Seven adenomas were responsive to both AICAR and SS-14. Cotreatment exceeded the effect of single treatments in three adenomas out of seven.
Overnight treatment with AICAR induced a clear-cut increase in phospho-(threonine-172) AMPK (activated AMPK) whereas SS-14 did not alter significantly AMPK phosphorylation in cultured human adenomas.
In GH3 cells, AICAR reduced the activity of p70S6 kinase, which plays an important role in cell growth. SS-14 did not affect significantly AMPK phosphorylation and p70S6K activity but it was able to enhance the inhibitory effect of AICAR on p70S6K.
Our studies suggest that AMPK activators and SS-14 may cooperate in the control of a subset of GH-secreting pituitary adenomas *in vitro*.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1353

**The relationship between Ki-67 labeling index and p53 with age, gender, number of surgeries, cure after surgery and cavernous sinus invasion in pituitary adenomas of patients with acromegaly**

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Introduction
Evidence suggests a relationship between high tumoral Ki-67 LI with persistence of tumor after surgery and lower response rate to medical therapy. Therefore, we aimed to determine the relationship between Ki-67 LI and p53 with age, gender, number of surgeries, cure after surgery and cavernous sinus invasion in pituitary adenomas of patients with acromegaly.
Methods
The paraffin-embedded tissue sections of 30 patiets with acromegaly, operated at our center during 2003–2010 via transsphenoidal route were immunostained for Ki-67 and p53, using avidin–biotin–peroxidase complex method. Clinical and radiological data were obtained through chart review. High Ki-67 LI and diffuse p53 immunoreactivity was defined as immunopositivity for $\geq 4$ and $\geq 50\%$ of cells respectively.
Results
The current study included 30 patients (17 female, 13 male), ranging in age between 23 and 65 years old. According to MRI, 3 (10%) of the tumors were microadenoma and 27 (90%) were macroadenoma. Cavernous sinus invasion was present in 14 (47%) cases. Seven (23%) of the patient were operated for at least two times and 23 (77%) were operated on once. Six (20%) patients were cured at first surgery and 24 (80%) had active disease. Fourteen tumors (47%) had Ki-LI $\geq 4\%$ and 2 (6.7%) tumors demonstrated $\geq 50\%$ p53 immunoreactivity. We could not find a relationship between age, gender, cure at first surgery and number of surgeries with high Ki-67 LI and diffuse p53 immunoreactivity ($P > 0.05$). However, KI-67 LI $\geq 4\%$ and diffuse p53 immunoreactivity were significantly higher in tumors with cavernouse sinuse invasion ($P = 0.007$ and $P = 0.03$ respectively).
Conclusion
High Ki-67LI and diffuse p53 immunoreactivity are associated with cavernouse sinuse invasion in pituitary adenomas of patients with acromegaly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

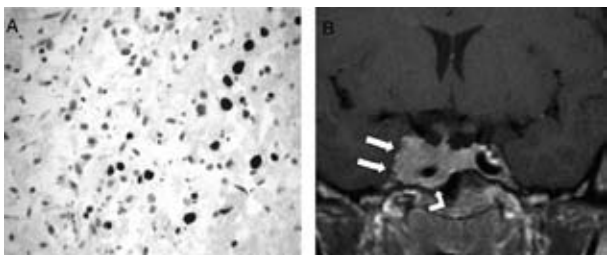


**Figure 1** (A) High Ki-67 LI (11%) in a pituitary adenoma of a petient with acromegaly. (B) Pituitary MRI of the same patient performed after surgery demonstrates right cavernous sinus invasion.

## P1354

**Characterization of mutant AIPV49M in somatotroph cells**

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Introduction
About 30% of all familial isolated pituitary adenoma (FIPA) and 50% of acromegaly families have a mutation in the aryl hydrocarbon receptor-interacting protein gene (AIP; Chahal, *TEM*, 2010). The functional role of AIP in somatotroph cells is unknown. Recently, it has been proposed that wild type AIP (wtAIP) is a tumor suppressor gene, role that is lost in the different mutant AIPs (mAIP; Leontiou, *JCEM*, 2008). In the adenomas, mAIPs are usually found as loss of heterozygosity with both alleles mutated. However, the point mutant V49M-AIP was found in a single giant male patient but there was no LOH, coexisting with a normal allele. Previous and different functional studies found that V49M-AIP was behaving as the wtAIP.
Aims
To study the effects of V49M-AIP in comparison to wtAIP in the proliferation and apoptosis of somatotroph cells.
Matherials and methods
We use the somatotroph rat pituitary cell line GH4C1 cell line that express basal levels of AIP. Thus they are a good model for heterozygosis. The cells were transiently transfected by nucleofection. As a standard of transfection efficiency a vector expressing GFP under a CMV promoter was co-transfected. Cells were counted with a cytometer. Apoptosis was detected by Hoechst staining.
Results
The nucleofection provided a transfection efficiency over 90% in all cases. In the presence of complete medium with growth factors we observed no difference in the proliferation between the empty vector, wtAIP or V49M-AIP. In deprived medium (0.1% serum) the cells transfected with the mutant V49M-AIP presented a 40% apoptosis. There was no relevant apoptosis in cells transfected with empty vector or with wtAIP.
Conclusions
In somatotroph cells V49M-AIP mutation does not affect proliferation but induces apoptosis. In cells expressing endogenous AIP this mutant seems to act as a toxic mutation. This would explain why this mutant is always in heterozygosis. The results also suggest that pro-tumoral effects of mutant AIP require other factors still unknown.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1355

**Lycopene and β-carotene induce growth-inhibitory and proapoptotic effects on pituitary tumor cells: the mechanism could involve Connexin 43**

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Pituitary adenomas account for ~10–15% of intracranial tumors and result in morbidity due to both altered hormonal patterns as well as side effects of therapy. Currently, great attention has been given to natural coumpouds from functional foods. Among these substances, we highlight that the consumption of carotenoids is associated with reduced risk of chronic diseases including cancer and vascular diseases. In this study we evaluate the influence of β-carotene and lycopene on cell viability, colony formation, cell cycle, apoptosis, intercellular communication and expression levels of Connexin 43 on mouse ACTH-secreting pituitary adenoma cells, the AtT-20 cells. Cells were cultivated with DMEM supplemented with 10% fetal bovine serum under an atmosphere of 5% $CO_2$ at 37 °C, and incubated with different concentrations of carotenoids (0.5,1.25, 2.5, 5,10,20 and 40 μM) for 48 and 96 h. Using MTT assay, it was showed an important decrease of cell viability after 48 and 96 h with both lycopene and β-carotene treatments. For colony formation assay in 21-day period there was a significant decrease in clonogenic capacity. By flow cytometry, the cell cycle analysis revealed that β-carotene induced an accumulation of cells in S and G2/M phases after 96 h; furthermore, lycopene increased the proportion of cells in G0/G1 phase and decreased in S and G2/M phases also after 96 h. Using the annexin V-fluorescein isothiocyanate apoptosis detection kit, it was demonstrated that lycopene and β-carotene 5 and 10 μM concentrations induced apoptosis after 96 h. To evaluate intercellular communication, microinjections were performed with Lucifer Yellow and it was observed that a few coupled cells in control cells but none lycopene and β-carotene treatment. By the other hand, the phospho-Connexin 43 expression was increased under cell treatment. These results show that lycopene and β-carotene reduced cell viability, induced apoptosis, blocked the clonogenic capacity and promoted cell cycle arrest in AtT-20 cell culture by a mechanism that probably involves phospho-Connexin 43. This data suggests that these compounds may be in the future a new pharmacological approach for the treatment of pituitary adenomas.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1356

**Somatostatine receptors expression in various pathological types of clinically non-functioning pituitary adenomas**

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Clinically non-functioning pituitary adenomas account for about one-third of pituitary tumors. The majority of them are pathologically classified as gonadotropinomas or null-cell adenomas without hormonal expression. The rest represent silent corticotroph adenomas and plurihormonal tumors. Conservative therapy with dopamine agonists and somatostatine analogues is effective in some cases only depending on the expression of somatostatine (SSTR) and dopamine 2 receptors (D2R).
Objective
The aim of this study was to quantitatively estimate SSTR2, SSTR3 and SSTR5 receptor expression in clinically non-functioning pituitary adenomas and correlate the results with immunohistochemical profile of adenoma.
Methods
Quantitative real-time RT-PCR technique.
Results
Quantitative analysis was performed in 78 clinically non-functioning pituitary adenomas for sst2, sst3 and sst5. All adenomas expressed SSTR2 and SSTR3. SSTR5 was expressed in 42% of adenomas. High variability of expression for each sst type was present. SSTR2 mRNA was expressed from 1174.8 to 146 680.8 copies/5 µl cDNA, SSTR3 62.9–46 914.3 and SSTR5 mRNA 0–43 776.6 copies/5 µl cDNA.
The median of relative quantity (after normalization to housekeeping gene) for SSTR2,3 and 5 was 5.5 and 0.3 for null-cell adenomas (SSTR5 not expressed); 0.5, 0.2 and 0.01 for gonadotrophs; 0.65, 0.1 and 0.14 in silent corticotrophs and 1.7, 0.7 and 0.05 in plurihormonal adenomas respectively.
Conclusion
SSTR2 and SSTR3 expression was not statistically different in regard to histological type of adenoma. SSTR5 expression was significantly higher in silent corticotroph adenomas. There was a correlation between SSTR2 and 3 expression. A very heterogeneous level of SSTR expression may be the reason why experimental use of somatostatine analogs and dopastatins is not clinically effective in the majority of CNFAs.
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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1357

**Lymphocyte subpopulations in Sheehan's syndrome**

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There are a limited number of studies investigating the immunological alterations accompanying the Sheehan Syndrome. The aim of this study was to evaluate lymphocyte subsets in these patients.
Flow cytofluorometry was used for the immunophenotyping of peripheral blood leukocytes from 15 patients (mean age 61.6±11.3, range 34–75 years) and 25 healthy controls (mean age 56.7±10.6, range 34–80 years). The percentages of $CD3^+$, $CD19^+$, $CD16^+/56^+$, $CD8^+28^+$, $CD8^+28^-$, $\gamma\delta TCR^+$, $CD8^+$; and the ratio of $CD8^+28^-/CD8^+28^+$ were similar ($P>0.05$) between patients and controls. Whereas the leucocyte counts ($P=0.004$), the percentage of CD3DR ($P=0.000$), $CD4^+CD25^+$ ($P=0.003$), the ratio of CD3DR/CD3 ($P=0.000$) were higher, the percentage of CD4 ($P=0.000$) and the ratio of CD4/CD8 ($P=0.006$) were lower in patients with Sheehan's syndrome compared to healthy controls (Table 1).
In conclusion, some peripheral lymphocyte cell subsets show marked variation in patients with Sheehan's syndrome in comparison to matched healthy subjects, which may have implications for altered immune regulation in these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1358

**Modulation of intrapituitary CRH gene expression**

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Corticotropin-releasing hormone (CRH) is known to be produced and secreted by normal rat corticotropes and to contribute in a paracrine manner to *POMC* synthesis and ACTH secretion in these cells. The regulation of intrapituitary CRH however still has to be clarified.
Aim
This study was to establish whether *CRH* gene expression can be measured in rat pituitaries *in vitro* and whether CRH modulates its own gene expression within the pituitary.
Methods
Rat anterior pituitary primary cultures were incubated with 10 nM CRH and RNA extracted after 24 and 48 h incubation. Control wells were incubated with serum-free DMEM. RNA was reverse-transcribed and subjected to real-time PCR using probes specific to rat *CRH* and *HPRT*. Data were analyzed as $2^{-\Delta\Delta C_t}$ and are expressed as fold increase over control wells.
Results
Low levels of *CRH* gene expression were detected in rat pituitary primary cultures. Wells incubated with 10 nM CRH presented increased *CRH* levels at both 24 h (1.7-fold ±0.4 higher than control wells) and 48 h (2.1-fold ±0.1 higher than control wells).
Conclusions
This study shows that *CRH* mRNA can be detected at low levels in normal rat pituitary cells, in keeping with its paracrine role. Interestingly, incubation with exogenous CRH induced an up-regulation in *CRH* gene expression, indicative of a positive intrapituitary feed-back loop. Further studies are needed in order to fully clarify the modulation of locally produced CRH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1359

**Resveratrol induces apoptosis in GH3 pituitary adenoma cells of the rat**

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Objective
Resveratrol is a phytoestrogen with various antiproliferatic and proapoptotic effects. One of the underlying mechanisms is downregulation of apoptosis inhibitors, such as Survivin. In human pituitary adenoma cells, Survivin expression is increased as compared to healthy pituitary gland tissue. This *in vitro* study aims to analyze the effect of Resveratrol on GH3 pituitary adenoma cells of the rat.
Methods
GH3 cells were cultivated in F-12K medium containing 2.5% fetal bovine serum and 15% horse serum. Ethanol served as solvent for Resveratrol. GH3 cells were incubated with Resveratrol concentrations from 20 to 100 µM for 48 to 72 h. Culture medium and Ethanol served as controls. Cells were counted using a hemocytometer. Apoptosis was quantified using an ELISA. Relative expression of Survivin as compared to β-2-microglobuline (B2MG) was measured using quantitative real time PCR (qRT-PCR). *T* served as statistical test, whereas $P<0.05$ was supposed to be statistically significant.
Results
GH3 cell counts significantly decreased with growing concentrations of Resveratrol ($P<0.005$). ELISA significantly demonstrated apoptosis in two cell passages treated with 100 µM Resveratrol ($P<1\times10^{-8}$). In two cell passages, qRT-PCR detected a relative increase in Survivin expression (SE) in the Ethanol control and a relative decrease in SE after treatment with 100µM Resveratrol as

compared to culture medium (culture medium SE: 0.009, 0.008; Ethanol control SE: 0.011, 0.014; SE after Resveratrol treatment: 0.007, 0.007).
Conclusions
Resveratrol decreases GH3 cell counts in a dose-dependent manner by inducing apoptosis, which may possibly be mediated by Resveratrol-dependent down-regulation of Survivin. Further investigation of the potential effects of Resveratrol on pituitary adenoma cells is warranted.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1360

**Effects of Brazilian palm tree extract on ACTH-secreting mouse pituitary tumor cells**

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Cushing Disease (CD) is a serious endocrine disorder that is primarily caused by an ACTH-secreting pituitary adenoma. Current treatments of CD present low efficiency in controlling the tumor mass growth and reducing the release of ACTH. This work investigates a possible new therapy for the treatment of pituitary tumors. Using a nanoparticle extract (nanoOse) of a typical Brazilian palm tree, we verify the *in vitro* effects of this extract on a ACTH-secreting mouse pituitary tumor cell line AtT20. AtT20 cells were maintained in a culture incubator at 37 °C in humidified air containing 5% $CO_2$ in a DMEM culture medium supplemented with 10% fetal bovine serum (FBS). AtT20 cells were treated with nanoOse at concentrations ranging from 100 to 300 μg/ml, and after that it was performed these experiments: morphological analysis by phase-contrast microscopy, cell viability MTT assay, cell proliferation assay using violet crystal, colony formation assay using the CFU method and cell necrosis index by LDH release assay. Experimental results show that even with the lowest concentration (100 μg/ml), nanoOse treatment was able to modify the morphological characteristics of AtT20 cells, to decrease 50% of cell viability and proliferation, to lose their clonogenic capacity and also to increase the cell necrosis index.These results indicate that nanoOse treatment negatively modulate AtT20 tumor cell progression, and suggest that this extract could be a new therapeutic strategy for the ACTH-secreting pituitary adenoma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Pituitary - Clinical

## P1361

**Case series of diabetic ketoacidosis as the first presentation of 860 patients with acromegaly**

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Background and aims
Excess GH causes insulin resistance resulting in impaired glucose metabolism. Although diabetes mellitus (DM) is common in patients with acromegaly, diabetic ketoacidosis (DKA) is rarely associated with acromegaly. Actually only 11 DKA patients have been anecdotally reported as the first presentation of acromegaly so far. Here we present nine cases of DKA as the first presentation of 860 consecutive patients with acromegaly and their clinical characteristics.
Materials
Data of 860 consecutive acromegaly patients with pituitary surgery in our hospital from 1980 to 2011 were collected.
Results
Nine cases were found to be complicated with DKA before diagnosis of acromegaly, seven males and two females. None of them had been diagnosed as DM. Their average age was 38.8±14.2-year-old, HbA1c 12.1±1.8%, plasma glucose 41.4±21.0 mmol/l, arterial blood pH 7.27±0.09 (means±s.d.). All patients were negative for antibodies related to type 1 DM. The precipitating factor of DKA in six cases was an excessive ingestion of sugar-containing soft drink. After pituitary surgery, plasma glucose levels were in good control without insulin in all cases; furthermore six patients needed no oral hypoglycemic agents. Their insulin secretion capacities were well preserved estimated with 75 g oral glucose tolerance test after surgery.
Conclusion
As we reported, acromegaly enhanced insulin resistance but some acromegalic patients could not compensate insulin secretion accordingly (*Eur J Endocrinol* **164** 467, 2011). Thus, an excessive ingestion of soft drink might predispose them to metabolic failure due to further glucose toxicity. Increased lipolysis by GH could also be involved in the development of DKA. In conclusion, patients with acromegaly may be prone to severe metabolic failure, such as DKA that is the first presentation in 1% of acromegaly. Acromegaly should be added to the list in background diseases of DKA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1362

**Markers of recurrence and long-term morbidity in craniopharyngioma**

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Context
Craniopharyngiomas are often associated with an unfavorable prognosis but data on their long-term consequences are sparse.
Objective
To identify markers of recurrence and factors associated with compromised social rehabilitation and altered quality of life (QoL) in a large cohort of patients with either childhood onset (CO) or adult onset (AO) craniopharyngioma.
Methods
Retrospective analysis was performed for 171 patients treated for craniopharyngioma in two academic centers in France between 1972 and 2009. For each subject, data were collected concerning clinical presentation, imaging features, visual sequelae, endocrine and metabolic impact, treatment modalities (surgery, radiotherapy), recurrence-free survival rate and social insertion, as well as answers to the WHOQOL-BREF questionnaire.
Results
A total of 65 CO and 106 AO patients were reviewed. If CO was diagnosed before the age of 10 years, this was associated with a higher incidence of obesity, blindness and panhypopituitarism, and only 40.7% of subjects had adequate work or school attendance compared to 72.4% of patients with later disease onset. Initial symptoms of intracranial hypertension (SIHT), pterional surgery and multiple surgery were associated with obesity and poorer social insertion. No determinant of QoL was identified. In the subgroup of patients treated in the 90's and later, the progression rate was 59.4% in patients with residual tumor on MRI and the recurrence rate was 19.8%. Recurrence/progression correlates significantly with male gender, early onset (before 10 years) and SIHT, but only SIHT at presentation remained a significant predictor with multivariate analysis.
Conclusions
Craniopharyngioma continues to be associated with severe outcomes. Higher morbidity rates are found in patients with early onset disease (before 10 years),

**Table 1**

| | <10 years | 10–18 years | P | >18 years | *P* (CO Vs AO) |
|---|---|---|---|---|---|
| At least three anterior pituitary insufficiencies | 100% | 82.3% | 0.03 | 73.8% | 0.01 |
| Diabetes insipidus | 96.4% | 82.3% | 0.12 | 68.0% | <0.01 |
| Major visual acuity and field impairment | 53.8% | 50.0% | 0.77 | 48.0% | 0.66 |
| Blindness | 37.0% | 5.9% | <0.01 | 13.6% | 0.30 |
| Epilepsy | 32.1% | 18.7% | 0.23 | 15.5% | 0.14 |
| Median BMI | 33.1 | 26.2 | <0.01 | 27.7 | 0.80 |
| BMI >30 | 67.9% | 24.2% | | 41.5% | |

initial SIHT or in whom pterional surgery was required. Markers of recurrence are difficult to identify, with SIHT being the most powerful predictor.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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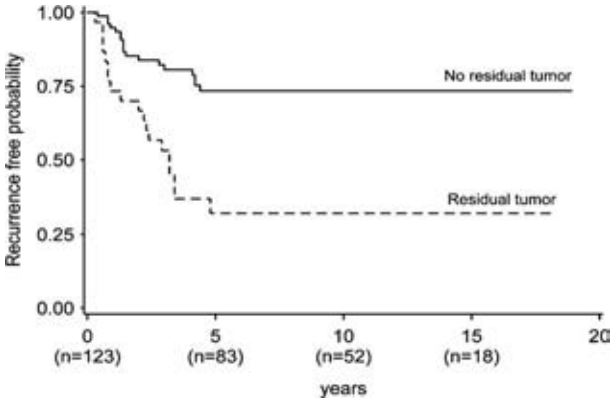


## P1363

### Familial isolated pituitary adenoma: review of four families

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Introduction

Pituitary adenomas are frequent brain tumors, with prevalence of about 1:1000. Most occur sporadically. The familial forms represent 5% of cases. These can be found associated with other endocrine neoplasia (MEN 1, Carney complex, MEN 4) or as a clinical isolated entity – FIPA. This is characterized by the presence of pituitary tumors in two or more family members, in the absence of features of other endocrine syndromes. AIP gene mutation, which may or may not be present, has been linked to FIPA. A review of four FIPA families was made. The Table 1 summarizes their main characteristics.

Conclusion

FIPA are characterized by the predominance of prolactinomas and somatotrophynomas (GH; 75%). They can be divided into families of homogeneous phenotype (only one tumor type, ex. isolated familial somatotrophynomas (IFS)) or heterogeneous (all types can occur, including non-functioning, but as a rule, at least one prolactinoma or GH producing tumor). This is a heterogeneous entity in which virtually all phenotypic combinations are possible. FIPA patients, especially with AIP gene mutation (positive in 15% of the heterogeneous phenotype and 50% of the IFS), are younger, have larger, more aggressive and resistant to therapy tumors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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**Table 1**

| Family | Number studied | Number affected | Family link | Age of diagnosis | Gender | Tumor type | Therapy | AIP |
|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 2 | Siblings | 40 | ♀ | ACTH, PRL | Surgery | NG |
| | | | | 46 | ♀ | PRL | Bromocriptine | NP |
| 2 | 18 | 2 | Siblings | 31 | ♀ | PRL | Bromocriptine | NP |
| | | | | 34 | ♂ | PRL | Bromocriptine | NP |
| 3 | 24 | 2 | Mother and daughter | 28 | ♀ | GH, PRL | Surgery, RT | NP |
| | | | | 63 | ♀ | NFA | Surveillance | NP |
| 4 | 16 | 2 | Siblings | 38 | ♂ | NFA | Surgery | NP |
| | | | | 43 | ♀ | PRL | Bromocriptine | NP |

♀, female; ♂, male; PRL, prolactin; NFA, non-functioning pituitary adenoma; NG, negative; ND, non-determined; RT, radiotherapy.

## P1364

### A prospective study of cardiac valvular status in patients treated with cabergoline for endocrine disease

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Introduction

Since the 1990's cabergoline has become the treatment of choice in prolactinomas, allowing rapid and efficient hormonal and tumoral control in most cases. Evidence of cardiac valculopathy was demonstrated in patients treated by dopamine agonists for Parkinson disease, which led to curtailment of their use in this disease. Retrospective studies in hyperprolactinemia patients treated with cabergoline did not show such an effect, probably due to much lower doses commonly used. However, prospective data with long-term follow-up are generally not widely available.

Aim

To undertake a novel prospective study of cardiac valvular function in patients treated by cabergoline for endocrine disease.

Methods

One hundred and four patients (34M, 70F; mean age 50.43 years) treated with cabergoline for endocrine disease (hyperprolactinemia $n=98$, other $n=6$) were included. All patients and controls underwent a complete transthoracic echocardiographic study (TES) using the same equipment. All TES were performed by two experienced echocardiographers, with special attention towards valvular status and were interpreted by a third echocardiographer. The mean interval between TES while on cabergoline was 28.66 months $\pm$11.26 months.

Results

The mean total duration of cabergoline treatment was 75.81 months ($\pm$75.25 months) and the mean total dose of cabergoline was 253.33 mg ($\pm$515.76 mg) at last follow-up. Cardiovascular risk factors included: dyslipidemia ($n=29$), obesity ($n=28$), hypertension ($n=22$), diabetes ($n=10$), smoking ($n=16$). Three patients developed mild aortic insufficiency while one case had improved aortic valvular function on follow-up. One case developed a mild tricuspid insufficiency and one case developed a mild pulmonary valvular insufficiency, while three cases had normalization of pre-existing grade one valve dysfunction deficiency. No variation in mitral function was found (Fig. 1).

Discussion

In this prospective study, we found no relevant alteration in cardiac valvular function with cabergoline treatment. This suggests that findings from retrospective analyses are correct and that cabergoline remains a safe chronic treatment at the doses commonly used in endocrine disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1**

| | **Mitral insufficiency grade 0** (absent) | **Mitral insufficiency grade 1** (mild) | **Mitral insufficiency grade 2** (moderate) | **Mitral insufficie grade 3** (severe |
|---|---|---|---|---|
| Irst echo | 59 | 41 | 4 | 0 |
| Control | 59 | 41 | 4 | 0 |
| | Tricuspid insufficiency grade 0 | Tricuspid insufficiency grade 1 | Tricuspid insufficiency grade 2 | Tricuspid insuff grade 3 |
| Irst echo | 64 | 36 | 4 | 0 |
| Control | 63 | 37 | 4 | 0 |
| Irst echo | Aortic insufficiency grade 0 ~93 | Aortic insufficiency grade 1 11 | Aortic insufficiency grade 2 0 | Aortic insufficie grade 3 0 |
| Control | 91 | 13 | 0 | 0 |
| | Pulmonary insufficiency grade 0 | Pulmonary insufficiency grade 1 | Pulmonary insufficiency grade 2 | Pulmonary inst grade 3 |
| Irst echo | 90 | 14 | 0 | 0 |
| Control | 92 | 12 | 0 | 0 |

## P1365

### IGF1 as a risk factor for insulin resisitance and glucose intolerance in acromegaly

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Introduction
In normal subjects GH is a hyperglycemic hormone as opposed to insulin-like growth factor 1 (IGF1). Active acromegaly is associated with insulin resistance (IR) and glucose intolerance although both GH and IGF1 are elevated. Our objective was to assess whether GH, IGF1 or both are risk factors for IR and glucose intolerance in acromegaly.
Methods
Basal serum IGF1 and GH, glucose and insulin during an oral glucose tolerance test were measured in 111 patients with active ($n=97$) or cured ($n=14$) acromegaly. 53 patients were assessed before any treatment and 58 after surgery and/or radiotherapy. Patients treated with somatostatin analogs, GH-receptor antagonists or antidiabetic drugs were excluded. IR was assessed by various basal and stimulated indices. Glucose intolerance (impaired fasting glucose ($n=20$), impaired glucose tolerance ($n=11$) and/or diabetes mellitus ($n=12$)) was defined according to American Diabetes Association criteria.
Results
Homeostatic model assessment 2-insulin resistance (HOMA2-IR) index correlated more closely with IGF1 ($r=0.64$, $P<0.0001$) than nadir ($r=0.25$, $P=0.008$) or random GH ($r=0.28$, $P=0.002$). Also, IGF1 correlated better than nadir or random GH with all other IR indices (fasting insulin, HOMA1-IR, QUICKI and Matsuda index). In multivariate logistic regression analysis IGF1 (OR=1.55, $P=0.01$) but not nadir (OR=1, $P=0.9$) or random (OR=0.99, $P=0.9$) GH was a significant risk factor for glucose intolerance after adjusting for age, sex, weight and acromegaly duration.
Conclusions
In acromegaly IGF1 correlates more closely than GH with IR. IGF1 levels but not GH are associated with glucose intolerance.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1366

### An insight into the mechanisms underlying the sympathoinhibition of acromegaly

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By direct measurement of muscle sympathetic nerve activity (MSNA) we previously demonstrated a markedly decreased adrenergic tone in newly diagnosed acromegalic patients. The present study was aimed at confirming this finding in a larger group of patients and establishing the pathophysiological role of insulin resistance, GH and IGF1 levels, circulating leptin and extracellular water (ECW) in this abnormality.
Study design
Fifteen *de novo* patients with active acromegaly without obstructive sleep apnoea and cardiac hypertrophy, and 15 healthy subjects matched for age, sex and BMI were recruited. After evaluating ECW by BIA and glucose metabolism, and measuring circulating GH, IGF1 and leptin, direct recording of sympathetic outflow via the microneurographic technique was performed.
Results
A marked sympathetic inhibition was confirmed in patients compared to controls (MSNA $18.3\pm8.10$ vs $37.3\pm6.48$ bursts/min, $P<0.0001$) in spite of a significatly increased HOMA index ($4.2\pm2.39$ vs $1.6\pm0.19$, $P<0.001$). ECW was comparable in patients and controls. A clear-cut reduction of plasma leptin ($1.6\pm1.04$ vs $6.5\pm2.01$ μg/l, $P<0.0001$) was recorded in the patients. In these latter MSNA was not correlated with ECW, GH or IGF1, but was positively correlated with leptin ($P<0.0001$). Leptin was the only independent predictor of MSNA in a multiple regression analysis.
Conclusions
Acromegalic patients display a decreased sympathetic outflow in spite of insulin resistance. The lack of correlation between ECW and MSNA does not support the hypothesis of a sympathoinhibition due to baroreceptor activation by volume expansion. A putative role of the GH–IGF1 system as direct central inhibitor of sympathetic drive is not likely, given the lack of correlations between these two parameters and MSNA. On the contrary, the highly significant correlation with leptin points to hypoleptinemia as a major contributor to the sympathoinhibiton of acromegaly, consistent with previous studies in obese animal models.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1367

### Aneurysmal subarachnoid haemorrhage is a rare cause of acute glucocorticoid deficiency and long term hypopituitarism

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Subarachnoid haemorrhage (SAH) is a well reported cause of hypopituitarism but the precise incidence is controversial. We aimed to prospectively determine the incidence of acute and long term hypopituitarism in SAH.
We prospectively recruited 100 patients (61% female, median age 53 (range 16–82)) with non-traumatic aneurysmal SAH. Each patient had plasma sodium, urea, osmolality, glucose, and 0900 h cortisol (PC) measured on days 1, 2, 3, 4, 6, 8, 10 and 12 following SAH. Results were compared with 15 patients admitted to ITU following vascular surgery. A PC $<300$ nmol/l in a patient in ITU was regarded clinically as inappropriately low. Survivors attended for insulin tolerance testing at $\geq 6$ months following SAH. Those in whom insulin tolerance testing was contraindicated underwent glucagon stimulation testing. If patients refused either of these tests, a short synacthen test was offered.
14% of SAH patients had at least one PC $<300$ nmol/l; in 4/14 (28.6%) hyponatraemia due to acute cortisol deficiency ensued, which responded to hydrocortisone treatment. In contrast, all controls had PC $>500$ nmol/l on day 1, and $>300$ nmol on days 2–12. 11% of SAH patients developed acute cranial diabetes insipidus (CDI); mortality in this group was 100%.
39/89 (43.8%) of SAH survivors attended for dynamic pituitary testing. The median time to testing was 15 months (range 7–30 months). 24/39 (61.5%) underwent insulin tolerance testing. 4/39 (10.3%) underwent glucagon stimulation testing and 11/39 (28.2%) underwent short synacthen testing. 2/39 (5.1%) were ACTH deficient, one of whom previously had low PC. 7/39 (17.9%) were GH deficient; one GH deficient patients previously had low PC. No patients were gonadotropin, TSH, or prolactin deficient. None had long term CDI.
In the largest prospective study of its kind, acute glucocorticoid deficiency occurs in 14% of SAH patients, and causes hyponatraemia in 28.6% of these. Long term hypopituitarism is uncommon following SAH and predominantly manifests as GH deficiency.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1368

### Hyponatraemia in aneurysmal subarachnoid haemorrhage is due to the syndrome of inappropriate antidiuresis and acute glucocorticoid deficiency

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Hyponatraemia is the most common electrolyte abnormality following subarachnoid haemorrhage (SAH). Retrospective data suggests that the Syndrome of Inappropriate Antidiuresis (SIAD) is the most common cause,

although glucocorticoid deficiency and rarely cerebral salt wasting may also cause hyponatraemia.
We prospectively studied 100 patients (61% female, median age 53 (range 16–82)) with non-traumatic aneurysmal SAH. Each patient had plasma sodium (pNa), urea, osmolality, glucose, 0900 h cortisol (PC) and vasopressin (AVP), and urinary sodium and osmolality measured on days 1, 2, 3, 4, 6, 8, 10 and 12 following SAH. Fluid balance and haemodynamic parameters were recorded daily. A PC $<$300 nmol/l was regarded clinically as inappropriately low.
49% developed pNa $<$ 35 mmol/l, including 14% with pNa $<$130 mmol/l. 36/49 (73.4%) developed hyponatraemia between days 1 and 3 post SAH. The median duration of hyponatraemia was 3 days (range 1–10 days).
In 35/49 (71.4%), hyponatraemia was due to SIAD as defined by standard diagnostic criteria. In 4/49 (8.2%) hyponatraemia was preceded by acute cortisol deficiency and responded to hydrocortisone treatment. Overall, 14/100 patients developed acute cortisol deficiency, of whom 4 developed hyponatraemia due to the cortisol deficiency and 1 developed hyponatraemia due to SIAD. In 5/49 (10.2%) hyponatraemia was due to volume depletion and in 5/49 hyponatraemia was due to inappropriate fluid resuscitation. There were no cases of cerebral salt wasting. Patients with SIAD had a failure of osmoregulation of AVP release, with detectable AVP levels despite hypoosmolality. Patients with SIAD had higher AVP levels than those with hyponatraemia due to acute cortisol deficiency or inappropriate fluid administration ($P=0.03$).
In the first prospective study of its kind, hyponatraemia occurs in over half of aneurysmal SAH cases, predominantly due to SIAD. Acute glucocorticoid deficiency is a infrequent but treatable cause of hyponatraemia. We found no evidence of cerebral salt wasting syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1369

**Pregnancy outcomes following cabergoline treatment in hyperprolactinemic patients: an observational survey study**

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Cabergoline (CAB) and other dopamine agonists are usually discontinued shortly after hyperprolactinemic patients become pregnant. Data on maternal and foetal exposure to CAB and on recurrence of hyperprolactinemia after pregnancy are still limited. The current survey study aimed at elucidating the safety of exposure to CAB during early pregnancy and the recurrence rate of hyperprolactinemia after pregnancy. Thus, 68 pregnancies in a cohort of 59 patients with hyperprolactinemia (age 37.2$\pm$5.5 years), including 51 patients with prolactin-secreting microadenomas, six with macroadenomas and two with non tumoral hyperprolactinemia, were evaluated. In all patients CAB therapy was recommended to be discontinued when pregnancy was confirmed. Pregnancies were monitored until delivery or termination according to routine clinical practice. Outcomes examined include the incidence of abortions, premature delivery and foetal malformations or abnormalities, as well as the recurrence rate of hyperprolactinemia after pregnancy. Pregnancies resulted in 10 (15%) spontaneous abortions and 58 (85%) live births. No neonatal malformations and/or abnormalities were recorded in our cohort. Only in five out of 59 patients treatment with CAB had to be restarted after pregnancy because of recurrence of hyperprolactinemia, whereas in 91.5% of cases no further therapy was required and patients were classified as in complete clinical and biochemical remission at last follow-up (48 months). In conclusion, foetal exposure to CAB at the time of conception and/or during pregnancy does not induce any increase in the risk of miscarriage or malformation. CAB withdrawal does not increase the risk of recurrence of hyperprolactinemia after pregnancy and pregnancy itself seems to increase the remission rate in CAB-treated hyperprolactinemia.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1370

**Sleep apnea in acromegaly: pathogenetic factors and long-term follow up**

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Background
Sleep apnea syndrome (SAS) is a common disease in acromegaly and it can persist during remission in up to 58% of the patients. Data regarding long term outcome of SAS in acromegalic patients are lacking. Moreover it is still unknown which component, either craniofacial deformation or soft tissue hypertrophy of the palate and upper airways, may play the major role in the pathogenesis of this complication.
Aim
To assess the presence of SAS in a series of acromegalic patients including active and controlled patients and to perform follow up in patients with SAS after biochemical control or during long-term remission. Moreover to evaluate site, degree and possible cause of upper airways obstruction by MRI and fiberoptic nasopharyngoscopy with the Muller maneuver (FNMM).
Patients and method
Polysomnography was performed in 58 acromegalic patients: 33 active and 25 controlled and was repeated in 25 patients with SAS of whom 16 after achieving biochemical control and 9 after long term remission (mean 6.6 years, S.D. $\pm$3.2). In 29 patients morphological study of the upper airways by MRI and FNMM was carried out.
Results
The prevalence of SAS was 64% in active and 52% in controlled patients. Among 16 active patients 8 (50%) showed SAS improvement and 2 (12.5%) recovered after biochemical control, whereas in 9 out of 13 (69.2%) controlled patients SAS persisted. Uvula alone or with tongue base was the main site of obstruction assessed by FNMM in 90% of patients. Uvula diameters correlated with the severity of airways narrowing at FNMM and tongue measure with the severity of the AHI.
Conclusions
SAS can improve after biochemical control of acromegaly, but can persist even after long term follow up despite recovery from acromegaly. Hypertrophy of soft palate and tongue is relevant factor responsible for occurrence and severity of SAS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1371

**Japan National Survey of metastatic pituitary tumor: preliminary report on 164 cases**

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Background and objective
The number of reports of metastatic (secondary) pituitary tumors has been increasing with progression of diagnostic and treatment skills for cancer patients. However, there were no nation-wide epidemiologic surveys or epidemiologic reviews about this topic. We have started a multi-institutional joint research in Japan to understand clinical features of metastatic pituitary tumors.
Subjects and methods
We distributed 1069 closely-monitored questionnaires to the institutions with members of the Japan Society for Hypothalamic and Pituitary Tumor, the Japan Society of Stereotactic Radiosurgery and the Japan Endocrine Society. As of end of 2011, we have identified 164 patients with metastatic pituitary tumor treated during the period from 1996 through 2011.
Results
The patients were predominantly male (89 vs 75). The mean age of onset was 59.1 years ranging 26–86. The mean latency between the diagnosis of metastasis and the diagnosis of initial tumor was 744.5$\pm$884.4 (S.D.) days in 58 reported patients. Metastatic lesion was reported to be found earlier than the initial sites in eight

patients. The main sites of primary lesions included lung (37.8%), breast (22%), kidney (7.3%), colon (6.1%), and miscellaneous (26.8%). The frequent symptoms were diabetes insipidus (24.3%), general fatigue (24.3%) and extra-ocular muscle palsy (18.3%). 36 patients had surgery for the tumor removal (22%), 108 patients (65.9%) had stereotactic radiation, and 25 patients (14.6%) underwent conventional radiation therapy. The mean survival time was 11.6 months in total 117 reported cases, in which the 3-year survival rate was 28.3%. The median survival time tended to be longer in stereotactic radiation group than in conventional radiation group; 11.6 vs 8.8 months, but not statistically significant ($P=0.26$, Logrank)
Conclusion
The Japanese Survey for Metastatic Pituitary Tumor is accumulating data on metastatic pituitary tumor, which must be conducive for early diagnosis and proper treatment of this pathologic entity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1372

**Effects of previous GH excess and current medical treatment for acromegaly on cognition**

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Context
In untreated acromegalic patients, decreased cognitive functioning is reported to be associated with the degree of GH and IGF1 excess. Whether previous GH excess or current medical treatment for acromegaly specifically affects cognition remains unclear.
Objective
To compare cognitive functioning in patients who are treated for acromegaly and non-functioning pituitary adenomas (NFA). In addition, we assessed the influence of prolonged medical treatment after initial transsphenoidal surgery (TSS) on cognition.
Methods
In this cross-sectional study 128 patients participated, who were previously treated for acromegaly ($n=50$; median (IQR) age: 53 (45–65) years) or NFA ($n=78$; age: 61 (55–70) years). In all patients, TSS was performed, followed by radiotherapy in 39%. Of the acromegalic patients, 28 had achieved cure, while 22 were still treated with long-acting somatostatin analogs and/or somavert. Memory and executive functioning were assessed by the 15 words test (15 WT) and the Ruff figural fluency test (RFFT), and reported as $z$-scores.
Results
All patient groups scored significantly poorer than the reference population on memory and executive functioning ($P<0.05$). However, cognitive test performance was not significantly different between patients with acromegaly and NFA (15 WT total memory $-0.90$ ($-1.60$; 0.05), $-0.95$ ($-1.60$; 0.13), $P=0.891$; RFFT unique designs $-0.52$ ($-1.47$; 0.30) and $-0.90$ ($-1.60$; 0.14), $P=0.232$) although acromegalic patients had a significantly higher IGF1 $z$-score mean$\pm$S.D., $0.24\pm1.02$ vs $-1.04\pm1.15$, $P<0.001$. Acromegalic patients with medical treatment at time of study had similar test results compared to patients who achieved cure for acromegaly (15 WT total memory $-0.85$ ($-1.63$; 0.23), $-0.90$ ($-1.53$; $-0.10$), $P=0.853$; RFFT unique designs $-0.83$ ($-1.51$; 0.00), $-0.29$ ($-1.42$; 0.74), $P=0.379$). The IGF1 $z$-scores between both acromegaly patient groups were comparable ($P=0.381$).
Conclusion
We found no association between previous GH excess and cognition. In addition, current medical treatment for GH excess was not related to memory and executive functioning.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1373

See OC10.2

## P1374

**Circulating total and high molecular weight adiponectin levels are lower in adult GH deficient subjects**

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Introduction
Regulation of adiponectin in GH deficiency (GHD), which presents visceral obesity, is still a matter of debate. No studies have evaluated circulating adiponectin isoforms in adult GHD subjects. Aim of this study was to evaluate total adiponectin (TA) and its isoforms (high molecular weight: HMW, medium molecular weight: MMW, low molecular weight: LMW) in hypopituitary adult subjects with and without GHD, in relation to weight, IGF1 and GH levels.
Methods
Cross-sectional study. 38 patients with hypothalamic-pituitary diseases (age, mean$\pm$S.E.M.: $48.8\pm3.7$ years, 13 F) and suspected of GHD were evaluated at fasting for: i) GHRH+arginine test, ii) baseline IGF1 (Immulite), TA, HMW, MMW, LMW (EIA).
Results
22/38 patients presented GHD according to BMI-dependent cut-off values. Patients with and without GHD had an overlapped BMI ($28.1\pm1.3$ vs $27.8\pm1.7$ kg/m$^2$). GHD patients showed lower TA ($5.6\pm0.9$ ng/ml) and HMW levels ($1.7\pm0.5$ ng/ml) compared to subjects without GHD (TA $9.0\pm1.4$ ng/ml, $P<0.01$; HMW: $4.6\pm1.1$ ng/ml, $P<0.004$). MMW and LMW levels showed a comparable trend, without reaching statistical significance. TA and HMW levels negatively correlated with BMI ($r$: $-0.393$, $P<0.007$, $r$: $-0.357$, $P<0.01$ respectively). HMW levels negatively correlated with GH peak ($r$: $-0.251$, $P<0.05$), even when controlled for BMI. No association was found with IGF1 levels. HMW and TA levels correlated with the GHD diagnosis, but, when controlled for BMI, the significance was maintained for HMW only ($r$: 0.290, $P<0.05$). Subjects in the lowest quartile of HMW had a low probability of not being GHD (OR 0.056, CI 95% 0.006–0.491, $P<0.01$).
Conclusions
In hypopituitary adult subject, the presence of GHD seems to be associated with reduced TA and HMW levels independently from BMI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1375

**Quality of life and mood disturbances in patients with acromegaly followed-up in a single center**

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Introduction
Acromegaly is a chronic disease associated with impairment of quality of life (QoL) and psychological status. The aim of the present study was to assess QoL and mood disturbance in patients with acromegaly.

Description of methods/design
It was a comparative, cross-sectional study conducted in the department of Endocrinology at a tertiary medical centre of northern Greece in 2011. AcroQoL questionnaire was used to assess QoL in acromegalics and the short-form of POMS to assess psychological distress in acromegalics compared with a group of patients with other chronic disease and healthy subjects.
Results
Cronbach's alpha analysis showed high reliability of the total AcroQoL (0.887) and POMS score (0.877). Forty patients with acromegaly (15 males, mean age 57.9±12.4 years) were included. Forty patients with chronic disease and 80 healthy subjects, all age- and sex-matched were also included.
Acromegalics showed a mean AqroQoL score of 83.4±17.6 (range 34–108). Males had better QoL than females ($P=0.034$). Moreover, women suffered from more anxiety ($P=0.043$), depression ($P=0.003$), fatigue ($P=0.001$) and confusion ($P=0.007$). No association between AcroQoL and age, hypopituitarism, size of adenoma, disease remission, IGF1, basal or nadir GH levels was observed. AcroQoL scores were negatively associated with POMS subscales, including tension, depression, anger, fatigue, confusion and distress.
Regarding POMS, better scores were noticed in patients with microadenoma ($P=0.019$) and in those who had not undergone radiotherapy ($P=0.035$). Compared with healthy controls, acromegalics suffered more from depression ($P=0.027$) and hostility ($P=0.044$), associations which remained significant after adjustment for age and sex. However, compared with chronic-disease controls there were no significant differences regarding POMS scale and subscales in acromegalics.
Conclusions
Acromegaly has a negative impact on psychological distress comparable to other chronic diseases, especially in those with macroadenomas and those having undergone radiotherapy. QoL is affected more in females.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1376

**Low total cortisol correlates closely with low free cortisol in traumatic brain injury and predicts mortality and long term hypopituitarism**

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Published data has demonstrated that low 0900 h plasma total cortisol (PTC) immediately following traumatic brain injury (TBI) predicts mortality. However, potential discrepancies exist between PTC and plasma free cortisol (PFC). We hypothesised that low PTC would correlate closely with PFC and predict mortality and long-term hypopituitarism.
One hundred patients (84 men, median age 33, range 18–75) with TBI (mean GCS±s.d.=8.59±4.2) were recruited. Each had PTC and CBG, albumin, electrolytes, glucose, and urine osmolality measured on days 1, 3, 5, 7, and 10 following TBI. GCS and fluid balance were recorded daily. Results were compared with 15 controls admitted to ITU following major vascular surgery. A PTC <300 nmol/l in a patient in ITU was regarded clinically as inappropriately low. PFC was calculated for 25% of TBI samples and all control samples using Coolens' equation[1]. TBI patients reattended for dynamic pituitary testing (using the insulin tolerance test unless contraindicated) ≥6 months after TBI.
All controls had PTC >300 nmol, whereas 78/100 TBI patients had at least one PTC <300 nmol/l. TBI patients in the lowest quartile of PTC had the highest mortality ($P=0.0187$). PTC correlated closely with PFC in both TBI patients ($r=0.99$, $P<0.0001$) and controls ($r=0.99$, $P<0.0001$). 40/100 developed transient cranial diabetes insipidus (CDI) and 11/100 developed persistent CDI. When compared with those who did not develop CDI, mortality was significantly higher in those with persistent CDI ($P=0.0003$) and transient CDI ($P=0.0002$). 32/79 (40.5%) of TBI survivors attended for dynamic pituitary testing. The median time to testing was 14 months (range 6–24 months). 6/32 (18.8%) were ACTH deficient, 6/32 were GH deficient, and 1/32 (3.1%) was gonadotropin deficient. No patients were TSH or prolactin deficient. Lower mean PTC was associated with the development of chronic hypopituitarism ($P=0.049$).
Acute hypocortisolaemia and acute DI are common in TBI and predict mortality. PTC measurement correlates closely with plasma free cortisol estimate. Acute hypocortisolaemia may predict long term hypopituitarism.

1. Coolens JL, Van Baelen H & Heyns W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. J Steroid Biochem. 1987 Feb;26 (2) 197–202.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1377

**Array-comparative genomic hybridization in congenital morphological alteration of the pituitary gland**

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Congenital morphological alteration of pituitary gland is a rare disease often associated with isolated or multiple hormonal deficiency. So far only few genes have been shown to be implicated in a small number of these cases.
Aim of this study was to evaluate putative genetic alteration by an array-based comparative genomic hybridization (array-CGH) in 19 adulthood patients, 16 males and 3 females, affected by congenital morphological alteration of the pituitary.
Eleven patients were panhypopituitaric, two were affected by GH, LH–FSH and TSH deficits; two by GH and LH–FSH; one by isolated GH; one by GH, ACTH and TSH; one by GH, LH–FSH and AVP and one patient by GH and AVP. Nuclear magnetic resonance study showed an hypoplasic adenohypophysis and ectopic neurohypophysis in 16 subjects and an hypoplasic adenohypophysis associated to an undetectable neurohypophysis in three.
The array-CGH showed unbalanced chromosomal rearrangements in 6 out of 19 patients; in 4 of these 6 patients, the rearrangements were already described as normal genomic variants. In a patient we found a microdelection of chromosome 2 (del2q37.2) not yet described, but present in his healthy father. In another patient was detected an undescribed 359Kb duplication on chromosome 11 (dup11q21), containing six genes including GPR83, ANKRD49, MRE11A, expressed in central nervous system. Unfortunately we could not confirm the origin of the duplication since the parents are still not available for the analysis.
In conclusion, we confirm that hypoplasic adenohypophysis and ectopic or undetectable neurohypophysis are associated with single or multiple hormonal deficits. The CGH-array experiments were not able to detect any significant genetic alteration in our cohort, although further studies are needed to evaluate the role of dup11q21 in pituitary malformation. Our plan is to follow up this search by including new patients and by implementing the study with the exome sequencing technique.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1378

**Low IGF1 levels are associated with a lower prevalence and incidence of anxiety disorders in primary care patients (DETECT cohort) and the general population (SHIP cohort)**

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Objective
*In vitro* and *in vivo* models revealed that the somatotropic system exerts central effects on the central nervous system. The objective of this study was to

investigate for the first time whether varying endogenous IGF1 levels are associated with mental health outcomes, namely affective disorders, in humans.
Design and methods
We included 6773 subjects from the DETECT study, a representative study on primary care patients in Germany with a 4.5-year follow-up period and 4079 subjects from the Study of Health in Pomerania (SHIP), a population-based study with a 5-year follow-up period. Main predictor was the baseline IGF1 value categorized as <10th percentile, between the 10th and the 90th percentile (reference) and >90th percentile. Outcome measures were affective disorders.
Results
In both cohorts (DETECT and SHIP), we found a strong association in the male population with low IGF1 levels and a lower prevalence and incidence of any anxiety disorder and general anxiety disorder (GAD), respectively, in all adjusted models (DETECT; any anxiety disorder: OR 0.59; 95% CI 0.36–0.95; SHIP; GAD OR 0.57; 95% CI 0.35–0.94) compared to subjects with IGF1 levels in the normal range. In the SHIP, but not in the DETECT cohort, this association was also seen for female subjects (SHIP; GAD: OR 0.53; 95% CI 0.34–0.83).
Conclusion
Low IGF1 levels are associated with lower rates of anxiety disorders in men and possibly in women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1379

**Epidemiology, histophatological characteristics and clinical manifestations of aggressive pituitary tumors, evaluated on the basis of KI-67 immunostaining: a single center experiense**

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Aggressive pituitary tumors are classically defined as pituitary tumors with large size, rapid growth and massive invasion of surrounding anatomical structures. This is a group of pituitary tumors with biological behavior between pituitary adenomas and carcinomas and includes a new group of pituitary tumor, defined atypical adenoma, characterized by the presence of invasive growth and combination of increased mitotic activity and a Ki-67 labeling index >3%. The aim of this retrospective study was to evaluate the epidemiology, histophatological characteristic, including tumor subtype and invasion of surrounding anatomical structures, and clinical characteristics of aggressive pituitary tumors, defined on the basis of a Ki-67 labeling index >3%. The analysis was performed in a cohort of 308 patients who underwent neurosurgery for pituitary tumor between 2007 and 2011. Twenty-three patients (14 women, 9 men, aged 17–62 years, mean 40 years) were classified as patients with an aggressive pituitary tumors (prevalence = 7.5%). The Ki-67 labeling index ranged from 4 to 23% with a mean of 6.9%. Among this group of tumors, 21 (91.3%) were macroadenomas. Moreover, five (21.7%) of the aggressive pituitary tumors and 21 with pituitary adenoma (7.3%) had evidence of invasion of the surrounding anatomical structures ($\chi^2 = 5.69$, $P = 0.01$). Twelve patients (52.1%) had hormone-secreting and eleven had nonfunctional pituitary tumors (47.8%). At the immunohistochemical analysis the tumor subtype were: eight (34.7%) null-cell tumors, six (26%) PRL-secreting tumors, four (17.3%) GH-secreting tumors, three (13%) ACTH-secreting tumors and two silent FSH–LH tumors (8.6%). Based on available post-operative data, 11 out of 18 (47.8%) patients with aggressive pituitary tumors and 29 out of 130 (22.3%) patients with pituitary adenoma had evidence of recurrence ($\chi^2 = 12.07$, $P = 0.001$). In conclusion, aggressive pituitary tumors, evaluated on the basis of Ki-67, were identified in 7.5% of patients underwent neurosurgery for pituitary tumors.These tumors are mainly nonfunctional macroadenomas. The evidence of a higher rate of tumor invasiveness and recurrence suggest the Ki-67 labeling index can be considered a reliable method to individuate an aggressive pituitary tumor.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1380

**Congenital hypopituitarism with ectopic posterior pituitary and pulmonic stenosis: hormonal and radiologic followup into adulthood**

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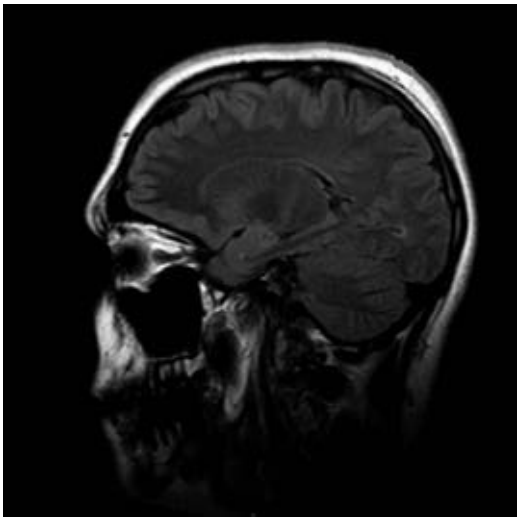
We describe the course of a now 24y/o M with congenital hypopituitarism, severe micropenis, cryptorchidism, hypoplastic testes, hypospadiasis and pulmonic stenosis. He presented with neonatal hypoglycemia. MRI showed small anterior pituitary and ectopic posterior pituitary near tuberous cinereum. Pulmonic stenosis detected after auscultation of a murmur, was mild grade on echo. Free $T_4$ 1 ng/dl (1–2.5) TSH 6.7 μU/ml (0.2–6) glucagon stimulation for GH and cortisol were blunted at 2.9 ng/ml and <1 mg/dl respectively prolactin 3.3 ng/ml (3–14.7). He received thyroxine and hydrocortisone from infancy. Total testosterone and gondotropin were undetectable (<10 ng/dl and <3 mIU/l respectively). He received intermittent testosterone to augment penis size. Inspite of testosterone level 315–921 ng/dl (241–827) he had subnormal penis size. Height increased at constant rate until age four when it fell 2.2 S.D. below mean. GH deficiency was confirmed with flat response to arginine and dopamine, peak GH 1.8 ng/ml. GH therapy was started and he attained nl adult height. Panhypopituitarism persists into adulthood with gradual diminution of pituitary size. MRI age 24 showed small sella only containing the infundibulum. Gland itself was not visualized. Adult hormone levels are undetecetable LH and FSH, IGF 92 ng/ml (83–456) ACTH 6 pg/ml (6–50) am cortisol <0.5 mg/dl prolactin 4.7 ng/ml (2–18) TSH <0.01 mIU/l (0.4–4.5) free $T_4$ 1.6 ng/dl (0.8–1.8) testosterone 280 ng/dl (25–1100) free testosterone 30.8 pg/ml (35–155). He remains on thyroxine, hydrocortisone, testosterone and adult GH. Previous associations of multiple pituitary hormone deficiency were with septooptic dysplasia, cranial nerve and cerebral midline defects. This is the first case demonstrating the association of congenital hypopituitarism and cardiac anomaly specifically pulmonic stenosis. One should consider screening patients with congenital hypopituitarism for cardiac anomalies at birth. Congenitally low gonadotrophins tend to improve with age. Contrary to this FSH and LH remained low such that he required testosterone to treat micropenis. This case illustrates that pituitary size and function continues to decline. Congenital hypopituitarism diagnosed initially in adults has been reported. Gradual diminution of our patient's pituitary size suggests need for close follow up when no or partial hormonal abnormalities are found on initial tests.
MRI age 24.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.



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## P1381

**Childhood onset craniopharyngiomas have normal selfrating quality of life but impaired neurocognitive function in adult life**

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Introduction
Hypothalamic damage in craniopharyngiomas (CP) is associated with poor functional outcome. Data on quality of life (QoL) in CP on complete hormone replacement, including GH, is missing. The aim of this study was to assess QoL and neurocognitive function in childhood onset (CO) CP on complete hormone substitution.
Methods and design
Forty-two (20 women) patients, aged $\geq$17 years were recruited from the South Medical Region of Sweden (population 2.5 million). The patients were surgically treated for a CO CP, between 1958 and 2000. Most (86%) patients were receiving GH therapy, panhypopituitarism was present in 32 patients and 48% had received cranial radiotherapy. Three self-rating questionnaires were applied: the Symptom Checklist-90 (SCL-90), the Interview Schedule for Social Interaction (ISSI) and the social network concept. All subjects were examined with a battery of neurocognitive tests with high sensitivity for subtle brain dysfunction.
Results
No statistically significant group differences were observed across any of the nine SCL-90 subscales. The CP patients had lower performance in neurocognitive tests, reaching statistical significance in 11 of the 20 test variables, including executive function and memory. The patient group had a lower summary measure of performance ($P=0.004$) with this difference becoming insignificant when extracting patient with tumour growth towards third ventricle (TGTV; $P=0.18$). While patients with TGTV, compared to controls had significantly lower mean total score ($P=0.006$). A significant negative correlation was recorded between mean $z$ score of neurocognitive performance and years since operation ($r=-0.331$, $P=0.049$).
Conclusions
A normal QoL was shown on this first study of GH substituted adult survivors of CO CP, which mirrors an adaptation to their present situation. Lower scores of neurocognitive performance were recorded and patients with TGTV had the lowest score. Therapeutic and rehabilitative efforts are highly warranted in the follow up of CP patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1382

**Orexin A concentration is reduced in acromegaly, regardless activity of the disease**

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It has been reported that orexins play an important role in the regulation of GH secretion. It is suggested that the defect in orexins synthesis could be responsible for the disturbances in GH synthesis and consequently aggravate metabolic disturbances. The aim of the study was to assess plasma orexin A levels in patients with acromegaly in relation to the activity of disease and hormonal and metabolic profiles. Fifty-five patients with acromegaly divided into three groups accordingly to minimal GH during OGTT and IGF1 concentrations were studied: 18 with surgically cured acromegaly (SCA), 17 during treatment with a long-acting octreotide were well controlled acromegalic group (WCA), 20 ones, who did not meet the criteria for the cure or disease control, were included in the active acromegalic group (AA). Twenty-nine healthy subjects were enrolled to the control group (CG). In all subjects the concentration of orexin A, GH, IGF1, lipids, glucose, insulin, and other hormones were analyzed. Adipose tissue content was studied by the DXA method.
Results
The concentration of orexin A was the highest ($39.29\pm11.51$ pg/ml) in CG and the lowest ($26.76\pm17.17$ pg/ml) in WCA group. Orexin A concentrations were statistically significantly lower in each group of acromegalics when compared to CG. There were no differences in orexin A among the groups of acromegaly patients. The tendency to negative correlation between orexin A and GH in 0 and 60 min during OGTT was observed, it was statistically significant in AA group. In AA group orexin A concentrations correlated negatively with serum lipids. In each group of the studied patients the tendency to negative correlation between orexin A and insulin was observed. Conclusions: Orexin A concentration is reduced in patients suffering from acromegaly, regardless the activity of the disease. Orexin A deficiency may increase the metabolic abnormalities in acromegaly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1383

**No evidence for detrimental effect of cabergoline therapy on cardiac valves in patients with acromegaly**

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Context
The effects of cabergoline on cardiac valves have been extensively studied in Parkinson's disease and hyperprolactinemia but not in acromegaly, a condition at risk of cardiac valve abnormalities.
Objective
We examined the prevalence of heart valve disease and regurgitation in a series of patients with acromegaly treated with cabergoline, by comparison with matched patients who had never received this drug.
Design and setting
Cross-sectional study in a single referral center.
Patients and methods
Forty-two patients who had received cabergoline at a median cumulative dose of 275.6 mg for a median of 34.5 months were compared to 46 patients with acromegaly who had never received cabergoline and who were matched for age, sex and disease duration. Two-dimensional and Doppler echocardiographic findings were reviewed by two cardiologists blinded to treatment.
Results
Demographic and clinical features were not significantly different between the groups. Cabergoline was not associated with a higher prevalence of valve abnormalities. Unexpectedly, a slightly higher prevalence of aortic valve regurgitation and remodeling was found in the controls relative to the cabergoline-treated patients ($P<0.02$ and $P<0.03$ respectively) but this was likely related to the presence of an aortic dilation, more prevalent in controls. A prospective study comparing incidence of valve abnormalities in patients with acromegaly treated with cabegoline compared with acromegalics never treated with this drug is ongoing and results will soon be available.
Conclusions
Cabergoline therapy in acromegalic patients is not associated with an increased risk of cardiac valve regurgitation or remodeling.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P1384

### Prevalence and incidence of pituitary adenomas; a population based study in Malta

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Context
Epidemiological data is important to correctly quantify the extent of disease and needed health care resources. There are no reports on both incidence and prevalence rates for pituitary adenomas (PAs) together in the same population.
Objective
The objective of the study was to establish the prevalence and incidence of pituitary adenomas with in-depth analysis of their various subtypes in a well defined population.
Design
This was a restrospective cross-sectional analysis of PA patients diagnosed prior to 31 July 2011 for prevalence estimates and those diagnosed between July 2000 and July 2011 for estimating incidence figures.
Methods
Prevalence rates /100 000 and standardised incidence ratios (SIR)/100 000/year were worked out.
Results
The prevalence rates for PAs overall was 75.7/100 000, for prolactinomas it was 35.0/100 000, for nonfunctioning PA 25.9/100 000 and for GH-secreting PAs it was 12.5/100 000. The SIR for PAs overall was 4.27/100 000 per year, for prolactinomas it was 2.05/100 000 per year, for nonfunctioning PA 1.79/100 000 per year and for GH-secreting PAs it was 0.31/100 000 per year. The overall prevalence for macroadenomas was 32.8/100 000 and SIR was 1.49/100 000 per year. The prevalence rate in males for PAs overall was 46.3/100 000 and SIR was 2.08/100 000 per year. For females the prevalence rate for PAs overall was 104.8/100 000 and SIR was 6.58/100 000 per year. Females had a lower proportion of macroadenomas than males (29.5 vs 75.0%; $P<0.001$) and macroadenomas tended to present at a later age compared to microadenomas (48 vs 34.5; $P<0.001$). The highest SIR was reached in the 30–39 age group at 7.42/100 000 per year. Those tumours presenting with apoplexy had a prevalence rate of 2.87/100 000 and a SIR of 0.15/100 000 per year.
Conclusion
This study brings together uptodate prevalence and incidence figures which complement each other. The quoted figures help to further quantify the extent of disease burden that PAs bear on health care resources.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1385

### Markers of disease activity correlate with cephalometric parameters in acromegalic patients

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Introduction
Acromegaly, a rare disorder resulting of tumor GH excess, is frequently associated with prognathism and facial dysmorphia. Characteristics and mechanisms responsible for the malocclusion and the craniofacial changes in acromegalic patients (ACR) are not clarified.
Aims
To evaluate the craniofacial changes in an acromegalic group.
Methods
We observed 59 individuals, 33 with ACR and 26 controls with nonfunctioning pituitary adenomas (NFA) matched for age and sex. Craniofacial skeletal variables related to the cranial base, maxilla and jaw assessment was performed through a basic graphics program Nemoceph.
Results
The mandibular length was significantly higher in ACR ($71.8\pm6.6$ mm) than in NFA (NFA $67.4\pm5.0$ mm, $P=0.007$). We observed a significant correlation between the length of the skull base and mandibular length, both in ACR ($r=0.42$, $P=0.01$), and NFA ($r=0.46$, $P=0.01$). We found a significant correlation between GH levels and jaw length in ACR ($r=0.357$, $P=0.05$). The IGF1, expressed as % upper limit of normal, was significantly correlated with the skull and jaw length and with borderline significance with mandible length ($r=0.351$, $p<0.05$/$r=0.377$, $P=0.033$/$r=0.334$, $P=0.06$ respectively). GH levels did not correlate with other cephalometric parameters, however we observed a statistically significant relationship between the amount of IGF1 and mandibular length adjusted to delayed diagnosis. The ACR have a mean convexity smaller than ANF ($2.30\pm5.66$ vs $4.62\pm3.25$ mm, $P=0.106$), which may represent an effective growth at the level of the chin. In our study, GH hypersecretion was associated with an increase in mandibular length, probably by stimulation of growth at chin level, also considered an extremity.
Conclusion
GH and IGF1 excess induce craniofacial changes. The role of normalization of the GH/IGF1 in the reduction of these comorbidities needs to be evaluated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1386

### Management of giant cystic invasive prolactinoma-the role of medical therapy revisited

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Introduction
Giant prolactinomas are uncommon tumours and typically exceed 40 mm. These tumours are locally invasive but still respond to dopamine agonist therapy. The medical management of cystic giant prolactinomas with dopamine agonists is controversial as the non-solid components of these aggressive tumours are believed to respond poorly to drug therapy. We present a case of a giant cystic invasive prolactinoma masquerading as a multilobular schwannoma that was successfully treated with cabergoline.
Case report
A 35-year-old man presented with severe headaches, left sided lancinating facial pain and bitemporal hemianopia. MRI brain revealed 52 mm large irregular mixed solid and cystic mass involving the pituitary fossa with optic chiasmal compression. MR angiography showed encasement of the internal carotid, left anterior and middle cerebral arteries. The differential diagnosis included cystic craniopharyngioma or multilobular schwannoma. Serum prolactin was markedly elevated at 87 900 mIU/l (normal: 53–360 mIU/l) and associated with hypogonadotrophic hypogonadism (serum testosterone: 3.0 nmol/l, LH 1.4 IU/l, FSH 4.3 IU/l). Thyroid, adrenal and posterior pituitary function was normal. The marked hyperprolactinaemia was suggestive of macroprolactinoma.
Cabergoline was commenced and titrated to maintenance dose of 3 mg/week which resulted in a rapid fall in serum prolactin. There was complete resolution of the visual field deficit, headaches and facial neuropathic pain in twelve weeks. Repeat pituitary MRI scanning demonstrated a progressive and dramatic reduction in the size of the pituitary mass. Nadir serum prolactin was 141 mIU/l.
Conclusion
Giant cystic prolactinomas can be locally invasive and mimic other primary intracranial neoplasms. Surgery has historically been the mainstay of treatment of cystic pituitary tumours but this case demonstrates that carefully supervised medical therapy with dopamine agonists is an effective first line option for cystic macroprolactinomas. This strategy obviates the risks of pituitary surgery with its attendant complications and risk of postoperative hypopituitarism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1387

### Surveillance study on the prevalence of manifestations, complications and illness associated to acromegaly

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Introduction
Acromegaly is a rare disease caused by chronic exposure to excessive levels of GH, usually related to the presence of a pituitary adenoma, and associated to

somatic and visceral hypertrophy, metabolic alterations, respiratory and cardiovascular complications, and increased risk of neoplasias.
Materials and methods
The prevalence of manifestations, complications and associated illness was evaluated in 137 acromegalic subjects (52 M, 85 F; age at diagnosis mean ± S.D. 51 ± 13.4 years), diagnosed between 1980 and 2011 and followed at our Endocrinology Division, using a specific standard protocol. The study cohort included patients with active disease and cured, treated medically, surgically and/or by radiotherapy. Medical history was collected using electronic medical records and a questionnaire administered to patients.
Results
Data analysis revealed the presence of disease manifestations (carpal tunnel in about 20% of the patients, sleep apnea in 10%, thyroid nodular hyperplasia in 68%) similar to expected and a remarkable rate of complications (hypertension in 55% of the patients, cardiomegaly in 24%, alterations of glucose metabolism in 37%, alterations of lipid metabolism in 44%, decreased bone density in 37%, dolicocolon in 15%, colic diverticulosis in 20%, kidney stones in 23%, gallbladder sludge or stones in 47%) and neoplasias (114 total, mostly affecting gut and genitourinary system). Prevalence of disease manifestations and complications was higher in patients with higher mean GH and IGF1 levels (corrected for age) at diagnosis and follow up, even if the correlation did not reach statistical significance ($P > 0.005$). Prevalence of neoplasias was independent from IGF1, GH and HbA1c levels at diagnosis and follow up, and pituitary tumor size.
Conclusions
Attentive surveillance for manifestations, complications and associated diseases is fundamental at diagnosis and follow up in acromegaly patients, independently from biochemical values, with the aim to provide a prompt diagnosis and treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1388

**Diabetes insipidus could be the initial sign of primary hypophysitis**

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Introduction
The natural history of primary hypophysitis is incompletely understood and best treatment remains controversial.
Methods
We performed a retrospective study of 19 patients (mean age of 47 years, fifteen women and four men) who were diagnosed with primary hypophysitis in Samsung Medical Center in Seoul, Korea, from January 2001 to October 2011.
Results
Two patients had recent pregnancy and underlying autoimmunity. Most common clinical feature was polyuria and polydipsia (78%) and most common endocrinological finding was also diabetes insipidus (DI, 73%). The initial presumptive diagnosis was pituitary adenoma or lymphoma in eight patients (42%) and inflammatory hypophysitis in eleven (57%). The first eight patients underwent transsphenoidal surgery but only four (21%) proceeded to total tumor removal. Four patients (21%) received steroid therapy of whom one patient relapsed with pressure symptoms and later underwent surgery. Half of the patients under steroid treatment suffered from side effects such as facial swelling and body weight gain. Only one patient (25%) did not respond satisfactorily to steroid therapy and later underwent surgery. Altogether, the histological diagnoses were confirmed in nine patients, seven lymphocytic hypophysitis (77%), each one of granulomatous and xanthomatous hypophysitis (11% respectively). All patients who underwent surgery required long-term hormone replacement, three (75%) relapsed and needed additional radiotherapy or steroid therapy. Eleven (57%) patients who had no pressure or visual symptoms received only conservative management with hormone replacement (i.e. vasopressin for DI). All these eleven patients have been regularly followed up without recurrence.
Conclusion
Diabetes insipidus could be the initial sign of primary hypophysitis and controlled satisfactorily without aggressive treatment.
Follow-up status of patients with primary hypophysitis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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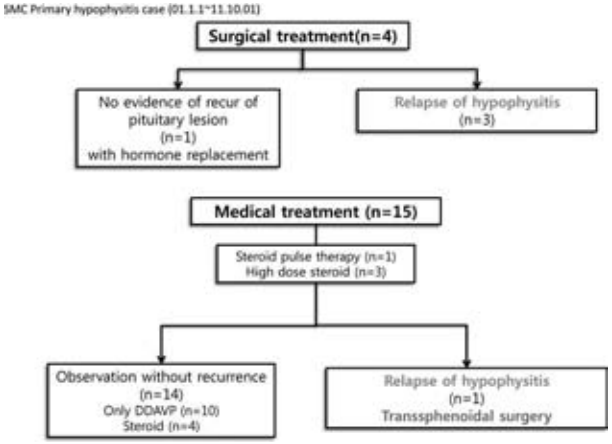
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.




## P1389

**Evaluation of metabolic parameters in patients with craniopharyngiomas using visceral adiposity index**

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Background
Craniopharyngiomas are benign tumors of the parasellar and sellar region. Patients with craniopharyngioma, treated with surgery, have comorbidities such as obesity and multiple pituitary deficiencies.
Aim
To evaluate the metabolic parameters of patients with craniopharyngioma treated with surgery.
Methods
We recruited 20 patients with craniopharyngioma ($M = 11$, $46.6 \pm 12.6$) and 20 patients ($M = 10$, $53.5 \pm 8.8$) with non functioning adenomas (NFA) followed-up at the Department of Endocrinology of the University 'Federico II' of Naples as controls. The majority of patients have multiple pituitary deficiencies. In all patients the following parameters were assessed: blood glucose, HbA1c, insulin, total-, HDL-, and LDL-cholesterol, triglycerides and BMI. We also calculated the visceral adiposity index (VAI) that is a sex-specific mathematical index, indirectly expressing visceral adipose function and insulin sensitivity.
Results
Patients with craniopharyngioma showed lower levels of HDL ($1.14 \pm 0.24$ vs $1.40 \pm 0.28$ mmol/l, $P < 0.04$) and higher blood glucose ($93.1 \pm 9.72$ vs $85.7 \pm 9.72$ mg/dl, $P < 0.021$) compared with patients with NFA. There was no significant difference between two groups in BMI ($32.65 \pm 5.92$ vs $29.47 \pm 5.03$), total- cholesterol ($191.9 \pm 43.09$ vs $209.1 \pm 33.05$ mg/dl), LDL-cholesterol ($111 \pm 42.88$ vs $122.5 \pm 28.19$ mg/dl), triglycerides ($1.8 \pm 1.49$ vs $1.66 \pm 0.48$ mmol/l), insulin ($7.43 \pm 5.97$ vs $9.9 \pm 5.79$ ng/ml) and HbA1c ($5.55 \pm 0.5$ vs $5.55 \pm 0.8\%$). No significant difference was found in the VAI index between two groups ($2.55 \pm 2.32$ vs $2.12 \pm 0.93$; $P = 0.441$). However, VAI index was higher in craniopharyngioma ($2.55 \pm 2.32$ vs 1.92) and in NFA patients ($2.12 \pm 0.93$ vs 1.93) according with a standard population age-matched.
Finally, there are no significant differences in presence of metabolic syndrome in two groups of patients (35 vs 15%, $P = 0.27$).
Conclusions
Our study shows no significant differences in metabolic parameters between patients with craniopharyngioma and patients with NFA. However, a difference was in VAI between two groups of patients and the general population. Thus, VAI can be considered an accurate index of visceral adiposity in these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1390

**Oxidative stress and reduced anti-oxidative status, along with endothelial dysfunction in acromegaly**

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Introduction
Acromegaly is characterized by high cardiovascular morbidity and mortality. Oxidative stress and endothelial dysfunction are underlying mechanisms of atherosclerosis. The aim of this study was to evaluate the blood redox status and endothelial function by means of nitric oxide (NO) levels in patients with acromegaly.
Description of methods/design
Total antioxidant capacity (TAC), catalase activity and glutathione concentration (GSH), as measures of anti-oxidative capacity, total oxidized glutathione (GSSG) and thiobarbituric acid reactive substances (TBARS), as indices of oxidative stress, and NO levels were assessed in 15 patients with acromegaly (age 55.4 $\pm$2.7 years; six males) and 15 age- and sex-matched controls (age 58.4$\pm$2.1 years; seven males).
Results
Active disease was present in 12 patients: 11 on current pharmacotherapy and one newly diagnosed. Three acromegalics were in remission after successful treatment.
Compared to controls, acromegalics had significantly lower levels of catalase activity (8.2$\pm$1.5 vs 51.3$\pm$7.5 μmol/ml per min, $P<0.001$), GSH (0.97$\pm$0.14 vs 1.41$\pm$0.09 mmol/l, $P=0.006$), GSSG (0.27$\pm$0.05 vs 2.04$\pm$0.34 mmol/l, $P=0.002$) and NO levels (6.0$\pm$0.8 vs 43.0$\pm$7.7 μmol/l, $P<0.001$), but higher TBARS (16.3$\pm$2.3 vs 10.1$\pm$2.8 nmol/ml, $P=0.019$). After adjustment for confounders, differences in catalase activity, NO levels and TBARS remained significant ($P=0.004$, $P<0.001$ and $P=0.025$, respectively).
No association between IGF1/GH and oxidative stress markers was noticed, except for a positive correlation between nadir GH and GSSG ($r^2=0.563$, $P=0.036$).
Conclusion
Acromegaly is associated with increased levels of oxidative stress coupled by diminished antioxidant capacity that may be implicated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1391

**Evaluation of clinical presentation, treatment approach and outcome of a cohort of patients with acromegaly: a single centre experience**

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Introduction
Acromegaly is a rare disease with a high morbidity and mortality rate.
Our aim was to characterise the population with acromegaly that is currently under supervision at our Department.
Materials and methods
We included 104 patients with acromegaly (mean age at the diagnosis 44.0 $\pm$13.0; with 71.2% females).
The referred population was analysed on what concerns disease's duration, clinical manifestations and complications, other pituitary hormone hyper/hyposecretion, adenoma size, histological type, treatment option and adverse effects.
Results
On average, these patients have been followed for 15.8$\pm$8.8 years. At diagnosis, 99% were symptomatic (86.7% with morphologic complaints). 39.1% had concomitant hyperprolactinemia and 32.6% hypopituitarism (86.7% hypogonadism, 26.7% hypothyroidism and 16.7% hypoadrenalism). At least one complication was developed in 88.3%.
A pituitary adenoma was found in 99% cases (77% macroadenomas). A familial syndrome was identified in 1.9% (one MEN1; one Carney complex).
Taking into account therapeutic options, 94.2% underwent at least one surgery (75.5% one, 19.4% two and 5.1% three surgical interventions). Regarding the first surgical approach (87.8% transsphenoidal), an adverse outcome occurred in 22.4% patients (77.3% hypopituitarism; 18.2% diabetes insipidus). Biochemical and significant size reduction was respectively achieved in 29.9 and 59.8%; the disease persisted in 40.2%. A full/partial recovery from other pituitary hormone hyper/hyposecretion was accomplished in 65.2%.
Pharmacotherapy was performed in 80.8% (9.5% as primary treatment); one third displayed a side effect.
From the 19.2% subjected to radiotherapy (85% conventional), 50% progressed to hypopituitarism.
Conclusion
In this cohort the majority of patients was symptomatic at diagnosis, revealing typical morphologic features. Furthermore, 77% had a macroadenoma, which could account for worse surgical outcome. Still, the disease is under control in 59.8% and improvement of presurgical hyperprolactinemia/hypopituitarism was noticed in 65.2% cases.
An endocrine, cardiac, respiratory, renal, gastrointestinal or osteoarticular complication was detected in 88.3% patients, decreasing their quality of life and life expectancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1392

**Permanent panhypopituitarism is a rare complication of acute bacterial sinusitis**

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Permanent pan-anterior hypopituitarism is a rare complication of bacterial sinusitis, with only six cases reported worldwide. However, all were reported before the advent of MR imaging and most did not have what is now regarded as gold standard evaluation of pituitary function. Also, all previous cases noted an interval of months between resolution of sinusitis and development of hypopituitarism. We report two cases of previously healthy Caucasian adults who developed pan-anterior hypopituitarism immediately following acute severe bacterial sinusitis.
The first patient presented with a 2 week history of frontal headache, sinus pain and fever and a three day history of right periorbital oedema. Clinical examination revealed partial bilateral third cranial nerve palsy, right sided sixth cranial nerve palsy and bilateral palsy of the ophthalmic branch of the trigeminal nerve. CT and MR brain scanning showed severe maxillary, sphenoid and ethmoid sinusitis. MR venogram showed no cavernous sinus thrombosis. The patient had endoscopic sphenoidotomy with decompression. There was no intraoperative hypotension. Histology revealed inflammatory disease. Following discharge he felt constantly fatigued, had reduced libido and facial hair growth. An insulin tolerance test was performed indicating pan-anterior hypopituitarism. He had no symptoms of diabetes insipidus. Repeat MR scanning revealed an atrophic pituitary gland.
The second patient had a long history of chronic sinusitis. MR scanning was performed to evaluate the sinuses and brain; this revealed a 1.5 cm mass in the pituitary fossa which was thought represent a pituitary macroadenoma. No focal neurological signs were present. Trans-sphenoidal surgery was performed which revealed pus in the pituitary fossa, biopsy was performed which revealed chronic inflammatory changes on histology. Again, there was no intraoperative hypotension. Post operative insulin tolerance testing revealed pan-anterior hypopituitarism. Diabetes insipidus was not present.
Pananterior hypopituitarism is a rare complication of severe sinusitis, which should be remembered if no response is seen to standard antibiotic treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1393

**Carboplatin is a novel cause of the syndrome of inappropriate antidiuresis**

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The syndrome of inappropriate antidiuresis (SIAD) is characterised by plasma hyponatraemia and plasma hypoosmolality with a high urinary sodium in a

clinically euvolaemic patient. We describe only the second reported case of SIAD due to carboplatin administration, with consequent acute severe hyponatraemia. A 61-year-old lady with no background history of note received neoadjuvant carboplatin and paclitaxel for stage III ovarian carcinoma. Five days after the administration of chemotherapy she developed headache, vomiting and generalised weakness. Biochemical investigation revealed a plasma sodium of 109 mmol/l, with a plasma osmolality of 235 mOsm/kg and a high urinary sodium (75 mmol/l). She was clinically euvolaemic; blood urea was 5.8 mmol/l and serum creatinine was 42 μmol/l, indicating adequate hydration status. Thyroid function was normal and 0900 serum cortisol was normal at 472 nmol/l, indicating adequate glucocorticoid reserve. After seven days of fluid restriction to 1 l/day, she made a full clinical recovery and her sodium improved to 132 mmol/l. Her chemotherapy regime was subsequently changed to cisplatin/paclitaxel with no further complications and normal plasma sodium levels.

Although cisplatin is well known to cause SIAD, this is only the second report worldwide of carboplatin induced SIAD. Patients with cisplatin induced SIAD (which is common) are often changed to carboplatin to avoid this complication; however this report shows that carboplatin can also cause severe hyponatraemia. Patients receiving carboplatin should have their plasma sodium urgently measured if they develop severe nausea, headache or altered mental status.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1394

### Evaluation of ejaculatory function in acromegalic men: preliminary study

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Introduction
Acromegaly is a systemic disease frequently characterized by hypogonadotropic hypogonadism, endothelial dysfunction and diseases classically associated with an increased incidence of erectile dysfunction (ED) (hypertension, dyslipidemia, impaired glucose metabolism) and premature ejaculation (prostatic disease). Furthermore, the high prevalence of ED may negatively affect ejaculatory function. The purpose of this study was to evaluate the ejaculatory function of patients with acromegaly.
Patients and methods
Seventy-two patients (47.8 ± 8.6 years) were enrolled into the study. Among these patients, 48 were in remission (7 after surgery and 41 in medical therapy) and 26 in active disease (21 newly diagnosed and 5 in medical therapy). All patients were subjected to the questionnaire for the assessment of sexual function (IIEF-15), to a questionnaire for the detection of premature ejaculation (PEDT-premature ejaculation diagnostic tool) consisting of five questions and to a transabdominal prostatic ultrasound. Patients were divided into two groups according to normal (group A) or high (group B) levels of IGF1.
Results
ED was present in 23 of 72 patients (32%) and premature ejaculation (PE) was present in 13 patients (18%). No statistically significant difference was found between groups A and B with respect to the scores of all IIEF scales, the score of PEDT and the prevalence of erectile dysfunction and premature ejaculation (9/49 vs 4/23). The prevalence of PE in acromegalic patients with ED (4/23, 17%) was not different from that of patients without ED (9/49, 18%). The score of desire was significantly lower in patients with PE compared to patients without PE (7.6 ± 1.6 vs 6.4 ± 2, $P < 0.005$). Prostate disorders (prostatitis and hypertrophy) were observed in 50% of patients. The prevalence of PE was not significantly different between patients with and without prostatic diseases.
Conclusions
Premature ejaculation does not have a high prevalence in patients with acromegaly, despite the high presence of prostatic diseases. Further studies may clarify these observations.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1395

### Evaluation of male sexual function in active acromegalic patients and after disease remission: preliminary study

M. Galdiero[1], P. Vitale[1], L. Grasso[1], F. Lo Calzo[1], F. Cariati[1], F. Lotti[2], M. Maggi[2], A. Colao[1] & R. Pivonello[1]
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Introduction
Acromegaly is frequently characterized by hypogonadotropic hypogonadism, endothelial dysfunction, hypertension and impaired glucose and lipid metabolism, classically associated with impaired sexual function (SF). There are no literature data on the SF in acromegaly. The purpose of this study was to evaluate the SF of patients with acromegaly.
Patients and methods
Twenty-two patients (47.8 ± 8.6 years) were enrolled into the study. Among these patients, 48 were in remission (7 after surgery and 41 in medical therapy) and 26 in active disease (21 newly diagnosed and 5 in medical therapy). All patients were subjected to the questionnaire for the evaluation of the SF (IIEF-15), together with the evaluation of the clinical profile and of glycolipid and hormonal status. Patients were divided into two groups according to normal (group A) or high (group B) IGF1 levels.
Results
ED was found in 23 patients (32%). In particular, 15 of 49 (30.6%) patients of group A showed ED (14 with mild ED and one with moderate ED) whereas 8 of 23 (35%) patients of group B showed ED (four with mild ED and four with severe ED) ($P$ = NS). There was no significant difference in the scores of all IIEF-15 scales subjects between group A and group B. Testosterone ($P < 0.05$), 17β-oestradiol ($P < 0.005$) and LH ($P < 0.05$) levels were lower while PRL ($P < 0.05$), HbA1c ($P < 0.05$) and insulin ($P < 0.005$) levels were higher in group B than in group A. In patients with ED, BMI ($P < 0.05$) and waist circumference ($P < 0.05$) were significantly higher while the score of desire ($P < 0.001$) was significantly lower than those without ED. The score ED-IIEF correlated positively with orgasm ($P < 0.05$), desire ($P < 0.01$) and the presence of testosterone replacement therapy ($P < 0.05$) and negatively with BMI ($P < 0.05$) , PRL ($P < 0.05$), history of previous cardiovascular events ($P < 0.05$) and GH levels ($P < 0.05$). BMI was the major predictor of the score-DE of IIEF. Overall satisfaction correlated negatively with PRL ($P < 0.05$) levels and use of drugs altering sexual function ($P < 0.05$).
Conclusions
Acromegaly is associated with a high prevalence of ED that does not seem to be completely resolved by normalization of IGFI after disease remission.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1396

### The clinical outcome of pituitary adenomas in the cavernous sinus

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Introduction
Pituitary adenomas with cavernous sinus involvement (CSI) are associated with serious clinical sequelae but are often incompletely excised during surgery. Contradictory viewpoints exist regarding management of residual tumors in the cavernous sinus with some authors suggesting use of adjuvant radiotherapy post-operatively for long term tumor control. But radiotherapy has been associated with hypopituitarism and increased mortality due to cerebrovascular causes. We investigated the clinical outcome of pituitary adenomas in the cavernous sinus.
Methods
Patients with pituitary adenoma and CSI (diagnosed radiologically ± surgical exploration) and subsequently managed with surgery alone (S), radiotherapy alone (R), surgery with adjuvant radiotherapy (S + R) or medical management (M) were prospectively observed for adverse clinical events. Adverse clinical events were defined as i) impingement on cranial nerves (III, IV, V1, V2 and VI) situated in the cavernous sinus and ii) increase in size of the residual tumor of more than 30%. Data are as mean ± S.D.
Results
Sixty patients (31 males, 29 females) with pituitary adenomas (31 non functioning pituitary adenoma, 16 acromegaly, 4 cushing's disease, 9 prolactinoma) with age at CSI diagnosis 51.4 ± 14.5 years, were followed up post CSI for 67.4 ± 50.1

months. Thirty-four patients were managed with S, 6 with S + R, 2 with R and 18 with M. Patients in all four groups had stable disease and no adverse clinical events were noted over this follow-up period.
Conclusion
The outcome with post-operative radiotherapy for residual pituitary adenomas was similar to management with surgery or radiotherapy alone. Moreover, medical management alone, in selected cases, also yielded similar results. From this data, it appears that risk of progression of the residual pituitary adenoma in the cavernous sinus is low and routine radiotherapy for prophylaxis in this subgroup may not be justified.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1397

**Outcome of transsphenoidal surgery for Cushing's disease dependent on tumor size: a single center experience**

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Introduction
Transsphenoidal surgery (TSS) currently presents treatment of choice for Cushing's disease (CD). Dependent on tumor size, remission rates after initial TSS range from 66 to 94%. In ACTH-secreting pituitary macroadenomas, remission rates are reported to be lower. Visible adenomas on preoperative MRI or intraoperatively and neurosurgical expertise might contribute to successful TSS.
Design
A retrospective, single center analysis in 51 CD patients.
Results
All patients with ACTH-secreting pituitary micro- or macroadenomas underwent TSS as initial treatment for CD. Overall, 45.9% of the CD patients were in remission after initial TSS. In the subset of microadenomas, a remission rate of 48% was observed, followed by a lower remission rate in the group of macroadenomas (41.7%).
54.1% of the patients (52% with microadenomas, 58.3% with macroadenomas) experienced a relapse after initial TSS. Mean time until relapse was $27.58 \pm 26.00$ months. 29.7% of the patients with persistent hypercortisolism after initial TSS (28% with microadenomas, 33.3% with macroadenomas) underwent second TSS. In 17.6% of these patients, second TSS was carried out in the same neurosurgical center where initial TSS took place. After second TSS, biochemical remission of the disease was documented in 67.6% of the patients (72% with microadenomas, 58.3% with macroadenomas). A subset of patients (27%, 24% with microadenomas, 33.3% with macroadenomas) experienced a relapse after second TSS. Mean time until second relapse was shorter ($13.25 \pm 16.76$ months).
68.6 and 13.7% of the CD patients, respectively, underwent TSS in a center of neurosurgical expertise. There was no significant correlation between lack of neurosurgical expertise and relapse rate after initial or repeated TSS.
Conclusion
CD patients with macroadenomas presented with lower remission rates after initial or repeated TSS and a higher chance of experiencing a relapse postoperatively. Interestingly, lack of neurosurgical expertise did not correlate with a higher relapse rate after initial or repeated TSS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1398

**Effects of medical therapy of acromegaly on glucose metabolism**

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Introduction
Acromegaly is associated with alterations of glucose metabolism. The effect of somatostatin analogues (SMS) and pegvisomant (PEG) on glucose metabolism is still argument of debate.
Study design
The purpose of this historical-prospective study was to compare, in a cohort of 47 patients with active acromegaly, the effects of SMS and PEG alone or in combination on glucose metabolism. All subjects were evaluated at baseline and at least 6 months after therapy changes with fasting chemistry evaluation and OGTT. All patients were initially treated with SMS; 21 were controlled (SMS-contr); those uncontrolled received pegvisomant in combination with SMS (PEG-SMS), and, thereafter only PEG (PEG).
Results
Fasting blood glucose was higher in groups receiving SMS (SMS, SMS–PEG) than at baseline or PEG even after correction for biochemical control of disease ($P < 0.0001$). Mean glucose concentrations at OGTT's were higher in SMS groups ($P = 0.0004$; associated with a higher prevalence of IGT and DM) than in the PEG group. The insulin was reduced in all groups compared to baseline ($P < 0.05$) regardless of the type of treatment. The insulin sensitivity, reduced at baseline, improved after therapy (HOMA-IR, QUICK-I), especially in groups with biochemical disease control (SMS-cont, SMS-PEG and PEG, $P < 0.05$). Moreover, in subjects receiving SMS was observed a reduction in HOMA-$\beta$ values.
Conclusion
PEG, at variance with SMS, is not associated with changes in blood glucose levels. Either SMS or PEG therapy were associated with improvement in insulin sensitivity, probably due to the reduction in GH levels or to the reduction of its action. The deterioration in glycaemic control observed with SMS may be secondary to inhibition on direct insulin secretion as suggested by the reduction in the values of HOMA-$\beta$. In conclusion, the type of medication used in acromegaly may have a significant impact on glucose metabolism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1399

**Insulin resistance and the effect of treatment on insulin resistance in patients with prolactinoma**

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Aim
The relationship between hyperprolactinoma and insulin resistance has been shown in many studies. It is also known that hyperprolactinoma causes changes in carbohydrate and lipid metabolism. The aim of our study is to evaluate insulin resistance in patients with prolactinoma and see the effect of treatment on insulin resistance and karotis intima media thickness (CIMT).
Material and methods
Twenty-two female patients diagnosed biochemically and radiographically with prolactinoma were included in the study. None of the patients were treated previously. Median age of the patients was $30.0 \pm 10.2$ years. Cabergolin was given as treatment, starting with 0.5 mg/day and tapered necessarily. Boold samples were taken for prolactin, sensitive CRP(s-CRP), homocystein, LDL-cholesterol, HOMA score, prior to treatment and 6 months after starting treatment. The body mass index (BMI) was measured and CIMT was evaluated for each patient prior to and 6 months after the treatment.
Results
The prolactin levels before and after treatment was $145.5 \pm 66.4$ and $12.4 \pm 7.2$ µg/l respectively ($P < 0.001$). This decrease was not related to the decrease in BMI($r = -0.057$, $P = 0.808$). HOMA score before and after treatment was 1.25 (0.22–4.5) and 1.02 (0.24–4.1) respectively ($P = 0.024$). This decrease was not related to the decrease in prolactin levels ($r = -0.248$ vs $P = 0.279$). Homocystein levels before and after treatment was 13.8 (7.0–28.0) and 8.5 (2.3–26.4) ($P < 0.001$) respectively. CIMT before and after treatment was $0.58 \pm 0.15$ and $0.52 \pm 0.12$ ($P < 0.05$) respectively. The decrease in CIMT was not found to be related with the decrease in prolactin levels, HOMA score and BMI ($r = 0.250$, $P = 0.274$).
Conclusion
Treatment with cabergolin causes decrease in HOMA score and decrease in CIMT after 6 months of treatment independant from the decrease in BMI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1400

### Effects on GH/IGF1 hypersecretion and tumor size of long-acting somatostatin analogue (Sandostatin LAR) in patients with untreated acromegaly and in previously treated with surgery and/or radiotherapy

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Objectives
To comperate the efficacy of sandostatin LAR therapy in patients with untreated acromegaly and in previously treated with surgery and/or radiotherapy.
Material and methods
We studied 16 consecutive patients (seven women and nine men, aged 29–71 year). Fourteen patients harboured a macroadenoma and two microadenoma. The study was retrospective examination of untreated and previously treated patients with surgery and/or radiotherapy based on the macedonian acromegaly registry. Six patients had received surgery + radiotherapy and seven patients had received only surgery prior to receiving medical therapy. Three patients due to their co-morbidity were unable to receive surgery and as a primary treatment received sandostatin LAR.
Results
Sandostatine LAR was administered i.m. at a dose 20 mg every 28 days for 6 months. Three patients were inoperable due to severe LVH and received SSA as a first line therapy. This treatment induced a significant decrease of GH ($2.1 \pm 0.4$ ng/ml) and IGF1 ($156 \pm 6.2$ ng/ml) in all 16 patients. After six months of treatment, the percent IGF1 suppression was $74.1 \pm 4.5\%$ and serum GH/IGF1 values were normalized in 13 patients. Tumour shrinkage occurred in 18.7% of patients. Among 16 patients two improved glycaemic control based on fasting and postprandial plasma glucose levels.
Conclusion
OCT-LAR is an effective agent in alleviating symptoms, suppressing GH, normalizing IGF1 and inducing tumour shrinkage in many acromegalic patients. Overall, OCT-LAR is well tolerated and should be recommended for nonsurgically cured acromegalics, and also be considered as primary therapy for selected cases, mainly for those with a low probability of surgical cure.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1401

### Management of hyponatremia in the hospital: interim results from a prospective, observational, multi-center, global registry

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Background
Hyponatremia (HN) is the most common electrolyte disorder of hospitalized patients. It occurs in up to 28% of in-patients, is more common in the elderly and patients with multiple co-morbidities, increases the in-hospital risk of death by 1.47-fold, and is associated with significantly higher mortality risk following discharge. The HN Registry is the first large-scale, international effort to document the clinical characteristics, choice of therapies, and impact of HN in the hospital setting.
Methods
After informed consent or waiver, medical records of patients meeting the registry entry criteria, principally age >18 years and euvolemic or hypervolemic HN (serum sodium ($(Na^+)$) <130 mmol/l) were abstracted. Accrual to date is ~59% of the projected enrollment of 3500 patients. Data are summarized by sample size (*n*) and percentage (%) for categorical data, and mean and standard deviation for continuous data.
Results
A total of 812 of the 2051 patients enrolled at 215 (US = 143, EU = 72) sites between study initiation in September 2010 and December 2011 had sufficient data for analysis. The mean entry and discharge ($Na^+$) values were $126.8 \pm 7.3$ and $131.8 \pm 5.0$ mmol/l respectively for all patients and $125.9 \pm 7.6$ and $132.1 \pm 4.6$ mmol/l respectively for SIADH patients. Additional results are below.
Conclusions
These interim data suggest that initiation of tolvaptan therapy is delayed and often used as therapy after fluid restriction or saline has failed. Hypertonic saline had the highest response rate but was associated with a greater risk of overly rapid correction (>12 mmol/l in 24 h) than other therapies. Some patients received multiple therapies and are therefore represented in multiple categories, limiting between group interpretations. Additional data, including results for other pharmacologic therapies, will be reported as sufficient data becomes available for meaningful analysis. An adjudication process will be performed to eliminate patients inadvertently enrolled with hypovolemic HN.
Results

Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
This work was supported, however funding details unavailable.

**Table 1**

| | **All patients** (*n*=812) | **SIADH patients** (*n*=265) |
|---|---|---|
| Treatment (*n*) - No treatment - Tolvaptan - Fluid restriction - Normal saline - Hypertonic saline | 36% (293) 8% (68) 40% (325) 32% (262) 7% (55) | 27% (71) 8% (22) 48% (126) 47% (124) 12% (32) |
| Time to treatment (days) - No treatment - Tolvaptan - Fluid restriction - Normal saline - Hypertonic saline | NA 3.43±3.48 1.45±2.72 0.77±2.05 1.65±1.99 | NA 4.22±4.30 1.71±2.68 0.63±1.27 1.66±2.16 |
| Duration of treatment (days) - No treatment - Tolvaptan - Fluid restriction - Normal saline - Hypertonic saline | NA 3.11±2.46 5.60±4.88 2.70±2.27 2.38±1.71 | NA 2.64±2.63 4.85±4.06 2.75±2.25 2.38±1.88 |
| HN correction after treatment (Na>130 mmol/l) - No treatment - Tolvaptan - Fluid restriction - Normal saline - Hypertonic saline | 45% (133/294) 74% (51/69) 58% (202/350) 68% (191/282) 89% (50/56) | 39% (28/71) 73% (16/22) 64% (85/132) 62% (84/135) 88% (29/33) |
| Increase >12 mmol/l within 24 h of treatment - No treatment - Tolvaptan - Fluid restriction - Normal saline - Hypertonic saline | 2% (7/293) 6% (4/67) 2% (6/341) 4% (12/270) 16% (9/55) | 1% (1/70) 9% (2/22) 2% (3/130) 3% (4/127) 19% (6/32) |

## P1402

### Cardiac magnetic resonance imaging detects myocardial fibrosis in patients with active acromegaly

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Background
Acromegaly affects almost all organs, including the cardiovascular system. Long-term exposure to high levels of IGH and insulin-like-growth-factor 1 (IGF1) may lead to cardiomyopathy.
Study design
Aim of our investigation was to study cardiac function in patients with active acromegaly using magnetic resonance imaging of the heart (cardiac-MRI) and echocardiography. Eleven patients with active acromegaly could be evaluated within a period of 6 months.
Results
Mean age of the patients (six woman, five men) was 51.5 years (range 23–78). As IGF1 levels have age-related normal ranges, *Z*-scores were used to compare the endocrine tumor activity. Mean IGF1 value was 416.2 ng/ml ± 228.9 with a mean *Z*-score for all patients with 2.7.
Echocardiographic evaluation revealed a hypertrophic left ventricle in five patients (45.5%) with an enlarged left atrium in six patients (54.5%). Interventicular septal thickness was enlarged in seven acromegalic patients (63.6%). Mean septal thickness was 12.5 mm (24) ±2.3 in men and 12.3 mm ±3.0 in women. Diastolic dysfunction measured by early (E) to late (A) atrial peak velocities (E/A ratio) could be seen in three patients (27.8%). Systolic left ventricular ejection fraction (LVEF) was within the low normal range (mean 57.2% ±3.6) in all but one patient.
Cardiac MRI confirmed left ventricular hypertrophy in five patients. In four patients (36.4%) a late enhancement could be found in MRI imaging as a sign of myocardial fibrosis. Intramyocardial oedema coud nor be detected. All but one patient with pathologic findings in cardio-MRI or in echocardiography had *Z*-scores for IGF1 levels above 2.2.

Conclusion
Cardio-MRI gives additional information to standard echocardiographic examinations in the cardiovascular work-up of patients with acromegaly. For the first time cardiac fibrosis could be detected in a relevant proportion of patients with acromegaly by MRI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1403

**Pregnancies in a large cohort of patients on growth hormone replacement therapy**

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Introduction
Growth hormone replacement (GHRT) during conception and pregnancy is an off-label treatment. Reports on pregnancy outcomes in women with growth hormone deficiency (GHD) are derived from single center studies with small sample sizes and show conflicting results.
Methods/design
We evaluate pregnancies reported in the KIMS (Pfizer International Metabolic Database) patients on GHRT. KIMS includes 4651 women aged 15–50 years (followed for 19348 patient-years; median 3.10 years) and 7438 men aged above 16 years (followed for 36074 patient-years; median 3.89 years).
Results
There were 151 pregnancies reported in 131 patients of which 116 were in women (aged 22–41 years) and 15 in partners of male patients (aged 26–68 years). Only 14 women (10%) and one man had stopped GHRT before conception. Reported GHRT during pregnancy in females was as follows: continuation in 24% of the cases, withdrawal in 56% and dose reduction in 2%. Within the cohort that stopped GH during the pregnancy, 46% gave birth to healthy babies, 12% reported fetal loss, and outcome is not reported in 42% of the cases. Within the cohort that continued GH during pregnancy, 41% gave birth to healthy babies, 34% reported fetal loss, and outcome is not reported in 24% of the cases. At the time of abstract submission, birth of 82 healthy children (including twins and triplets) has been confirmed; 14 in women who continued GH during pregnancy and 6 with paternal exposure to GH.
Conclusions
In summary, here we provide the first demographic data on pregnancy rates in a large cohort of patients receiving GHRT. It appears that in the clinical practice setting, nearly all patients taking GH replacement continue treatment during the time when they seek fertility, and one-fourth continue it during pregnancy, but further data are needed and the work is ongoing.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
This work was supported, however funding details unavailable.

## P1404

**Pasireotide LAR vs octreotide LAR in patients with acromegaly: double-blind, crossover, extension period to a randomized, double-blind, multicenter, phase III study**

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Introduction
In a large, randomized, double-blind, phase III trial in patients with acromegaly, pasireotide LAR was significantly more effective than octreotide LAR at inducing GH $<$ 2.5 μg/l and normal IGF1 after 12 months of therapy (core study). The crossover phase of this trial allowed patients without full biochemical control at month 12 to switch treatments. This abstract reports the results of patients who switched therapy.
Methods
Medically-naïve patients with active acromegaly (GH $>$ 5 μg/l or GH nadir $\geq$ 1 μg/l post-OGTT, and IGF1 $>$ ULN) who were *de novo* with a visible adenoma on MRI or post-surgical, and who completed 12 months' therapy with pasireotide LAR 40–60 mg/28day or octreotide LAR 20–30 mg/28day, could enter a double-blind extension phase. Patients with GH $<$ 2.5 μg/l and normal IGF1 at month 12 could continue on their randomized therapy, whereas patients with GH $\geq$ 2.5 μg/l and/or IGF1 $>$ ULN (age and sex related) could switch treatment at month 13 to either pasireotide LAR 40 mg/28day or octreotide LAR 20 mg/28day, with dose titration allowed at 3-month intervals. Significance testing was not planned or performed during the extension phase.
Results
80.1% (141/176) and 85.7% (156/182) of pasireotide LAR and octreotide LAR recipients completed the 12-month core study. 81 patients switched to pasireotide LAR; 38 patients switched to octreotide LAR. GH $<$ 2.5 μg/l *and* normal IGF1 was achieved by 21.0% (17/81) and 2.6% (1/38) of patients 6 months after switching to pasireotide LAR and octreotide LAR, respectively; GH $<$ 2.5 μg/l was recorded in 43.2% (35/81) and 31.6% (12/38); normal IGF1 was recorded in 30.9% (25/81) and 7.9% (3/38). The most common AEs (pasireotide LAR and octreotide LAR) were hyperglycemia (25.9 and 7.9%), diarrhea (21.0 and 15.8%), nasopharyngitis (16.0 and 18.4%) and headache (18.5 and 10.5%).
Conclusions
In patients with uncontrolled acromegaly after 12 months' therapy with octreotide LAR, switching to pasireotide LAR improved biochemical control. The safety profile of pasireotide LAR was similar to that of octreotide LAR, with the exception of hyperglycemia. Pasireotide LAR may provide a pituitary-targeted alternative treatment option for patients inadequately controlled with currently available somatostatin analogues.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P1405

**Long-term use of pasireotide in Cushing's disease: 24-month safety results from a randomized phase III study**

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Introduction
Rapid and sustained decreases in UFC and significant improvements in signs and symptoms were seen in a large, randomized, 12-month phase III study of pasireotide in Cushing's disease. The safety profile of pasireotide was found to be similar to that of other somatostatin analogues, with the exception of hyperglycemia-related AEs (reported in 72.8% of patients). This abstract reports safety data from a 12-month extension to this phase III trial.
Methods
Patients with persistent/recurrent or *de novo* (if not surgical candidates) Cushing's disease and UFC levels $\geq$1.5xULN were randomized to pasireotide 600 μg ($n$=82) or 900 μg ($n$=80) sc bid. Patients who had mean UFC $\leq$ ULN or were achieving clinical benefit from pasireotide at month 12 (end of core) were eligible to enter the extension. Dose titration was permitted at the investigator's discretion (min: 300 μg sc bid; max: 1200 μg sc bid). Data on AEs were collected throughout the extension.
Results
Fifty-eight patients entered the extension phase and 19 discontinued prior to month 24. After 24 months' pasireotide treatment, UFC was decreased by a mean of 59.5% (95% CI: −68.6, −50.5). During the core and extension phases, 98.1% of patients experienced $\geq$1 AE, and 25.9% experienced $\geq$1 SAE. There were no deaths. The safety profile in the extension phase was similar to that in the core; the majority of reported AEs were mild-to-moderate GI events. During the 24-month treatment period, 40.1% and 29.6% of patients had an AE of hyperglycemia and diabetes mellitus respectively. Mean $HbA_{1c}$ increased from 5.8% at baseline to 7.2, 6.8 and 6.8% at months 12, 18 and 24 respectively. In addition to the patients who reported these events during the core study, 2 additional patients experienced

SAEs during the extension, 3 reported mild-to-moderate cholelithiasis, one reported a newly occurring QTcF >480 ms, and 2 had a >30 ms increase in QTcF. Three patients discontinued because of an AE during the extension. Further 24-month efficacy results are reported in a separate abstract (Schopohl *et al.*).
Conclusion
The long-term safety profile of pasireotide was similar to that reported following 12 months' pasireotide treatment, including the proportion of patients with hyperglycemia-related AEs. These results support the use of pasireotide as a long-term treatment for Cushing's disease.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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## P1406

**Pasireotide treatment is associated with improvements in hypertension: 12-month results from a large phase III study in Cushing's disease**

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Introduction
Patients with Cushing's disease (CD) have an increased risk of hypertension (HTN). phase III data have shown that pasireotide leads to rapid reductions in UFC levels and significant improvements in CD symptoms. We now present further analyses of these data, evaluating the effects of pasireotide on HTN in patients with CD.
Methods
Patients with persistent/recurrent or *de novo* (if not surgical candidates) CD and UFC ≥ 1.5 times the upper limit of normal (ULN) were randomized to pasireotide 600 μg ($n=82$) or 900 μg ($n=80$) sc bid. Systolic (SBP) and diastolic blood pressure (DBP) were evaluated by single measurements at baseline ($n=162$) and month 12 ($n=78$). Baseline HTN was defined as at least one of: history of anti-HTN medications; medical history of HTN; SBP > 130 mmHg; or DBP > 90 mmHg. Addition of medications for HTN was allowed per investigator discretion.
Results
At baseline, 77.8% of patients had HTN ($n=126$), of whom 97 took anti-HTN medication during the study. Mean SBP decreased from 133.5 mmHg at baseline to 126.1 mmHg at month 12 (−6.1; 95% CI: −9.8, −2.4). Mean DBP decreased from 86.3 to 82.8 mmHg (−3.7; 95% CI: −6.2, −1.2). When stratified by UFC response, these improvements were only significant in those with UFC ≤ ULN at month 6. Significant improvements from baseline to month 12 were seen in patients with baseline HTN (SBP: −8.0 (−12.4, −3.6); DBP: −4.7 (−7.7, −1.7)). There were no significant changes in SBP or DBP in patients without baseline HTN (SBP: 0.2 (−6.1, 6.4); DBP: −0.4 (−4.6, 3.9)). Decreases in DBP tended to be larger in patients with higher baseline DBP (Fig. 1); similar trends were seen with SBP. A >5 mmHg decrease in DBP was seen in 42% (33/78) of patients at month 12. This was higher in those with baseline HTN (50% (30/60)) than in those without (17% (3/18)). Of those with baseline HTN, a >5 mmHg decrease in DBP was seen in 63% (10/16) of patients who did not take anti-HTN medication during the study and in 46% (20/44) of those who did. Adverse events of hypotension and adrenal insufficiency were reported by 11 and 9 patients, respectively; others were as expected for a somatostatin analogue, except degree of hyperglycemia.
Conclusions
In addition to rapid and sustained decreases in UFC, pasireotide treatment is associated with significant decreases in SBP and DBP in patients with CD.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
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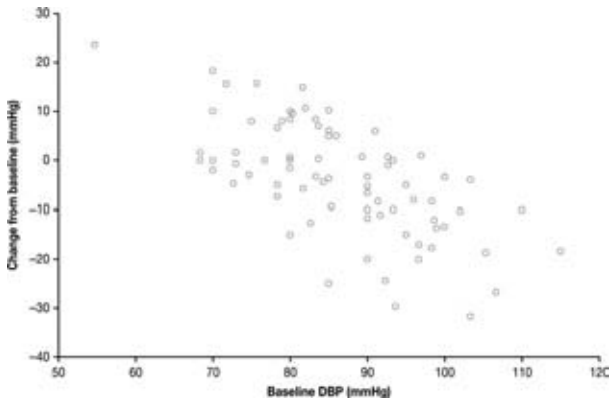


**Figure 1** Changes in DBP by baseline DBP

## P1407

**GH Secretion response to triple secretagogue stimulus (ghrh, ghrelin and arginine) is gender and BMI dependent in healthy postmenopausal women and older men**

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Objective
Identify predictors of pulsatile GH secretion following a triple secretagogue stimulus (TSS, Ghrelin and GHRH preceded by an infusion of Arginine) in a cohort of healthy postmenopausal women and healthy older men.
Hypothesis
Even in older subjects, total GH secretory capacity is gender dependent.
Design
23 men and 19 women participated in 6 separate, overnight 16-h infusion sessions at Clinical Research Center. At the end of each visit, all volunteers received TSS infusion (arginine, 30 gm for 30 min IV infusion followed by GHRH and ghrelin boluses, both at 1.0 μg/kg). GH response was monitored by sampling blood (0.75 ml) every 10 min at the start of the arginine infusion and for 2 hours afterwards.
Methods
We used deconvolution analysis to characterize pulsatile GH release after triple secretagogue infusion. We analyzed time to peak, peak GH and GH mass/burst responses in both men and women by multivariate analysis, using baseline GH, gender, BMI, IGF-I, IGFBP1, and IGFBP3 as independent variables (results reported in table as mean ± SEM).
Results
Total and peak GH responses to TSS are two-fold greater in women than in men and inversely correlated with BMI in both genders (see figure).
Conclusion
Positive effect of female gender on GH secretion might reflect capability of estrogen to increase synergy between GHRH and Ghrelin and blunt inhibition by somatostatin, possibly via greater inhibitory effect of arginine on somatostatin in women. Negative effect of obesity implies reduced GHRH release and/or heightened somatostatin inhibition. These data are the first to show a major gender effect on total GH secretory capacity.

**Table 1** Basic characteristics and Results

| | Women ($n=19$) | Men (n=23) | *t*-test(p-value) |
|---|---|---|---|
| Age (years) | 64 ± 1.4 | 61 ± 1.9 | 0.210 |
| BMI (kg/m2) | 26 ± 0.7 | 28 ± 0.6 | 0.198 |
| Baseline GH (μg/L) | 0.63 ± 0.3 | 0.26 ± 0.07 | 0.155 |
| GH mass/burst (μg/L) | 209 ± 23 | 98 ± 11 | 0.001 |
| Peak GH (μg/L) | 99 ± 10 | 45 ± 4.8 | 0.001 |
| AUC (μg/L*min) | 5665 ± 581 | 2591 ± 290 | 0.001 |
| IGF-I (μg/L) | 150 ± 15 | 175 ± 15 | 0.368 |
| IGFBP1 (μg/L) | 40 ± 4 | 30 ± 3 | 0.044 |
| IGFBP3 (mg/L) | 3 ± 0.1 | 3 ± 0.2 | 0.068 |
| GH time to peak (min) | 11 ± 0.5 | 10 ± 0.5 | 0.212 |

Results analyzed by deconvolution analysis for GH pulses, stepwise regression analysis for GH response based on gender and BMI and paired t-test for basic characteristics analysis based on gender.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector

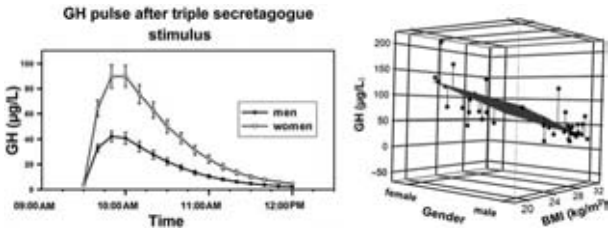


**Figure 1** Left panel: Average GH pulse after triple secretagogue stimulus in men and women. Right panel: Surface plot of GH response based on BMI and gender.

## P1408

### Pasireotide treatment is associated with clinically meaningful improvements in health-related quality of life in Cushing's disease: results from a large, randomized, double-blind phase III trial

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Introduction
Patients with Cushing's disease have significantly impaired health-related quality of life (HRQoL). Effective treatment is needed to treat Cushing's disease and improve HRQoL; however, there are currently no approved medical treatments for Cushing's disease. The effect of pasireotide on HRQoL in patients with Cushing's disease was evaluated as part of a randomized, phase III study.
Methods
Patients with persistent/recurrent or *de novo* (if not surgical candidates) Cushing's disease and UFC levels $\geq$1.5 times the upper limit of normal (ULN) were randomized to pasireotide 600 µg ($n=82$) or 900 µg ($n=80$) SC bid. Dose titration (max: 1200 µg bid) was allowed after month 3. UFC control was defined as UFC $\leq$ ULN, partial control as UFC $>$ ULN and $\geq$50% reduction from baseline, and uncontrolled as UFC $>$ ULN and $<$50% reduction from baseline. HRQoL was assessed at baseline and months 3, 6 and 12 using the 12-item CushingQoL questionnaire. Items were scored on a 5-point scale, resulting in a score of 12 (worst) to 60 (best) standardized to 1–100. A change in CushingQoL score of $>$10.1 has been estimated to be clinically meaningful.

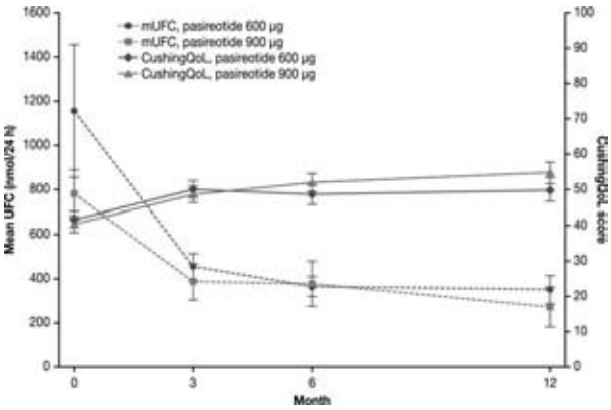


**Figure 1** Mean UFC and CushingQoL scores

Results
Overall, HRQoL increased from 41.1 at baseline ($n=159$) to 52.5 at month 12 ($n=76$) (mean increase: 11.1; 95% CI: 6.8, 15.5). In both dose groups, HRQoL improved from baseline along with rapid and sustained decreases in UFC levels (Fig.1). There was a clinically meaningful improvement in HRQoL at month 12 in patients whose UFC levels were controlled ($n=29$; mean improvement: 12.8; 95% CI: 7.1, 18.5) or partially controlled at month 6 ($n=17$; mean improvement: 10.7; 95% CI: 0.8, 20.5), but the improvement in HRQoL did not reach the 10.1 change threshold in the uncontrolled group ($n=30$; mean improvement: 9.9; 95% CI: 2.3, 17.6). The greatest HRQoL improvements ($\geq$20 points) were seen in patients with the greatest UFC decreases (from $>$10xULN to $\leq$5xULN; $n=5$). Significant correlations ($P<0.01$) were seen between changes in CushingQoL scores and changes in mean UFC ($r=-0.40$; $n=68$), BMI ($r=0.31$; $n=74$), weight ($r=0.32$; $n=74$), and Beck depression inventory score ($r=-0.59$, $n=72$).
Conclusion
Pasireotide treatment decreases UFC level and improves HRQoL. Clinically meaningful improvements in HRQoL are seen regardless of achieving fully normalized UFC.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
This work was supported, however funding details unavailable.

## P1409

### Increased risk of hypothalamic pituitary dysfunction amongst naso-pharyngeal cancer survivors with the use of concurrent chemo-irradiation

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Background
Radiotherapy is the mainstay of NPC (nasopharyngeal carcinoma) treatment and recently there is increased use of concurrent chemo-irradiation (CCRT) to improve survival. The irradiation field for NPC includes the base of skull, risking radiation damage to the hypothalamic–pituitary (HP) axis.
Aims
To evaluate the prevalence of HP dysfunction in NPC survivors post-irradiation and to compare the risk of developing HP dysfunction amongst patients who had CCRT versus radiotherapy alone.
Methods
We recruited 58 patients (33 males/25 females) with no known hormonal dysfunction, who completed radiotherapy for NPC more than 3 years ago. All patients had baseline cortisol, ACTH, GH, IGF1, $fT_4$, TSH, FSH, LH, oestradiol/testosterone and prolactin measured at 8am after an overnight fast. In addition 49 of the 58 patients underwent dynamic testing with the insulin tolerance test (ITT).
Results
All patients received a standard dose of external beam radiotherapy of 70 Gy to the posterior nasal space. 43 patients received concurrent chemo-irradiation while 15 had radiotherapy alone. Median age: 56 (48.75–63.25) years and median time post-irradiation 8 (6.0–11.25) years. Hypopituitarism was present in 84% of patients, 31% with involvement of a single axis, 29% with two axes, 18% with three axes and 6% with four axes. The prevalence of GH, corticotroph, gonadotroph, thyrotroph deficiency and hyperprolactinaemia was 80, 41, 19, 3 and 25% respectively. The development of HP dysfunction was significantly associated with the use of CCRT when compared with the radiotherapy alone group, OR: 14.57 (2.42–28.6), $P=0.01$.
Conclusion
HP dysfunction post-irradiation is widespread amongst NPC survivors with the most common being GH-deficiency. Radiation-induced damage appears to be profoundly increased with the use of radio-sensitising chemotherapy. As these endocrinopathies result in significant morbidity/mortality we recommend periodic assessment of pituitary function post-irradiation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1410

### Long-term pasireotide use leads to improvements in the biochemical parameters of Cushing's disease: 24-month results from a randomized phase III study

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Introduction
The large, randomized, phase III study of pasireotide in Cushing's disease found that pasireotide treatment resulted in rapid and sustained decreases in UFC levels and in significant improvements in signs and symptoms over 12 months of treatment. A 12-month extension of this trial has recently completed, and the results are reported here.
Methods
Patients with persistent/recurrent or *de novo* (if not surgical candidates) Cushing's disease and UFC levels $\geq$1.5 times the upper limit of normal (ULN) were randomized to pasireotide 600 μg ($n=82$) or 900 μg ($n=80$) SC bid. Patients who had mean UFC $\leq$ ULN at month 12 or who were achieving clinical benefit from pasireotide were eligible to enter an additional 12-month extension. Dose titration was permitted at the investigator's discretion (min: 300 μg SC bid; max: 1200 μg SC bid). UFC, serum cortisol and plasma ACTH were recorded every 3 months during the extension.
Results
Fifty-eight patients entered the extension, and only these patients are included in the analyses below. A mean decrease in UFC of 54.7% (95% CI: −71.8, −37.6) was observed from baseline to month 12, which was sustained throughout the extension phase; mean decreases of 55.7% (95% CI: −71.1, −40.3) and 59.5% (95% CI: −68.6, −50.5) were seen at months 18 and 24, respectively. Using a last observation carried forward analysis, 34.5% of patients had UFC $\leq$ ULN at month 24. A significant decrease from baseline in mean serum cortisol was observed at month 12 (−15.5%; 95% CI: −22.4; −8.6) and at month 24 (−17.8%; 95% CI: −26.9; −8.7). A similar trend was observed for plasma ACTH, with a reduction from baseline to month 12 of 16.2% (95% CI: −29.1, −3.4) and to month 24 of 13.9% (95% CI: −25.5, −2.2). Adverse events (AEs) during the extension phase were consistent with those reported during the core study and were predominantly gastrointestinal and hyperglycemia related. Further safety results are reported in a separate abstract (Bertherat *et al.*).
Conclusion
Long-term pasireotide use resulted in sustained improvements in UFC, serum cortisol and plasma ACTH over 24 months. These results support the use of pasireotide as a long-term treatment for Cushing's disease in responsive patients.
Declaration of interest
I fully declare a conflict of interest. Details below:
Funding
This work was supported, however funding details unavailable.

## P1411

### Hyperprolactinemic patients are prone to increased protein oxidation: a risk for low grade inflammation?

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Objective
Hyperprolactinemia is reported to be associated with impaired glucose metabolism, and low-grade inflammation. There is some data regarding increased oxidative stress- particularly protein oxidation in the conditions like metabolic syndrome or diabetes, but not in hyperprolactinemic subjects in the literature. We aimed to investigate advanced oxidation protein product (AOPP) levels-an established marker of protein oxidation and antioxidant thiol levels in hyperprolactinemic patients with prolactinomas.
Patients and Methods
Thirty- four patients (24 women and 10 men) with prolactinoma, aged 37.50 ± 10.98 years and twenty (age, gender and BMI matched) healthy volunteers as a control group were enrolled to the study. The patients with macroadenomas and mass effects were underwent to transsphenoidal adenomectomy, while the others received medical therapy with a dopamine agonistic agent- cabergoline (1–8 mg/week). AOPP and thiol levels were measured at baseline and after 3–6 months following normalisation of prolactin levels.
Results
Advanced oxidation protein product levels were higher in patients compared to healthy controls at baseline ($P=0.002$), a significant decrease was observed in patient group following prolactin normalisation. Thiol levels were found to be increased in hyperprolactinemic patients and remained higher ($P=0.016$, $P=0.008$ respectively) after treatment.
Conclusion
Our results revealed the presence of protein oxidation in hyperprolactinemic patients. We conclude that thiol levels increase to protect these patients from oxidative stres. The controlling duration may not be enough long to observe normalization of antioxidant thiol levels. Further studies are required in order to determine the role of oxidative stress in the pathogenesis of impaired glucose metabolism, and low-grade inflammation seen in hyperprolactinemic patients.
Key Words: Hyperprolactinemia, oxidative stres, AOPP, thiol.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Comparison of patients and healthy volunteers before and after treatment.

| | Levels of AOPP (μmol/l) | Levels of thiol (μmol/l) |
|---|---|---|
| Patients before treatment $n=34$ | 400.03 ± 208.15 349.61 (258.97–582.84) | 215.21 ± 60.64 225.61 (188.26–250.76) |
| Patients after treatment $n=29$ | 316.32 ± 175.41 277.68 (179.61–394.29) | 215.41 ± 46.40 207.32 (185.98–254.57) |
| Healthy volunteers $n=20$ | 275.85 ± 143.52 261.87 (169.77–287.19) | 177.56 ± 53.59 179.88 (153.96–216.46) |
| *P* Before treatment - after treatment | 0.002 | 0.682 |
| *P* Before treatment - healthy volunteers | 0.003 | 0.016 |
| *P* After treatment - healthy volunteers | 0.314 | 0.008 |

## P1412

### Fifteen years of GH replacement improves body composition and metabolic parameters

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Objective
Few studies have determined the effects of more than 5-10 years of GH replacement in adults on body composition and metabolic parameters.
Design/Patients
In this prospective, single-center, open-label study, the effects of 15 years of GH replacement on body composition and metabolic parameters were determined in 156 hypopituitary adults (93 men) with adult onset GH deficiency (GHD). Mean age was 50.5 (range 22–74) years at study start. Body composition was measured using dual-energy X-ray absorptiometry (DXA).
Results
The mean initial GH dose of 0.55 (SEM 0.03) mg/day was gradually lowered to 0.40 (0.01) mg/day after 15 years. The mean serum insulin-like growth factor 1 (IGF1) SDS increased from −1.53 (0.10) at baseline to 0.74 (0.13) at study end ($P<0.001$ vs baseline). Body mass index (BMI) increased from 27.7 (0.4) to 28.3 (0.4) kg/m$^2$ ($P<0.01$). Lean soft tissue increased to 3% above baseline level after 15 years ($P<0.001$). Body fat decreased 9% during the first year of treatment ($P<0.001$ vs baseline), then increased and had returned to baseline level after 15 years. Total cholesterol decreased from 6.0 (0.1) to 5.5 (0.1) mmol/l and LDL cholesterol decreased from 4.0 (0.1) to 3.3 (0.1) mmol/l (both $P<0.001$ vs baseline). HDL cholesterol increased from 1.2 (0.03) to 1.4 (0.04) mmol/l ($P<0.001$ vs. baseline). There was an increase in fasting plasma-glucose from 4.4 (0.1) at baseline to 4.8 (0.1) mmol/l at study end ($P<0.001$). However, HbA1c decreased from 5.0 (0.1) % to 4.6 (0.1) % ($P<0.001$).
Conclusions
Fifteen-year GH replacement in GHD adults induced a transient decrease in body fat and sustained improvements of lean soft tissue and lipid profile. Fasting plasma glucose increased whereas HbA1c was reduced.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1413

**Incidence of hypogonadism and evaluation of its impact on cardiovascular risk factors and quality of life in acromegalic patients**

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Acromegaly and hypogonadism represent independent cardiovascular risk factors, worsening either different metabolic parameters or quality of life (QoL).

In our cohort of 41 acromegalic males (20–82 years), 35 resulted affected by hypogonadotropic hypogonadism, while only six were eugonadal. Twenty-nine out 35 had low testosterone levels at diagnosis and six developed a post-surgical hypogonadism (testosterone cut-off: 350 ng/dl). Among hypogonadal subjects, only ten initiated hormone replacement therapy (HRT) while 25 do not. At diagnosis and at the last follow-up, we evaluated in the entire cohort: i) QoL by the AcroQoL questionnaire; ii) hormonal (GH, IGF1, prolactin, total testosterone) and iii) clinical and metabolic parameters (BMI, blood pressure, total cholesterol, HDL, LDL, triglycerides, fasting glucose, HbA1, uric acid). These parameters were correlated with disease control and testosterone levels at diagnosis and at the last follow-up.

Based on the latest criteria for disease control, at the last visit 28 patients were controlled, 4 uncontrolled and 9 partially controlled. As expected, IGF1 levels at the last visit were significantly lower than at diagnosis ((IGF1 SDS group 1:1.8$\pm$1.1, group 2 IGF1 SDS: $-0.9\pm1.3$ ($P<0.01$)), whereas testosterone levels were significantly increased, compared to the levels at the diagnosis, even in patients who did not underwent to HRT ($P<0.05$). AcroQol showed a corresponding score's improvement, while clinical and metabolic parameters were similar in both groups.

In conclusion, the incidence of hypogonadism in acromegalic subjects is very high at diagnosis, however, it improves significantly with disease control, independently from the therapeutic strategies. Hypogonadism may be partially related to the presence of the adenoma and improves with the treatment of the disease (improvement of testosterone levels also in subjects without HRT). Finally, the main factor that can reduce cardiovascular risk seems to be the achievement of disease control.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1414

**GH secretory function is well preserved in surgically cured acromegalics**

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Objective
To know the GH secretory function in surgically cured acromegaly and its impact on quality of life of the patients.
Subjects and methods
Sixty-seven acromegalics were judged to be surgically cured according to the Cortina consensus criteria and underwent postoperative insulin tolerance test (ITT) provoking hypoglycemia with nadir blood sugar under 50 mg/dl. All the patients underwent surgery under combined microscopic and endoscopic observation by skilled pituitary surgeons with experiences of more than 500 transsphenoidal surgeries. The incidence of GH impairment and GH deficiency (GHD) was determined based on the peak GH during ITT. QOL was evaluated by two sets of questionnaire (SF-36 and JAHQ: Japan adult hypopituitarism questionnaire) studies.
Results
The postoperative incidence of impairment of GH secretory function was 6.0% (4/67) for severe GHD; peak GH during ITT $<1.8$ ng/ml, 6.0% (4/67) for moderate GHD; 1.8 $<$ peak GH $<3.0$ ng/ml, and 13.4% (9/67) for slightly impaired GH; 3.0 $<$ peak GH $<6.0$ ng/mL. GH secretory function was perfectly preserved, peak GH $>6$ ng/mL, in 74.6% (50/67) of the subjects. Physical component of SF-36 QOL score of the patients positively correlated with peak GH during postoperative ITT. Both physical function score and social & mental QOL scores of Japan adult hypopituitarism questionnaire (JAHQ) tended to correlate with peak GH during postoperative ITT.
Conclusion
The incidence of GHD (peak GH during ITT $<3$ ng/ml) was low as 12% in our surgically cured acromegaly. The low incidence seems owing to the sophisticated surgical technique for selective adenomectomy under combined microscopic and endoscopic observation. Considering patients' QOL positively related to the peak GH during postoperative ITT, pituitary surgeon must pay further attention to preserve pituitary function during adenomectomy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1415

**The effect of GH replacement therapy on exercise capacity, fat mass, ectopic lipids intramyocellular and intrahepatocellular lipids and insulin resistance in hypopituitary patients with GH deficiency**

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Introduction
Increased levels of ectopic lipids (IMCL and IHCL) have been related to insulin resistance. Aerobic exercise affects IMCL. GHRT is known to increase exercise capacity, insulin resistance and decrease fat mass. The effect of GHRT on ectopic lipids is not known.
Methods
Ten patients with GHD and ten sedentary control subjects (CS; matched for gender, age, BMI and waist) were recruited. $VO_{2max}$ was assessed using an exercise test on a treadmill. Insulin sensitivity was determined by a two-step hyperinsulinaemic euglycaemic clamp. By MR-imaging visceral (VAT) and subcutaneous (SCAT) fat compartments were quantified. IHCL and IMCL were measured before and after a 2-h aerobic exercise at 50–60% of $VO_{2max}$ using MR-spectroscopy. Free fatty acid concentrations (FFA) were determined during exercise. Identical investigations were performed after 6 months GHRT.
Results
Four female, 6 male GHD patients and matched CS volunteered for this study. $VO_{2max}$ tended to be reduced in GHD compared to CS and significantly increased after GHRT. Basal FFA and AUC FFA during exercise were similar in GHD, CS and following GHRT. SCAT and VAT was similar in GHD and CS. GHRT significantly decreased SCAT and VAT. Pre-exercise ectopic lipid levels were similar in CS, GHD and GHRT. 2h-aerobic exercise resulted in a significant decrease in IMCL (δ-IMCL) and increase in IHCL (δ-IHCL) in CS, GHD and GHRT. GHRT did not significantly impact on δ-IMCL and δ-IHCL. HOMA-value tended to be decreased in GHD compared with CS. GHRT resulted in a non-significant increase in HOMA. Endogenous glucose production and glucose disposal rate were similar in GHD, CS and following GHRT.
Conclusions
-GHRT results in an increase in $VO_{2max}$ and a decrease in SCAT and VAT.
-Aerobic exercise acutely impacts on ectopic lipids in GHD, CS and GHRT.
-Ectopic lipids (IMCL and IHCL) are similar in GHD, CS and following GHRT.
-GHD is not associated with insulin resistance when compared with CS matched for waist.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1416

### Prevalence of central adrenal insufficiency during lifespan in Prader–Willi syndrome

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Introduction
The etiology of the increased mortality seen in Prader–Willi syndrome (PWS) is not completely known. In this context, it has been suggested that central adrenal insufficiency (CAI) due to hypothalamic dysfunction may be responsible for unexplained deaths. However, data on hypothalamic-pituitary–adrenal (HPA) axis in PWS are still conflicting. Because adrenal insufficiency is a life-threatening disease, its diagnosis should be precise, urgent and reliable. In this light, low dose short synachten test (LDSST) seems to be highly sensitive in the evaluation of the integrity of the HPA in patients with CAI. Aim of this study we investigated the prevalence of CAI during a LDSST in a group of children and adults with PWS.
Methods
132 subjects with genetically confirmed PWS, 66 males, were evaluated. 79 patients were younger than 18 years (PED) (mean age 7.4 + 4.8 years) whereas 53 were adults (ADU) (mean age 27.8 + 6.9 years). Baseline morning ACTH and cortisol were measured, following which, the LDSST started with the i.v. injection of 1 μg tetracosactrin. A peak cortisol response at 30 min $<$ 18.1 μg/dl was considered for diagnosing CAI.
Results
Basal ACTH and cortisol levels were 19.8 ± 11.8 ng/l (mean ± S.D.) (nv: 8; –50 ng/l) and 12.7 ± 6.2 μg/dl (nv: 5-25 μg/dl) in PED, and 22.4 ± 17 ng/l and 12.2 ± 5.1 μg/dl in ADU. The mean peak cortisol after LDSST was 27.0 + 7.5 μg/dl in PED and 22.3 + 4.9 μg/dl in ADU ($P < 0.001$). Pathological cortisol peak response to the LDSST was registered in seven PED (8.8%) and eight ADU (14.8% $P < 0.01$).
Conclusions
Our results support the view that CAI may be part of the PWS. Moreover, it seems that HPA axis gradually declines with age. On the basis of these data, we suggest to perform adrenal testing as soon as possible in all PWS. In case of normal results, further retesting should be scheduled periodically.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1417

### Limited effects of temozolomide monotherapy on aggressive pituitary tumors. -Based on our own experiences of three cases-

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Back ground
Encouraging responses of pituitary adenomas and pituitary carcinomas to temozolomide treatment have been well described and loss of immunopositivity of *O*6-methyl-guanine-DNA methyltransferase (MGMT) reportedly serves a predictor of good response. Recently some nonresponsive cases, however, also appeared. We describe here our own experiences of temozolomide treatment on three aggressive pituitary tumors.
Subjects and methods
Three pituitary tumors, a prolactin producing carcinoma, a prolactin producing atypical adenoma, and a growth hormone producing atypical adenoma were treated with 25, 3, 5 cycles, respectively, of temozolomide with dose of 200 mg/m$^2$ × 5 days/ month. The immunohistochemical expression of MGMT in these tumor specimens was also evaluated.
Results
All the patients well tolerated to the treatments and no serious adverse effects were recorded. The prolactin producing carcinoma with sparse positivity (8%) to MGMT responds well to the treatment until 19 cycles but recurred under the continuation of the treatment. But the prolactin producing atypical adenoma negative to MGMT and the growth hormone producing atypical adenoma with sparse positivity (5%) to MGMT did not show any response to the three cycle or five cycles of treatment.
Conclusion
Considering good tolerance to the regimen, and good response in at least one carcinoma case of our series, and prior encouraging reports, temozolomide treatment may be a choice for aggressive pituitary tumors resistant to multimodality of treatment. But, true response ratio of aggressive pituitary tumors to this treatment, predictive factors of response and escape from effect, and long term effect should be elucidated by prospective and longitudinal multicenter study.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1418

### The impact of long-acting somatostatin analogs treatment on glucose tolerance and insulin resistance in acromegaly.

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Introduction
Impaired glucose tolerance and insulin resistance are frequently associated with acromegaly. The aim of this study was to assess the impact of long-acting somatostatin analog treatment on glucose homeostasis in acromegalic patients.
Patients and methods
In this prospective study 16 naïve acromegalic patients (eight females, eight males; aged 51.5 ± 10.9 years) were studied before and after 3-month therapy with long-acting somatostatin analog (i.e. octreotide LAR 20 mg i.m. every 28 days). Diagnosis of active acromegaly was established on the basis of widely recognized criteria. In each patient glucose and insulin concentrations were assessed during the 75 g oral glucose tolerance test (OGTT) before and after 3 months of treatment. HbA1c levels were evaluated baseline and after 3 months of therapy. To estimate insulin sensitivity we performed hyperinsulinemic euglycemic clamp and calculated homeostasis model assessment (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI).
Results
We did not found any statistically significant change in plasma glucose concentrations either fasting or during OGTT ($P > 0.05$). A significant reduction in HbA1c levels was observed (6.54 ± 1.72% vs. 6.02 ± 0.78%) after 3 months of treatment. A prominent decrease in insulin secretion during OGTT was found after octreotide LAR treatment compared to the baseline (4.4 ± 2.0 vs 12.1 ± 9.6 mIU/ml, $P < 0.001$). After 3 months of therapy there was an improvement in insulin resistance: a significant reduction in HOMA-IR (0.92 vs 2.27, $P < 0.05$) and a significant increase in QUICKI (0.39 vs 0.34, $P < 0.05$) values. In euglycemic clamp method we found a statistically significant increase in glucose disposal rate, i.e. *M* value (4.52 ± 2.34 vs. 2.37 ± 1.24 mg/kg per min).
Conclusions
We concluded that the treatment of acromegaly with octreotide LAR improve insulin sensitivity and HbA1c levels, even if it does not show any effect on glucose concentrations during OGTT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1419

**Immunohistochemical detection of sstr2 and 5 ligand binding domains in 110 pituitary tumors**

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Classical somatostatin analogues (SST-A), such as octreotide and lanreotide, bind mainly SSTR2 whilst the multiligand pasireotide binds with the highest affinity SSTR5. The selective immunodetection of ligand binding domain (LBD) of SSTR subtypes with specific monoclonal antibodies may be useful to explain the potential efficacy of different SSTA on pituitary tumours growth and/or secretory activity.
Patients and Methods
We applied new monoclonal antibodies (Y-SSTR MoAbs, Ypsilon biotechnology, Naples, Italy) recognizing the second extracellular loop of SSTR2 and SSTR5 for the immunohistochemical screening of SST-LBD expression in an archival series of 110 pituitary tumours: 43 PRL-secreting, 45 ACTH-secreting, 22 non secreting. We used formalin fixed/paraffine embedded tissue sections from pituitary adenomas and, as control, sections from normal tissue areas surrounding the same tumor. A standard streptavidin-biotin-labeled peroxidase immunostaining was performed. Negative controls were performed with non immune serum. The degree of immunopositivity was evaluated semi-quantitatively according to an arbitrary scale.
Results
A specific immunostaining reaction for SSTR2 was detectable in 90% of PRL-secreting tumors with medium-high intensity and in 80% of ACTH-secreting tumors with low-medium intensity. Immunostaining for SSTR5 was found in all but 2 of PRL-secreting adenomas with variable intensity from low-medium to high and in 100% of ACTH secreting adenomas with intensity from medium to high. No immunostaining for SSTR2 and SSTR5 was observed in non-secreting adenomas.
Conclusions
Y-SSTR2 and 5 MoAbs can be used to detect the presence of SST-LBD on the membrane of pituitary cells in classical paraffin embedded histological sections. The presence of SSTR LBD on adenomatous cells may address the choise of either classical or panligand SST analogues for the medical treatment of pituitary adenomas, when surgery is not indicated or fails.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1420

**Symptomatic hypotonic hyponatraemia after endoscopic transsphenoidal surgery: results from two large series**

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Introduction
The incidence of hyponatremia as a delayed complication of transsphenoidal surgery varies widely from study to study and much of the debate has focused on the symptomatic hypotonic hyponatremia (SHH) occurring usually after discharge.
Aim/Design
We studied the incidence, risk factors, course and management of postoperative SHH in two large series of endoscopic transsphenoidal surgery (ETS) for pituitary tumors.
Results
The first series included 1199 consecutive surgical procedures carried out during the last 13 years by a single surgeon in Italy (87% adenomas, 10% craniopharyngiomas/Rathke's cleft cysts, 3% miscellaneous lesions). 103 patients were excluded on the basis of exclusion criteria (postoperative triphasic diabetes insipidus, diuretics, drug-induced SIADH, scant lab data). A total of 1096 eligible patients were identified. Of these, 50 (4.5%) developed SHH, which occurred after discharge in any patients but three. Nadir of medians of SHH (126 mmol/l) occurred on postoperative day 8. Clinical data and laboratory tests were consistent with SIADH in all the patients. Age, tumor size and tumor type did not correlate with the development of SHH. Conversely females were more likely to develop SHH than males (F/M: 2.8/1). Treatment consisted of fluid restriction in 42% and hypertonic saline in the remaining 52%. No case of osmotic demyelination syndromes occurred. The second series included 316 consecutive surgical procedures done during a 34-month period ending March 2011 by a single surgeon in USA (72.8% adenomas, 15% craniopharyngiomas/Rathke's cleft cysts, 2.8% arachnoid cysts, 9.2% miscellaneous lesions). SHH developed in 16 patients (5%). Again, clinical and laboratory data were consistent with SIADH in all the patients. Nadir of medians of SHH (123 mmol/l) occurred on postoperative day 7. The major risk factor was the presence of a macroadenoma.
Conclusion
The incidence of SHH in patients undergoing ETS was about 5% in both series, but different risk factors were identified (i.e. gender in the first series, macroadenoma in the second series). Interestingly, the incidence of SHH in this study was identical to that found in a previous study designed by one of us for the standard microscope transsphenoidal approach some years ago.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1421

**Audit of pituitary dysfunction after traumatic brain injury: caution in interpretation of glucagon stimulation test in diagnosis of GH and ACTH deficiency.**

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Introduction
Traumatic brain injury (TBI) is the leading cause of death and disability in young adults. It is important to recognise pituitary dysfunction following TBI as it can cause significant morbidity.
Methods
179 TBI patients (137 males) age 41.0 ± 15.7 years (17.6–88.1 years) attended Charing Cross Hospital clinic (July 2009–August 2011). Median time since TBI was 0.27years (0.05–46.5 years, 26% > 1 years). 34% had an intracranial bleed and 9% needed craniotomy. 20% had an absolute contraindication for insulin tolerance testing (ITT) and 42% a relative contraindication. Glucagon stress test (GST) was performed in 135 patients to assess GH and ACTH deficiency. A cortisol < 350 nmol/L or GH < 5 μg/l was considered abnormal. To confirm deficiency, patients who failed GST had a GHRH-Arginine test (GHRH-A) to assess GH response; a Metyrapone suppression test (MST) to assess ACTH deficiency; or a water deprivation test (WDT ) to assess for diabetes insipidus.
Results
No patients had TSH deficiency (0/174) or diabetes insipidus (11/174 had normal WDT), one patient had syndrome of inappropriate ADH. Two patients had asymptomatic hyperprolactinaemia (2/169) and three had gonadotrophin deficiency (3/170). 30/135 had a GST peak cortisol < 350 nmol/l, but only 1/19 had confirmation of ACTH deficiency by failing a MST. None of the 14 patients with GST peak cortisol > 350 nmol/l failed a MST. 35/135 had a GST peak GH < 3 μg/l (25.9%) and 55/135 peak GH < 5 μg/l (40.7%), but only 3/12 failed GHRH-A. Initiation of GH replacement in appropriate patients resulted in symptomatic improvement.
Conclusions
GST has a high false positive rate in diagnosing both GH and ACTH deficiency, likely due to the low pre-test probability of true pituitary dysfunction in this group. Thus, a second confirmatory test should be performed. In our cohort, clinically significant pituitary dysfunction was confirmed in 10/174 patients (5.7%). It is important to identify these patients, as treating the hormonal dysfunction is therapeutically beneficial.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1422

**Body composition has a higher impact on peak GH during the pyridostigmine–GHRH test than the insulin tolerance test in healthy individuals**

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Objective
To evaluate the association between body-composition and peak GH, during two standard GH stimulation tests; the pyridostigmine-GHRH (PDGHRH) test and an insulin tolerance test (ITT).
Method
83 healthy subjects (56 men), aged 18–65 years with a median BMI of 24 kg/m$^2$ (range 17–35) underwent a PDGHRH test and an ITT in random order 1 week to 1 month apart. Women had both tests performed in the same phase of their menstrual cycle. Body composition was assessed by BMI, waist circumference, waist-to-hip (W/H) ratio, total fat percent (TF%) and total (TFM) and abdominal fat mass (abdFM) assessed by dual energy X-ray absorptiometry.
Results
Peak GH was significantly higher in response to PDGHRH stimulation compared to the ITT (mean 73.8 mU/l versus 42.1 mU/l; $P < 0.0001$). Men and women had similar peak GH values during both tests ($P > 0.5$). In men peak GH in response to the PDGHRH test was negatively correlated to BMI ($r = -0.64$), waist ($r = -0.62$), W/H ($r = -0.59$), TF% ($r = -0.69$), TFM ($r = -0.69$) and abdominal fat mass ($r = -0.68$) (all $P < 0.0001$). In women similar associations were present for BMI ($r = -0.69$), TFM ($r = -0.70$) and abdFM ($r = -0.71$).
Neither men nor women demonstrated significant correlations between peak GH in response to the ITT and body-composition measures.
Conclusion
Body composition had a more prominent inhibitory effect on the results of the PDGHRH test as compared to the ITT in healthy individuals. No gender differences were observed. Measures of central adiposity were not superior to BMI in predicting peak GH in response to the PDGHRH test in healthy individuals. The GH response by ITT seems more resistant to influence from adiposity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1423

**Progression of acromegalic arthropathy despite long-term biochemical control: a prospective, radiological study**

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Background
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Background
Arthropathy is an invalidating complication of acromegaly, despite persisting biochemical disease control, which has a high impact on the quality of life (QoL). The prognosis and determinants are currently unknown.
Objectives
To investigate radiographic progression of arthropathy during 2.5-year follow-up and to assess determinants of outcome in patients with long-term well-controlled acromegaly.
Methods
Fifty-eight patients (mean age 62, women 41%) with controlled acromegaly for a mean of 17.6years were included in a prospective cohort study. 40 patients(69%) were cured by surgery and, if necessary, additional radiotherapy, 18 (31%) patients were controlled by somatostatin (SMS) analogs. Radiographic progression of joint disease was defined by the Osteoarthritis Research Society International (OARSI) classification as a one point increase in joint space narrowing (JSN) or osteophyte scores on radiographs of the hands, knees, and hips obtained at the first study visit and after 2.5 years. Potential risk factors for progression were assessed.
Results
Progression of osteophytes and JSN was observed in 72% and 74% of patients, respectively. Higher severity of radiographic arthropathy features at the first study visit was associated with more radiographic progression over 2.5years. Higher age and presence of d3-GH receptor (d3-GHR) polymorphism predisposed for osteophyte progression. Patients with biochemical control by SMS analogs had more progression of osteophytosis than surgically cured patients (OR = 18.9, $p = 0.025$), independently of age, sex, BMI, insulin-like growth factor1 (IGF1) SDS at baseline and d3-GHR. This was also evident for progression of JSN, as were higher age and higher baseline IGF1 SDS.
Conclusions
Acromegalic patients have progressive arthropathy according to both osteoarthritis (OA) features, despite long-term biochemical control. Parameters reflecting GH/IGF1 activity were associated with progressive joint disease. Remarkably, biochemical control by SMS analogs was associated with more progression than surgical cure, which may indicate insufficient GH control according to current criteria and the need of more aggressive therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1424

**Cavernous Sinus Sampling (CSS) - a specific method for localization of minute ACTH secreting adenomas in Cushing Disease (CD)**

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Introduction
Diagnosis and exact localization of minute adenomas in Cushing disease (CD) can be problematic, leading to unsuccessful trans-sphenoidal pituitary explorations (TSS). In addition to the well established inferior petrosal sinus sampling (IPS) first described in 1989, direct intraoperative cavernous sinus sampling (CSS) and in 1993, preoperative CSS with very small catheters, have been published (1,2). The experience in relatively small series had been promising, but is rarely discussed as an alternative to IPS (3). Therefore, we present our good results with CSS in a reasonably large number of CD cases.
Methods
consecutive trans-sphenoidal surgeries (1999 to 2010) in patients with CD were included in this prospective study. All had the triad of high cortisol in night salivary cortisol, dexamethasone suppression and stimulation of ACTH and cortisol after CRH. 60 patients (15%) were referred to our neuro-radiologists for CSS according to the technique of Teramoto (2). The indications for CSS were unclear endocrine tests in 7 and inconclusive high standard magnetic resonance imaging (MRI) in all 60.
Results
In two patients (3%) CSS was technically inadequate. Since the Cushing triad was positive, TSS was performed. No central-peripheral (CP) gradient was found in 7 patients with equivocal tests. In two the ectopic source was confirmed. Thus, we cannot exclude some false negative CSS as published for IPS (4, 5). From 51 patients with a clear CP gradient, the lateralisation was correct in 41 (80%). Of 6 patients with no lateralisation in CSS, four had midline, and two had bilateral adenomas. No severe complications related to CSS were encountered.
Conclusion
With CSS non-pituitary ACTH dependent Cushing could be diagnosed in 7 patients. In 92% of cases with unclear MRI, CSS was of essential help for the accurate detection of ACTH microadenomas and seems to be superior to IPS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1425

**Effects of medical treatment on proliferation parameters MIB-1 and topoisomerase-II$\alpha$ in GH secreting pituitary adenomas**

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Introduction
In this present study, we assessed the effects of the last preoperative medical treatment (dopamine-agonists, somatostatin-analogous and GH receptor

antagonists) on the proliferation parameters Ki-67 and Topoisomerase-IIα in pituitary adenomas of patients with acromegaly.
Material and methods:
We retrospectively studied the clinical characteristics, neuroimaging, histology and immuno-histochemistry (Ki-67 and topoisomerase-IIα labelling index) of 365 specimens of GH expressing pituitary adenomas collected between 2002 and 2010. According to the last medical treatments before surgery, these cases were divided into five groups: no medication, dopamine agonist, somatostatin analogues, combination of both or GH receptor antagonist.
Results:
As compared with adenomas which had no medical treatment, the group of somatostatin analogues treated tumours as well as those who have been treated with GH-receptor antagonists showed significant lower values of Ki-67- and topoisomerase-IIα -labelling index ($P<0.001$, $P=0.001$ respectively), while the other groups showed no significant difference. However, no significant correlation was found between the dosages or the duration of the medical treatment. In the group of macro- and invasive adenomas higher values of both proliferation parameters were observed as compared with microadenomas ($P<0.001$) or non-invasive adenomas ($P<0.001$, $P=0.001$ respectively).
Conclusions:
Interestingly in this analysis as well as somatostatin-analogue and GH-receptor antagonist treatment is associated with a lower proliferation profile in GH secreting adenomas instead of the supported idea that peripheral GH-receptor blockade might lead to an increased tumour proliferation. From our point of view the reduced proliferation indices in the GH receptor group is the result of the previous attempt of somatostatin-analogue treatment which failed to control GH-secretion and therefore patients were put on GH-receptor antagonists.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1426

**Comprasion of cycline D1 gene (Ccnd1) polymorphism in invasive and non-invasive prolactinomas**

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Cycline D1 gene that plays a crucial role in a cell cycle, have been shown to be expressed more in nonfunctioning pituitary adenomas and functioning invasive pituitary adenomas compared to normal hypophisis tissue and noninvasive pituitary adenomas. A/G polymorphism in CyD1 gene exon 4/intron 4 regions have been demonstrated to affect clinical outcomes and survey in some tumors. Objective of this study was to investigate the effect of polymorphism of cycline D1 gene on the tumor invasiveness in prolactinomas.
A hundred and thirteen patients followed-up in the Uludag University, Department of Endocrinology and Metabolic Diseases, with the diagnosis of prolactinoma were included in the study. The patients were divided into two groups as noninvasive and invasive tumors according to radiological findings. The cyclin D1 gene A870G polymorphism was determined in all patients with PCR.
Rates of GG, GA and AA genotypes were found as 11.8, 55.9 and 32.4%; respectively, in the noninvasive group, while these rates were defined as 15.6, 44.4 and 40.0%; respectively, in the invasive group. Although the rate of AA genotype was found higher in the invasive than in the noninvasive group, the difference was not statistically significant. The rate of A allele was found as 60.3 and 62.2%, while the rate of G allele was defined as 39.7 and 37.7% in the noninvasive and invasive groups; respectively. A/G ratio in the total alleles was found as 1.52 in the noninvasive group and 1.65 in the invasive group.
Although A allele incidence was slightly higher in the invasive adenomas compared to the noninvasive adenomas, this difference also was not statistically significant. It did not affect the features of the tumor defined in the imaging findings, reveals the result of this could not be demonstrated as a factor in prognosis of the disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1427

**Control of GH and IGF1 in acromegaly in the UK: responses to medical treatment**

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UK National Acromegaly Register collects data on real-life clinical practice in 34 centres. We analysed all GH and IGF1 data to assess adequacy of control on medical treatment (Rx) with somatostatin analogs (SMS) and dopamine agonists (DA).
Methods
All GH records (basal, profile or GTT) in database were correlated with IGF1, Rx, surgery (TSS) and radiotherapy (RT), then processed to derive summary data for each patient and each course of Rx. GH considered controlled if $\leq 2$ μg/l or $\leq 5$ mU/l.
Results
GH records identified in 2572 patients (50% ♂). 23 107 patient-years of observation. 70% patients had TSS, 45% RT; 4206 courses of Rx included SMS in 40.6% and DA 41.4% of patients. Overall control of GH and IGF1 improved with time both on and off Rx, e.g. on Rx, post-TSS + RT, GH was controlled in 18/34/60% pre1990/1990's/2000's (IGF1 –/36/52%, both –/19/40%). Table shows responses in 2000's to all Rx courses + to latest course of duration >360 days (last). Overall SMS were more effective than DA, but differences were less marked where this was last chosen treatment. Control achieved with octreotide LAR and lanreotide autogel was similar at last treatment, but review suggested that maximum effective dose was not always used. For DA, control on cabergoline in 2000's was better than on bromocriptine in 1990's, but the responsive minority who continued bromocriptine long-term in 2000's achieved better control. For both SMS and DA, % age control of GH/IGF1 worsened substantially with increasing GH levels pre-course off-Rx. Overall, control was less good in courses on Rx before TSS/RT and better in courses after TSS/RT, probably representing a lower pre-course GH.
Conclusions
GH and IGF1 control is improving with time but control of both is still only achieved on Rx in a minority of courses. SMS and DA can both achieve control in a useful proportion of patients. Unsuccessful TSS/RT may still improve responses to subsequent medical Rx.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1**

| | # Courses | GH control (%) | IGF1 control (%) | Both control (%) | Basal GH (%) |
|---|---|---|---|---|---|
| SMS all | 923 | 57 | 51 | 39 | 48 |
| SMS last | 251 | 75 | 69 | 55 | 37 |
| Octreotide LAR last | 157 | 76 | 71 | 58 | 36 |
| Lanreotide autogel last | 52 | 75 | 63 | 47 | 38 |
| DA All | 398 | 50 | 36 | 26 | 75 |
| DA Last | 92 | 77 | 55 | 45 | 72 |
| SMS + DA All | 302 | 44 | 34 | 20 | 44 |
| SMS + DA Last | 66 | 50 | 51 | 32 | 38 |

## P1428

### Low tumoral mRNA IGF2 might balance the mechanism of tumorigenesis in GH-secreting adenomas

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Pituitary adenomas may show alterations of PI3K/Akt pathway. IGF2, binding the IGF1-R, is able to active it. LOI of IGF2 has been implicated in the pathogenesis of many tumours. Activation of PI3K produces phosphorylation of p27 and its cytoplasmic mislocalization. Loss of nuclear p27 was associated with worse prognosis. In this study we correlated IGF2 imprinting with activation of PI3K/Akt pathway in a series of 29 somatotropinomas. After surgery, tumors were embedded in RNAlater and stored at −20 °C. Macroadenomas ($n=25$) were defined as tumors >10 mm and 18 of them as aggressive, because of cavernous and/or sphenoidal sinus invasion and/or Ki-67 ≥3%. Tumoral total RNA was isolated and RT-PCR was performed. ApaI polymorphic site in exon 9 of IGF2 was used to evaluate allelic expression. qPCR showed the tumoral expression of IGF2 normalized with β-glucuronidase (high expression: IGF2 copies/β-glu copies >2). Immunohistochemistry analysis with p-Akt (Ser 473) and p27 (C-19) antibodies was perfomed in 21 tumours. Cytoplasmic or nuclear staining was assessed as negative (negative or weak, <10% of the cells) or positive (moderate or strong). Fourteen out of 29 patients (48.2%) exhibited LOI of IGF2 in the tumour. Thirteen of 14 tumours with LOI had IGF2 <2 vs 7/15 without LOI ($P=0.009$). Seventy-six % (19/25) of macroadenomas presented IGF2 <2 vs 25% (1/4) of microadenomas ($P=0.076$). Most aggressive tumours (15/18, 83%) showed IGF2 <2, whereas 6/11 (54.5%) non-aggressive tumours had IGF2 >2 ($P=0.043$). The pattern of nuclear p27 immunostaining seemed opposed to that of cytoplasmic p-Akt ($P=0.063$). Tumors with LOI of IGF2 tended to show loss of nuclear p27 staining ($P=0.063$), without reaching statistical significance. In conclusion, LOI seems not to alter IGF2 gene expression in GH-secreting tumours. Low levels of IGF2 mRNA would be related to aggressive clinical traits, suggesting that IGF2 might counteract somatotropic tumorigenesis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1429

### Synergistic effects of combined stimulation with CRH + ghrelin on ACTH and cortisol responses in patients with Cushing's disease: a pilot study

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Background

Several stimulatory tests for differential diagnosis of ACTH dependent Cushing's syndrome are avilable, CRH being the most valuable test in patients with Cushing's disease (CD). Exaggerated responses to ghrelin have also been shown in patients with CD mainly due to overexpression of GHS receptors on corticotrope tumor cells. However, the effects of combined stimulation with CRH + ghrelin on ACTH and cortisol secretion have not been tested before in these patients.

Objective

To compare ACTH and cortisol responses to ghrelin, CRH and CRH + ghrelin in patients with Cushing's disease.

Patients and methods

Six consequtive patients with diagnosed CD were included in the study. All patients were females, mean age 54.8 ± 16.7 years, with microadenomas on magnetic resonance imaging. Ghrelin test (1 μg/kg of body weight, i.v. bolus), CRH test (100 μg i.v. bolus) and combined CRH + ghrelin test were performed at least a week apart in random fashion. Blood was sampled at 0, 15, 30, 45, 60 and 90 min. Plasma ACTH (Cisbio Bioassays, France) and cortisol (Cisbio Bioassays, France) responses were measured and compared in all three tests. GH and prolactin (PRL) responses were also measured.

Results

Baseline mean ACTH and cortisol values were 31.5 ± 32.9 pg/ml and 562.5 ± 257.4 nmol/l respectively. No significant differences were found between peak ACTH and cortisol responses during ghrelin (ACTH 200.5 ± 344.9 pg/ml, cortisol 846.2 ± 351.9 nmol/l) and CRH stimulation (ACTH 90.7 ± 72.2 pg/ml and cortisol 1070.9 ± 141.9 nmol/l). However, significantly higher peak ACTH (359.6 ± 593 pg/ml, $P<0.05$) and cortisol (1312.8 ± 117.1 nmol/l, $P<0.05$) responses were found after combined stimulation with CRH + ghrelin compared to CRH test alone, suggesting a synergistic effect. Significant mean GH (peak 13 ng/ml) and PRL (peak 909.6 mU/l) responses were also noted after stimulation with CRH + ghrelin.

Conclusion

Combined stimulation with CRH + ghrelin exerts synergistic effect on ACTH and cortisol release in patients with CD. Combined stimulation with CRH + ghrelin is more powerful test than CRH alone.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1430

### Bone turnover in patients with active acromegaly in relation to glucose metabolism

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Objective

There is a complex cross-talk between bone and glucose metabolism. The surrogate markers of bone metabolism are osteocalcin (for bone formation) and CTx (for bone resorption). The osteoblast-derived protein osteocalcin has recently been shown to affect glucose homeostasis.

Aim of the study

To investigate the relationship between markers of bone metabolism and parameters reflecting bone composition, glucose homeostasis and insulin resistance in patients with chronic GH and IGF1 excess.

Patients

Twenty-three patients with active acromegaly were studied (11 males, age: 50.6 ± 3.4 years, BMI: 26.7 ± 0.9 kg/m$^2$, duration of disease 5.3 ± 0.6 years, IGF1 735 ± 46 ng/ml, GH level 15 ± 3 μg/l). Seven patients had microadenoma. Five patients were operated, one was treated with radiotherapy and none with somatostatin analogue. Eight patients had T2DM.

Methods

At baseline glucose, insulin, GH, IGF1, osteocalcin and CTx were measured. In nondiabetic patients an oral glucose tolerance test (OGTT) was performed. Peak and area under the curve (AUC) for glucose and insulin, indexes of basal insulin resistance (HOMA-IR) and basal insulin secretion (HOMA-%B) were analyzed. Body composition (body fat, lean body mass) was analyzed using dual-energy X-ray absorptiometry.

Results

In patients with active acromegaly both serum osteocalcin (49.9 ± 7.1 ng/ml) and CTx (1072 ± 139 pg/ml),as well as HOMA-IR (5.6 ± 0.6) were significantly increased,irrespective they had or did not have T2DM. HOMA-%B was significantly lower in diabetic patients (56.7 ± 30.6) compared with nondiabetic patients (137.4 ± 25.9; $P=0.05$). Osteocalcin positively correlated with CTx ($R=0.928$, $P=0.0001$). Osteocalcin and CTx inversely correlated with BMI ($P=0.01$) and CTx inversely correlated with percentage of fat mass ($R=-0.473$, $P=0.05$). Both bone markers correlated positively with IGF1 ($P=0.01$). Osteocalcin positively correlated with peak insulin during OGTT ($R=0.643$, $P=0.018$) and with AUCOGTT insulin ($R=0.522$, $P=0.05$).Bone markers were not associated with HOMA-IR, HOMA-%B or lean mass.

Conclusion

Active acromegaly is associated with high bone turnover. Serum osteocalcin is positively associated with IGF1 and insulin during OGTT. It is tempting to speculate that insulin and IGF1 promote osteocalcin production which in turn may act to improve glucose metabolism by stimulating beta cell secretion.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1431

**Relationship between gsp mutations and clinico-pathological features in GH producing pituitary adenomas**

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Purpose
To know the relationship between gsp mutations and clinico-pathological features in GH producing pituitary adenomas.
Subjects and methods
Somatotropinomas resected from 43 acromegalic patients, 18 males and 25 females, were examined for gsp mutation analysis. The mutation was detected in 25 of 43 (58.1%) tumours with alternations of Arg to Cys in codon 201 (68%), Arg to Ser in codon 201 (8%), Gln to Leu in codon 227 (20%) and Gln to Arg in codon 227 (4%).
Results
Incidence of gsp mutation tended to be higher in non-dot pattern (or densely granulated) adenomas (66.7%) than in dot in cytokeratin staining pattern (or sparsely granulated) adenomas (38.5%) ($P=0.083$). The tumour size was significantly smaller ($P=0.006$) and the GH-producing index was significantly larger ($P=0.007$) in adenoma with gsp mutations than those without gsp mutations. While all tumours with the mutation manifested an abnormal response to TRH challenge, this was the case in 50% of adenomae without gsp mutations ($P=0.001$). The ratio of abnormal response to LHRH also tended to be higher in adenoma with gsp mutations ($P=0.095$) We observed a good response to octreotide in 44.4% of adenoma with gsp mutations and in 18.2% of tumours without the mutation; the difference was not significant ($P=0.217$), possibly due to small sample size of cases who underwent octreotide test ($n=20$). The other clinico-pathological features (including age, sex, basal GH level and MIB-1 index) of the 43 patients were not significantly different, irrespective of their mutation status.
Conclusion
The existence of gsp mutation relates well to clinico-pathlogic features of growth hormone producing adenomas such as cytokeratin staining pattern, tumor size, and response to challenge test with TRH or LHRH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1432

**Decreased IGF1 levels and GH-resistant hepatic state to estrogens during the first trimester of pregnancy in non-acromegalic women**

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Introduction
In women with GH-secreting pituitary adenoma, a decrease of IGF1 levels has been reported during the first trimester of pregnancy, before placental GH (pGH) secretion inducing a progressive increase in IGF1 levels throughout gestation. This decrease has been related to hepatic GH-resistant state, via JAK2/STAT pathway, due to increased estrogen ($E_2$) levels.
Objectives
Evaluate IGF1 change in non-acromegalic women during the first trimester of pregnancy.
Patients and methods
Nine women (mean age $30.7\pm3.9$ years) were seen before and during pregnancy in the follow-up of benign thyroid disease. Plasma IGF1, GH, IGF-BP3, TSH and $E_2$ levels were measured before conception and during the first trimester of gestation ($6.7\pm1.5$ weeks of amenorrhea) before the onset of pGH secretion.
Results
Before conception, all women had normal GH/IGF1 secretion and euthyroid function tests (TSH before $=1.89\pm0.95$, 1st trimester $=1.79\pm1.17$ mU/l). During pregnancy, mean IGF1 levels decreased (before $=186\pm51$ ng/ml, 1st trimester $=141\pm44$ ng/ml, $P<0.05$) without significant change in GH (before $=2.03\pm2.01$ ng/ml, 1st trimester $=1.54\pm2.34$ ng/ml) or IGF-BP3 (before $=2.30\pm0.32$ ng/ml, 1st trimester $=2.07\pm0.31$ ng/ml) levels while $E_2$ levels increased (before $=56\pm35$ pg/100 ml, 1st trimester $=675\pm820$ pg/100 ml, $P<0.05$).
Conclusion
In this preliminary study we report a significant IGF1 decrease without change in GH and IGF-BP3 concentrations during the first trimester of pregnancy in non acromegalic women. Therefore, as previously reported in acromegalic women, the results suggest the existence of a GH-resistant state due to physiological $E_2$ secretion in normal pregnant women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1433

**Coexistence of macroprolactinaemia and hyperprolactinaemia in women with oligo-/amenorrhoea is associated with high risk of pituitary adenomas**

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Background
The so called 'big-big' prolactin, also known as macroprolactin is formed by prolactin-immunoglobulin complexes, is considered to be biologically inactive, but may cause elevation of serum prolactin (Prl) concentrations measured by standard assays. In women presenting with oligo- and/or amenorrhoea the cause of menstrual irregularity needs to be explained even in the setting of concomitant macroprolactinaemia. We have therefore attempted to assess the prevalence of pituitary pathology in women with macroprolactinaemia and oligo-/amenorrhoea.
Material and methods
We performed pituitary MRI scans in 60 women with oligo- and/or secondary amenorrhoea aged $31.0\pm6.7$ years (mean $\pm$ S.D.), range 18–45 years who were found to have raised Prl concentrations due to macroprolactinaemia, detected by the polyethylene glycol (PEG) precipitation method.
Results
After PEG precipitation of macroprolactin, 'free' Prl concentrations were still raised (i.e. above 530 mIU/l) in 35 (58%) women with macroprolactinaemia. Furthermore, pituitary microadenomas were detected in 9/60 (15%) and pituitary macroadenomas in 3/60 (5%) of women with macroprolactinaemia and oligo-/amenorrhoea. In all of these cases there was a concomitant elevation of 'free' Prl after PEG precipitation. The highest value of 'free' Prl was in a case of microadenoma (total Prl 19207 mIU/l, 'free' Prl after PEG precipitation 7738 mIU/l), while in the case of macroadenoma the highest concentration of 'free' Prl was 2798 mIU/l (total Prl 7441 mIU/l before PEG precipitation). Hence, in case of coexistence of macroprolactinaemia and raised 'free' Prl after PEG precipitation of macroprolactin, the chance of finding of either a micro- or a macroadenoma was as high as 34% (12 cases out of 35).
Conclusions
Hyperprolactinaemia and macroprolactinaemia may coexist in the same patient. If 'free' prolactin is still raised after PEG precipitation of macroprolactin, then the chance of finding of a pituitary micro- or macroadenoma in women with oligo-/amenorrhoea is about 30%. Therefore pituitary magnetic resonance imaging is mandatory in all such cases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1434

### Mortality rates in childhood and adult onset GH deficient patients enrolled in the Global Hypopituitary Control and Complications Study (HypoCCS)

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Preliminary data suggested an increased mortality in a French cohort after childhood somatropin treatment compared to the French reference population[1]. This cohort included patients (pts) with idiopathic-isolated GH-deficiency (GHD), and a similar cohort is also under follow-up for adult GH-replacement in HypoCCS.

We therefore assessed all-cause mortality rates (*n*/1000 person-years (PY), (95% CI), standardized to the age/gender structure of HypoCCS or the general population) in 7946 GH-replaced adult HypoCCS pts with available post-baseline information. Overall standardized (general population) mortality rate was 5.8 (4.3, 7.2) in the US HypoCCS cohort compared to 7.6/1000 PY in the US general population[2], and 5.2 (4.4, 6.1) in Europe, the rate in comparable populations ranging from 5.1/1000 PY in Italy to 6.4/1000 PY in Belgium[3]. As shown in the table, mortality rates were not different between onset-types overall and for non-idiopathic GHD, but were lower in childhood onset (CO) compared to adult onset (AO) pts with idiopathic GHD replaced with GH.

Of a total of 174 deaths, 14 occurred in CO pts and only in the non-idiopathic GHD group (acute illness/suicide (7), second/recurrent neoplasm (2), cerebral hemorrhage/carotid artery stenosis (2) or unknown reason (3)).

Although the Carel *et al*. study[1] raises the important question of increased adult mortality after somatropin treatment in childhood, the present analysis does not confirm an increased mortality in GH-replaced adult pts, either with CO or AO GHD, compared to reference populations. However, the present results are limited by selection bias and relatively short follow-up time.

1. J-C *et al*., *Endocr Rev* 2011, **32** LB-5.
2. Xu J *et al*., *National Center for Health Statistics*; 2010. May 20, **58**(19).
3. World Health Organisation Mortality Statistics. http://data.euro.who.int/dmdb/Help/inds.htm. Accessed: 22 March 2011.

Declaration of interest

I fully declare a conflict of interest. Details below:

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**Table 1** Standardized (HypoCCS population) mortality rates (*n*/1000 PY (95% CI)) in HypoCCS pts by onset-type

| Onset-type | **All** (*n*=7946) | **Idiopa-thicGHD** (*n*=1267) | **Idiopathic-isolated GHD**[a] (*n*=530) | **Non-idiopathicGHD** (*n*=6679) |
|---|---|---|---|---|
| CO (*n*=1528) | 3.2(0.2,6.2) | 0 | 0 | 5.4 (0.2,10.6) |
| AO (*n*=6418) | 5.4 (4.5,6.3) | 7.0 (3.9,10.1) | 3.7 (0,7.8) | 5.3 (4.3,6.2) |

[a]Sub-group of idiopathic GHD.

## P1435

### Experience in the use of tolvaptan in patients with syndrome of inappropriate antidiuretic hormone secretion (SIADH)

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Introduction

Hyponatremia is the most common electrolyte disorder in hospitalized patients. SIADH is characterized by euvolemic hyponatremia with sodium and urine osmolality inappropriately high. The selective antagonist of the vasopressin V2 receptor (tolvaptan) helps to eliminate water free of solute (aquaresis), being effective in treating this condition.

Objetive

Evaluate the efficacy and safety of tolvaptan in the treatment of SIADH.

Subjects and methods

Prospective observational study, sequential sampling of 9 patients with SIADH treated with tolvaptan. We evaluated causes associated with SIADH, clinical manifestations of hyponatremia, changes in plasma sodium during use of tolvaptan. Descriptive statistics, median (P25–P75) (SPSS 11.0).

Results

Age 65 (53–78) years, 33% male, 33% asymptomatic. Main symptoms: somnolence (50%), nausea (37%), disorientation (37%), headache (37%), lethargy (25%), visual hallucinations (12%). Causes associated with SIADH: cancer (3), hip surgery (1), brain pathology: subarachnoid hemorrhage (1), basilar artery acute thrombosis (1), pituitary surgery (1), meningioma surgery (1), transverse myelitis (1). Center protocol: indication of tolvaptan in patients with SIADH that do not improve with fluid restriction and sodium intake (i.v./oral), analytical control at 8 and 24 h, if rise $>8$ mEq (8 h) and $>12$ mEq (24 h) did not repeat new dose, indicated monitoring. Starting dose was 15 mg, increased to 30 mg in four patients and 45 mg in two. The duration of treatment was 17 (5–40) days, except for two patients who continues to this day. Plasma sodium value for initiation of tolvaptan 127 (122–127) mEq/l. Changes in sodium: 131 (122–132) ($n=7$) in the first 8 h, 132 (127–135) ($n=9$) at 24 h and 131 (130–133) ($n=8$) in the 48 h. One patient gained 15 mEq/l at 12 h and another reached 14 mEq/l in 24 h, in the last two patients discontinued treatment had to be reset by further decline. One patient was administered a single dose.

Conclusion

Treatment with tolvaptan in patients with SIADH is a safe and effective treatment, determining a progressive slow-rising serum sodium.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1436

### Blast concussion is associated with high frequency of pituitary dysfunction

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Introduction

Studies of traumatic brain injury from all causes have found evidence of chronic hypopituitarism, defined by deficient production of one or more pituitary hormones at least 1 year after injury, in 25–50% of cases. Most studies found the occurrence of posttraumatic hypopituitarism (PTHP) to be unrelated to injury severity. Growth hormone deficiency (GHD) and hypogonadism were reported most frequently. Hypopituitarism, and in particular adult GHD, is associated with symptoms that resemble those of PTSD, including fatigue, anxiety, depression, irritability, insomnia, sexual dysfunction, cognitive deficiencies, and decreased quality of life. However, the prevalence of chronic PTHP after blast-related concussion, or mild TBI (mTBI), an extremely common injury in modern military operations, has not been characterized.

Design

We measured concentrations of 12 pituitary and target-organ hormones in two groups of male US Veterans of combat in Iraq or Afghanistan. One group consisted of participants with blast-related mTBI whose last blast exposure was at least 1 year prior to the study. The other consisted of Veterans with similar military deployment histories but without blast exposure.

Results

In total, 11 of 26, or 42% of participants with blast concussions were found to have abnormal hormone levels in one or more pituitary axes, a prevalence similar to that after other types of TBI. Five members of the mTBI group were found with markedly low age-adjusted IGFI levels indicative of probable GHD, and three had testosterone and gonadotropin concentrations consistent with hypogonadism. Five of the blast concussion group exhibited abnormal vasopressin and/or oxytocin levels suggestive of posterior pituitary dysfunction. Indications of dysfunction in multiple hormonal axes were observed in five Veterans with mTBI. None of the deployment control subjects exhibited any hormonal abnormalities.

Conclusion

Blast mTBI is associated with a high frequency of PTHP. If symptoms characteristic of both PTHP and PTSD can be linked to pituitary dysfunction, they may be amenable to treatment with hormone replacement. Routine screening for

chronic hypopituitarism after blast concussion shows promise for appropriately directing diagnostic and therapeutic decisions that otherwise may remain unconsidered and for markedly facilitating recovery and rehabilitation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1437

**Efficacy of transsphenoidal surgery for Cushing's disease: the role of combined dexamethasone desmopressin test**

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Introduction
The treatment of choice in Cushing's disease (CD) is transsphenoidal surgery (TSS). The success of surgical procedure is of particular importance in terms of patient's prognosis. The aim of this study was the prospective evaluation of compatibility of coupled 1 mg dexamethasone suppression test and 10 μg desmopressin stimulation test (CDDT) with standard criteria of remission in CD.
Methods
The study population consisted of 36 patients with CD operated on using the same protocol and followed-up for a period of at least 18 months (median: 30 months). The adopted criteria of remission were: subnormal serum cortisol level on the 1st postoperative day, normalization of urinary free cortisol and serum cortisol $\leq$1.8 μg/dl following 1 mg of dexamethasone. In total, 28 patients (77.8%) agreed to undergo CDDT – 15 out of 23 cured (65.2%) and all 13 non-cured subjects. CDDT was performed between 12 and 18 months of follow-up. A positive result of CDDT was indicated by the increase in plasma ACTH and serum cortisol by more than 35 and 20%, respectively.
Results
In total, 15 patients (53.6%) were regarded as surgically cured from Cushing's disease. In 13 patients (46.4%) persistent hypertcortisolemia was confirmed. Positive result of CDDT was observed in 12 non-cured (92.3%) and in one cured subject (6.7%). Negative result was obtained in 12 cured (80%) and in one non-cured subject (6.7%). Equivocal result was observed in two cured subjects (13.3%). The qualitative results of CDDT were compared with results of standard hormonal assessment performed at the end of follow-up. They were found to be highly compatible ($\kappa=0.846$; $P<0.001$).
Conclusion
Negative result of CDDT can be treated as a confirmation of successful surgical treatment for CD. During follow-up period CDDT can serve as a valuable supplement to hormone assessment performed in basic conditions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1438

**Improved renal function after five years of GH therapy in GH deficient (GHD) adult survivors of childhood leukaemia**

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Introduction
Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy. The survival rate is now 85% which emphasis the importance of long-term treatment complications. GH-deficiency (GHD) is common among these survivors treated with cranial radiotherapy (CRT) and chemotherapy. Renal impairment has been reported in childhood (CO) cancer survivors and glomerular filtration rate (GFR) is decreased in hypopituitarism. GH therapy to CO GHD patients has been shown to increase GFR.
Methods
In 44 (21 women) ALL patients, treated with 24 Gy CRT and chemotherapy and 44 matched controls GFR (ml/min) was investigated. We used Cystatin C (CysC) to estimate GFR. The level of CysC in serum is less influenced by body composition than creatinine. The median age was 25 years (19–31 years) and 91% of the patients were GHD. In 16 GHD ALL patients the effect of 5 years of GH therapy on GFR was evaluated and compared to 16 matched controls.
Results
At baseline the ALL patients had significantly lower GFR compared to controls ($P=0.01$). After 5 years of GH therapy GFR improved among the ALL patients ($P=0.04$). Two patients had subnormal GFR ($<$60 ml/min) at baseline and GFR was normalized in these patients after GH therapy. After 5 years no significant difference in GFR between patients and controls was recorded ($P=0.2$). When stratified for gender, GFR improved significantly among the men ($P=0.01$), but not among the women ($P>0.3$).
Conclusion
GHD adult survivors of CO ALL have impaired renal function compared to matched controls 20 years after ALL diagnosis. Five years of a low dose of GH therapy improved renal function among the ALL patients, particularly among the men. Whether higher GH doses in women will improve their renal function needs further investigation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1439

**Thyrotropin-secreting pituitary adenomas: experience of a single centre**

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Thyrotropin-secreting pituitary adenomas (TSH-omas) account for less than 1% of all pituitary adenomas. Here we report retrospective data of 17 patients (seven M and 10 F) with TSH-oma followed at our centre from 1990 to present. Median follow-up time was 9.7 years. The mean age at diagnosis was $43\pm12$ years. Radiological evaluation revealed macroadenomas in 11 of 17 patients (71.6%). Macroadenomas were extrasellar in 58.4% of cases, while only one microadenoma had extrasellar extension. At baseline, TSH levels were measurable in all patients ($4.8\pm3.2$ μIU/ml) in the presence of elevated free thyroid hormones. Serum SHBG concentrations were in the hyperthyroid range in all patients. Serum α-GSU levels were normal in all microadenomas. All patients had abnormal TSH suppression during $T_3$ administration but in three patients, serum TSH did not increase after TRH injection. A mixed GH/TSH secreting adenoma was observed in two patients, whereas a mixed gonadotropin/TSH adenoma was seen in one. In total, 16 patients were treated with trans-sphenoidal adenomactomy (TNS), 10 with somatostatin analogue (SSA) and six with radiotherapy (RT). About 35% of patients received one treatment, 24% two and 41% three. Combination of therapies were more frequent in macroadenomas (63%). At last follow-up nine patients (56%) were cured, three patients had normal free thyroid hormones but did not respond to TRH test, four patients were on SSA and one was recently treated with RT. Macroadenomas were cured in 45% of cases, 36% after TNS and 9% after TNS + RT, microadenomas in 80%, all after TNS alone. Last MRI was negative for residual/recurrence of adenoma in 11 patients. In conclusion, our series confirms the importance of $T_3$ suppression test and measurement of serum SHBG for the diagnosis of TSH-omas and the efficacy of TNS surgery, in particular for microadenomas.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1440

### Detection of somatostatin receptors in aggressive non-functioning pituitary adenomas and effects of somatostatin analogs therapy in these tumors

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Clinically non-functioning pituitary adenomas (CNFPA) represent a heterogenous group of tumors. Most of CNFPA are aggressive, usually diagnosed relatively late and surgery often fails to achieve the complete excision of tumor and the recurrence rate is high. About 80% of CNFPA are characterized by intense expression of somatostatin receptors (SSTR) inspiring use of somatostatin analogs (SSTa) in the medical cure of CNFPA. Scintigraphy with visualization of SSTR2 expression and/or immunohistochemistry with selective recognition of all five SSTR subtypes are used for justify the long-acting SSTa treatment of CNFPA.
The aim of the study was to correlate the expression of SSTR determined with immunohistochemistry or receptor scintigraphy techniques (group of 48 patients) and verify effects of long-acting SSTa in the treatment of CNFPA (11 patients).
Results
All 11 patients with CNFPA treated with SSTa indicated expression of SSTR2 in immunohistochemistry but only nine patients of this group were SSTR2 positive in scintigraphy. Absence of SSTR2 expression in scintigraphy was not always associated with absence of SSTR2 visualization in immunohistochemistry. Intense expression of SSTR1 and SSTR5 was observed in most CNFPA. In all patients treated with SSTa stabilization of the tumor growth and stabilization or improvement of visual disturbances were observed.
Conclusion
Both scintigraphy and immunohistochemistry methods must be combined to assess the presence of SSTR in CNFPA. The treatment of CNFPA with SSTa is connected with the positive effect on tumor size progression – stabilize the tumor mass. SSTR1 and SSTR5 expression in CNFPA justifies the trials to treatment CNFPA with broad spectrum SSTa, like SOM230.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1441

### Effect of a modulation of somatostatin release on TSH secretion in healthy volunteers

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Objectives
In normal subjects, inhibitory effect of a glucose load (OGTT) on GH secretion is probably mediated by an increase in hypothalamic release of somatostatin whereas the stimulatory effect of arginine on GH secretion is probably mediated by a decrease in somatostatin release. In humans and animals, somatostatin infusion inhibits basal and TRH-stimulated TSH secretion but little is known on the role of endogenous somatostatin in the regulation of TSH secretion. The aim of this study was to evaluate TSH response to situations known to modulate somatostatin release *in vivo* (OGTT and arginine infusion).
Methods
OGTT (75 g) or arginine infusion (30 g in 30 min) were performed in seven healthy male volunteers. In five subjects results were also obtained after TRH injection. Isotonic saline infusion was used as control. Tests were begun 60 min after a catheter has been inserted ($T=0$ min). Blood samples were taken every 15 min from $-60$ to 240 min for glucose, TSH, PRL, GH measurements. Basal level for TSH and PRL was chosen as the mean of the value at $T-15$ and 0 min. Results were expressed as % of basal value (mean$\pm$S.E.M.). GH values were expressed in ng/ml.
Results
Statistical analysis was performed employing one-way ANOVA followed by Bonferroni's multiple comparison tests. OGTT exerted a typical inhibitory effect on GH secretion (Nadir GH $<0.25$ ng/ml) and arginine a stimulatory effect (peak GH: $11\pm3.64$ and $7.46\pm1.51$ ng/ml when combined with TRH; $P<0.01$).
As expected, PRL secretion was stimulated by arginine and TSH by TRH. Effects of a glucose load and of arginine on TSH secretion were less clear. TSH nadir observed during OGTT was statistically different from basal values but not from the nadir obtained with saline. The stimulatory effect of Arginine on TSH secretion was not significant when compared to basal value but statistically significant when compared to he value obtained during saline perfusion. No changes were observed on TSH stimulated values.
Conclusions
cccc
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Modulation of somatostatin release *in vivo* by a glucose load or arginine infusion plays a minor role in basal or TRH-stimulated TSH secretion.

| | Nacl 0.9% | OGTT | Arginine | Nacl 0.9%+ TRH | OGTT+ TRH | Arginine+TRH |
|---|---|---|---|---|---|---|
| TSH (% of basal value) | 85.2±3.9 | 79.0± 5.1*,[a] | 117.0± 8.3† | 828±115* | 663±63* | 731±86* |
| PRL (% of basal value) | 75.7±5.9 | 76.5±3.5 | 339.5± 4.5* | 720±82* | 853±129* | 988±146* |

*$P<0.01$ vs basal values; †$P<0.01$ vs saline values.

[a]NS vs saline.

## P1442

### Are "*in silico*" predictions reliable regarding splice-site mutations? – Studies in the aryl hydrocarbon receptor-interacting protein (AIP)

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Background
It is often difficult to define the clinical relevance of a novel gene variant. *In silico* analyses of variants located close to exon–intron-junctions are utilised to predict the result of these basepair changes. We have previously identified two splice-site variants in AIP and confirmed the predicted changes for c.249G>T, p.G83AfsX15 and c.807C>T. We identified the c.469-2A>G heterozygous variant located at the end of intron-3 in a childhood-onset acromegaly patient. This change has been previously described and was suggested to result in the loss of exon-four based on *in silico* analysis.
Methods
RNA has been extracted from our patient's peripheral blood sample and it was amplified using RT-PCR with primers located on exon-2 and exon-6. After electrophoresis DNA bands were extracted from agarose gel and sequenced. *In silico* prediction was carried out by the ALAMUT software (www.interactive-biosoftware.com).
Results
The agarose gel revealed two bands in the patient sample and a single band in the control sample. The common band showed the expected wild-type sequence. The additional lower-size band revealed the loss of 84-nucleotides at the 5′ end of exon-4, while the rest of exon-4 was normal. This change is not predicted to result in a frameshift.
Conclusion
The disruption of intron-3 acceptor splice-site resulted in the appearance of a cryptic splice-acceptor site in the middle of exon-4. The resulting RNA is predicted to yield a shorter protein missing 27 amino acids in the second alpha-helix of the first tetratricopeptide repeat motif of the AIP molecule. This variant has been identified in our childhood-onset acromegaly patient and previously in an adult-onset case, but has not been reported in normal subjects, except for family members of the above patients. We suggest that experimental confirmation of *in silico* predictions of gene variants is helpful for appropriate genetic counselling and clinical management of these families.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1443

**Effectiveness of cyberknife in the management of pituitary adenomas and craniopharyngiomas**

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Introduction
CyberKnife (CK), an innovative LINAC-based robotic device for frameless stereotactic surgery, is an emerging approach in the management of tumours of the sellar region.
Patients and methods
In total, 14 patients (six men, mean age 52.7 years) with sellar tumours (three craniopharyngiomas and 11 pituitary adenomas) were evaluated. Pituitary adenomas (PA) were non-functioning (7), GH-secreting (2), one GH/TSH-secreting (1) or PRL-secreting (1). Before CK, hypopituitarism had been diagnosed in six patients. CK was performed as first line treatment in one patient with non-functioning adenoma, in one with GH/TSH-secreting adenoma and in one with craniopharyngioma. Tumours were treated with a median coverage of $96.11 \pm 2.2\%$ (range, 93.7–98.6%), a median conformity index of 1.37 (range, 1.2–1.63), and a median treatment isodose line of 70% (range, 60–84%). Patients were treated with multiple (2–5) fractions with a BED of 85–105 Gy for alfa/beta 2. The mean follow-up period was 17.7 months (range 4–48 months).
Results
CK induced tumour disappearance in three cases with non-functioning PA (CK was first line treatment in one) and decrease in one with non-functioning PA and in two with craniopharyngioma (CK was first line treatment in one). Five out of eight patients with previously normal pituitary function developed hypopituitarism (one GH deficit and four multiple impairments). Among six patients with previous hypopituitarism, two developed panhypopituitarism while no worsening of pituitary function was seen in the other cases. One acromegalic patient, resistant to medical therapies, reached IGF1 normalization.
Conclusions
This mid-long term evaluation shows that CK induced tumor shrinkage in 43% of cases and in 2/3 of those treated as first line approach. However, hypopituitarism occurred in 62% of patients with normal pituitary function and adjunctive pituitary deficiencies were demonstrated in 33% of the others. Acromegaly control was reached in the patient resistant to other therapies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1444

**Evaluation of cardiovascular risk factors and metabolic profile in hyperprolactinemic subjects**

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Objective
Hyperprolactinemia has been reported to be associated with abnormalities of glucose metabolism and cardiovascular inflammatory markers. However, the metabolic effects of high prolactin levels are not adequately clarified. The aims of this study were to evaluate the effects of hyperprolactinemia on endothelial function, insulin sensitivity and inflammatory markers in prolactinoma diagnosed subjects.
Methods
In total, 58 hyperprolactinemic subjects, 18 men and 40 women, aged $38.9 \pm 10.6$ years with newly diagnosed pituitary adenomas were recruited from our outpatient clinic. In total, 30 healthy subjects, without any known disease, of similar age, gender and body mass index (BMI) were included to serve as control group. The study was approved by the Hospital Ethical Commitee and informed consent was obtained from all patients. All subjects underwent measurements of height, weight, waist and hip. BMI and homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Metabolic variables were obtained after an overnight fasting. Metabolic syndrome (MS) was diagnosed using the Adult Treatment Panel III criteria. Endothelial function was determined by measuring carotid intima media thickness (CIMT) on high resolution external ultrasound.
Results
Serum levels of glucose, insulin, HOMA-IR, total cholesterol, triglyceride and waist circumference were significantly higher in the patient group in comparison with the controls. No significant difference were observed in blood pressure measurements. Although high sensitive C-reactive protein levels were similar between groups ($P = 0.094$), CIMT measurements were significantly higher in hyperprolactinemia patients ($P = 0.002$). The prevalance of obesity, overweight, MS, and IR was 43.1%, 29.2 and 27.6% respectively in patient group.
Conclusion
Based on the results of this study, hyperprolactinemia is associated with endothelial dysfunction and decreased insulin sensitivity, which are early markers of atherosclerosis. Metabolic control must be considered in the clinical management of patients diagnosed prolactinoma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1445

**Pituitary tumor apoplexy: overview of 14 cases diagnosed during the last 12 years at a central hospital**

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Introduction
Pituitary apoplexy is a potentially life-threatening syndrome due to acute infarction and/or hemorrhage of the pituitary gland. In many cases it is the first form of presentation of a pituitary adenoma. The purpose of the study is to analyze the clinical presentation, diagnosis and treatment of this syndrome.
Patients and methods
A retrospective analysis of the patients diagnosed with pituitary tumor apoplexy at Santo Antonio's Hospital from 2000 to 2011 was done. Their medical records were reviewed.
Results
In total, 14 cases of pituitary tumor apoplexy were reviewed: 10 males and four females (mean age 47.8 years, range 21–71). They were followed for up to 10 years (mean 3.9 years, range 1–10). Only one case occurred on a previously known pituitary macroadenoma; all other occurred as first manifestation of the tumor. At the presentation, headache occurred on 11 cases (78.6%), visual changes on 10 (71.4%) and nausea/vomits on eight (57.1%); one patient was asymptomatic. MRI was done on 11 cases (78.6%); pituitary tumor hemorrhage and/or necrosis was correctly identified on all. Surgical treatment with transfenoidal surgery was done on 12 cases (85.7%), on average 4.5 days after hospital admission (range 1–13 days). Visual changes improved on all surgically treated. By immunostaining criteria, of the 12 surgically removed tumors, five (41.7%) were null-cell adenomas, three (25%) were GH-positive, two (16.7%) were FSH-positive and two were GH and PRL-positive.
Overall, nine patients needed long-term hormone replacement: steroid and thyroid hormones were required in eight cases (88.9%), testosterone therapy in five (55.6%). One patient needed long-term desmopressin therapy.
Conclusions
Pituitary apoplexy diagnosis relies on the presenting symptoms and it is usually confirmed by an imaging method, preferably MRI. Transfenoidal surgery is the most used technique and a sooner intervention carries a better prognosis. A significant percentage of patients need long-term hormone replacement.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1446

**Copeptin is not associated with menstrual cycle hormones**

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Background
Copeptin (CP), a derivate from the antidiuretic hormone (ADH) precursor pre-pro-vasopressin, stochiometrically mirrors ADH secretion. CP is increasingly evaluated as a diagnostic and prognostic biomarker in different diseases. It is therefore important to recognize possible confounding factors when interpreting CP levels. In healthy regularly menstruating women, there is a small but measurable physiological variability of hormones involved in fluid regulation. ADH plasma levels have been found to be lowest at menstruation, increasing during the follicular phase with a peak at ovulation and a drop in the luteal phase. We investigated the variability of CP during the menstrual cycle (MC) and its correlation to MC hormones.
Methods
In total, 15 healthy women with regular MC (from 26 to 33 days) were included in this study. Ovulation was confirmed by progesterone (prog) levels on day 21 of the MC before entering the study and during the study. Blood collection was performed on days 3, 5, 8–16, 18, 21, 24 and 27 of their MC. Serums were assayed for prog, estradiol ($E_2$), LH, and CP. Mixed linear regression analysis for repeated measures was performed to study the changes of CP, prog, $E_2$ and LH during the MC, and to test the correlation of CP with sex hormones during the MC.
Results
Mean MC length in all subjects was $28.5 \pm 2.2$ d. $E_2$, prog, and LH exhibited characteristic changes during the MC (all $P < 0.05$). All cycles were ovulatory (peak prog $54 \pm 15$ nmol/l). CP levels did not change significantly throughout the MC, and were not associated with changes in prog, $E_2$ or LH-levels (all $P$=ns).
Conclusion
CP levels remain stable during the MC and are not influenced by changes in sex hormones. This implicates that it is not necessary to consider MC phases when using CP as a biomarker in premenopausal women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1447

**Acylated ghrelin as provocative test for the diagnosis of gh deficiency in adults**

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ITT is the test of reference for the diagnosis of adult GH deficiency (GHD), but also GHRH in combination with arginine (ARG) or GH secretagogues (GHS) are considered equally reliable tests. Testing with GHS alone is, anyway, a potent stimulus exploring the integrity of hypothalamic pathways controlling somatotropic function.
We therefore aimed to clarify the diagnostic reliability of testing with ghrelin, the natural GHS.
We studied the GH response (every 15 min from $-15$ to $+120$ min) to acylated ghrelin (1 μg/kg i.v. at 0 min) in 78 patients with history of pituitary disease (49 M, 29 F; age [mean ± SD]: $52.1 \pm 18.7$ years; BMI: $26.7 \pm 5.3$ kg/m$^2$). As gold standard for the diagnosis of GHD we assumed the lack of GH response to GHRH + ARG and/or ITT. We identified the best GH cut-off to ghrelin test, defined as the one with the best sensitivity (SE) and specificity (SP), using the ROC analysis.
The best GH cut-off to ghrelin test was 7.3 μg/l in lean subjects with a SE and SP value of 88.2 and 90.9%, 2.9 μg/l in overweight subjects with a SE and SP value of 92.6 and 100%, and 0.6 μg/l in obese subjects with a SE and SP value of 50 and 100%. The diagnostic accuracy was 89.3, 94.1 and 62.5%, respectively.
In conclusion, these findings indicate that testing with acylated ghrelin would represent a reliable diagnostic tool for the diagnosis of adult GHD, at least in lean and overweight subjects provided that appropriate cut-off limits are assumed. Obesity strongly reduces the GH response to ghrelin, its weight-related cut-off limit, and its diagnostic reliability.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1448

**National incidence and prevalence of TSH-secreting pituitary adenomas in Sweden**

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Introduction
TSH-secreting pituitary adenomas (TSHoma) are rare. Epidemiological data are scant and there are no reports on national incidence. The aim of this study was to estimate the national incidence and prevalence of TSHomas in Sweden.
Methods
This is an observational study from all tertial referral centers where the Swedish Pituitary Registry and WHO ICD coding were used to identify patients diagnosed with TSHomas in Sweden 1990–2010. Medical records were studied for identified patients until latest follow-up (median 5.0 years (range <1–20)). Incidence, prevalence, demography, tumor characteristics, treatment outcome and thyroid hormone level at diagnosis were studied.
Results
The age-standardized national incidence of the 28 TSHoma patients was estimated to 0.15/million inhabitants/year for the period, with an increasing incidence over time (0.05/million/year in 1990–1994 to 0.26/million/year in 2005–2009). The national prevalence of patients followed for TSHomas in 2010 was 2.8/million inhabitants. Most patients ($n=22$) underwent surgery, three had radiotherapy and six had somatostatin analogues. In total, 18 patients were considered cured at latest follow-up; 25% remained uncontrolled. Subjects treated for putative thyroid illness prior to diagnosis ($n=8$) had TSH levels more than two times compared with those with intact thyroid at diagnosis of TSHoma, $P=0.013$. Women had longer time to diagnosis than men, median 4 vs <1 years, $P=0.026$ and had more often surgery, 94.1 vs 54.5%, $P=0.022$.
Conclusion
This is the first estimation of a national incidence of TSHomas. Additional epidemiological studies are needed to compare this result with other geographical areas. The study implies an increased incidence of TSHomas, in parity with reports on other pituitary adenomas.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1449

**High mean platelet volume and lipid abnormalities in prolactinoma patients without insulin resistance**

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Introduction
Hyperprolactinemia has been associated with dyslipidaemia, hypercoagulability, impaired endothelial function and decreased insulin sensitivity. Recent data show that prolactin hormone (PRL) could contribute to atherogenesis. The aim of our study was to investigate the relationship between MPV and PRL, androgen hormones, lipid profiles in premenopausal prolactinoma patients, who did not have insulin resistance (IR).
Methods
Thirty-nine newly diagnosed premenopausal prolactinoma patients (mean age $28,6 \pm 6,6$ years, mean body mass index (BMI) $23,6 \pm 1,9$ kg/m2, mean HOMA-IR $1,33 \pm 0,4$.) and twenty normoprolactinemic, age- and BMI- matched healthy control females were involved in the study. Blood samples were taken for total blood count including MPV, FSH, LH, estradiole, total testosterone (TT), free testosterone (FT), androstenedione (A), dehydroepiandrosterone-sulfate

(DHEAS), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), insulin and fasting glucose (FG). IR was calculated by HOMA-IR(fasting glucose (mg/dl) x serum insulin (μIU /ml) /405).
RESULTS
There was no difference between prolactinoma and control group regarding to age, BMI and and waist circumference ($P>0.05$, for all). PRL, FT and MPV in women with prolactinoma were significantly higher than the control group ($P<0.01$, $P<0.01$, $P=0.013$). No significant differences were observed in the groups according to HDL-C, LDL-C, triglyceride, HOMA-IR, gonadotropin and the other androgen hormone levels ($P>0.05$, for all). Both PRL and triglyceride levels showed a positive correlation with MPV levels ($r=0.727$, $P<0.001$, $r=0.357$, $P=0.026$, respectively) and a negative correlation between HDL-C and MPV levels was found in our study group ($r=-0.437$, $P=0.005$).
Conclusion
Our study demonstrated the positive correlation between MPV levels with both PRL and triglyceride levels. Also a negative correlation between HDL-C and MPV in prolactinoma patients. Hyperprolactinemia and hypertriglyceridemia might be cause platelet reactivity through elevating MPV levels in prolactinoma patients. Similarly, it could be said that, high HDL-C may be protect from atherosclerosis by lowering MPV in prolactinoma patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1450

**Asymptomatic nonfunctioning pituitary macroadenomas take advantage of surgery**

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Introduction
Pituitary incidentalomas (PIs) defined as pituitary tumors discovered by systematic neuroimaging constitute an increasingly clinical problem. Most of PIs are nonfunctioning tumors (NFPIs) with systematic investigation revealing visual and/or endocrinological impairment in some patients while others remained asymptomatic. If the therapeutic management is well codified for functioning PIs and symptomatic NFPIs, a debate still remain for asymptomatic NFPIs between surgery and a "wait and see" approach.
Design
To answer this point, we conducted a retrospective study on 76 patients with newly diagnosed symptomatic or asymptomatic NFPIs operated on from 2005 to 2011 by three experimented neurosurgeons. We compared age, tumor size and surgical results (quality of resection, endocrinological and ophthalmological results, morbidity) of the two patient groups.
Results
After the initial exploration, 48 (63%) patients were symptomatic and 28 were asymptomatic. Mean age ($60.9\pm13.1$ versus $52.9\pm11.9$ years) and tumor height ($25\pm6$ versus $20\pm7$ mm) were significantly higher in symptomatic compare to asymptomatic patients ($P<0.001$, $P<0.01$, respectively). There was no significant difference regarding the Knosp's classification. Gross total removal was significantly better in asymptomatic (82%) than in symptomatic patients (58%; $P=0.03$). Postoperative pituitary function was normalized/improved (41%), unchanged (48%) or worsened (11%) in the 27 symptomatic patients with initial endocrinological impairment. It was worsened in 19% of the symptomatic patients without preoperative endocrinological impairment versus 14% in asymptomatic patients ($P=0.7$). The postoperative ophthalmological function was normalized/improved (88%) or unchanged (12%) in the 35 symptomatic patients with initial ophthalmological impairment whereas there was no worsening in all other patients. No significant difference was found regarding postoperative morbidity: cerebrospinal fluid leakage (5%), meningitis (1%), epistaxis (1%) and definitive diabetes insipidus (2.5%).
Conclusions
The knowledge that natural history of NFPIs makes them symptomatic and the quality of the resection without worsening of visual function give arguments to propose surgery for asymptomatic macro NFPIs by experimented neurosurgeons.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1451

**Mortality and survival in a large series of adult patients with hypopituitarism followed for 10 years**

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Hypopituitarism is associated with increased morbidity and mortality compared to healthy population. However, the factors influencing prognosis are still not well known.
Objectives
To determine the causes of mortality in adult patients with hypopituitarism and compare the mortality rate with general population
To identify the factors associated with mortality and survival.
Patients
Two hundred and nine adult patients followed because of hypopituitarism during a 10-year period.
Results
The most frequent cause of hypopituitarism was a pituitary or peri-pituitary tumour (55.5%), 87.3% operated and 61% irradiated. FSH/LH was the most frequent deficiency (80.4%), followed by TSH (72.2%), ACTH (60.3%), GH (60.3%) and ADH (19.6%).
Prevalence of obesity was 44.8% (BMI) and 56.6% (waist circumference). 15.4% had diabetes (59.3% well controlled), 34.3% hypertension and 38.8% dyslipidemia. Cardiovascular, chronic respiratory, cerebrovascular disease and malignancy were diagnosed in 16.9, 2.5, 4.8 and 7.2% respectively.
Thirty-two patients died due to cardiovascular disease (46.8%), infections (28.1%), malignancy (15.6%) and cerebrovascular disease (9.4%). Standardized mortality rate was 8.05, higher in males (8.92 vs 7.43) and young patients (84.93 vs 5.26). Previous radiotherapy ($P=0.02$), acromegaly ($P=0.033$), higher BMI ($P=0.04$) or waist circumference ($P=0.032$), diabetes ($P=0.03$), uncontrolled diabetes ($P=0.026$) and cancer ($P<0.0001$) were associated with mortality.
Stepwise multivariate analysis showed that reduced life expectancy was related to older age, previous radiotherapy, uncontrolled diabetes and malignancy, while tumoral causes prolonged survival.
Discussion
Mortality in these patients was eight times higher than general population. Lower survival since diagnosis was related to older age, non-tumoral causes, malignancy, uncontrolled diabetes and previous radiotherapy. Diagnosis of acromegaly, higher BMI and waist circumference were related to mortality, although in these cases differences in survival were not detected.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1452

**Gastric and colonic pathology at patients with active acromegaly**

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We examined gastroenterocolonic tract condition at 92 patients with active acromegaly (male 30, female 66, age 26–78 y.o., most of the patients 45–59 y.o.). Gastroduodenoscopy ($n=92$) and colonoscopy ($n=74$) were performed. Median GH levels were 22.7 (12.5; 51) mMe/l, median IGF1 levels – 640 (507; 800) ng/ml. Esophagitis was diagnosed at 22 (23.9%) patients, incompetence of cardia – 33 (35.8%) patients. There was one case of gastric cancer (operated before). Helicobacter pylori was found at 78% of patients (predominantly 2–3 degree). Gastroduodenitis was found in all patients (100%), additionally chronical or acute stomach erosions were revealed at 20 (21.7%) patients. Stomach polyps were found

at 28 (30.4%) patients (hyperplastic polyps $n=5$). Polyps localized predominantly at distal parts of the stomach (mostly antrum), size up to 5 mm 14 cases, 6–10 mm – 11 cases, >10 mm – three cases. Duodenal ulcer was found at six patients, duodenal erisions – in seven cases. Duodenal polyps were found at 2 other patients (hyperplastic polyp $n=1$).
Colonic pathology were found in 64 patients (86.5%), including sigmoiditis ($n=13$, 17.6%), colitis ($n=7$, 9.5%), colonic diverticula ($n=22$, 30%), dolichocolon ($n=21$, 28.4%). Colonic polyps were found in 39 (52.7%) cases and consisted of hyperplastic polyps ($n=26$, 35.1%) and adenomatous polyps ($n=13$, 17.6%), including 7 (9.5%) cases of joint presence of hyperplastic and adenomatous polyps, and adenocarcinoma ($n=1$, 1.4%). Polyps were single in 20 cases (27%), 2–8 polyps in 8 (10.8%) cases, and >8 polyps in 12 (16.2%). Polyps were in size up to 5 mm in two cases, 6–10 mm in 20 cases, 11–15 mm in 14 cases, and >15 mm in three cases. We did not found association of stomach and colonic polyps in any case. Median GH levels were higher at patients with stomach polyps comparing with colonic polyps (33 (12.5;58) vs 18.8 (12.5;40) mMe/l) however the difference was not statistically significant. Median IGF1 levels were similar in both groups (655 (507; 812) vs 600 (500; 710) ng/ml respectively).
Thus, gastro/colonic pathology is common among acromegalic patients. Stomach polyps like colonic polyps are also associated with acromegaly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1453

**Pituitary dysfunction in adult patients after cranial radiotherapy for non-pituitary tumors: a long-term follow-up study**

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Background
Hypopituitarism after cranial radiotherapy for non-pituitary tumors is well recognized. However structured endocrine assessments are not incorporated into routine clinical practice
Aim of the study
To evaluate pituitary function in adult patients irradiated for non-pituitary tumors at our center
Patients and Methods
Cross-sectional evaluation of pituitary function of all available patients treated with irradiation at our center for non-pituitary cerebral or nasopharyngeal tumors and hematological malignancies
Results
497 patients were identified from the archives of Radiotherapy and Endocrinology. Of these, 60 patients(12%) had been subjected to endocrine testing. 392 were excluded for evaluation. 231 were diseased, 102 had progressive disease, 26 did not meet inclusion criteria, 13 were unavailable and 20 declined evaluation. A total of 105 patients were eligible for this study. 63 treated for cerebral tumors, 15 for nasopharyngeal tumors, 25 for hematological malignancies, and two for cerebral metastasis. Median age at radiotherapy was 29(2–74) years and median radiation dose 54 (6–74) Gy. Median follow-up was 11.5 years (0.5–38). Endocrine evaluation included morning hormonesampling and ITT ($n=63$), metyrapone($n=4$), CRH ($n=26$), ACTH ($n=15$), or GHRH/Arginine ($n=19$). Any hypopituitarism was present in 51/105(48.6%) of cases. The prevalence of GHD was 29%. ACTH-deficiency in 25%, TSH-deficiency in 16% and LH/FSH-deficiency in 20%. Hyperprolactinemia was present in 25%. A higher irradiation dose (>25 Gy) was associated with a significantly higher prevalence of hypopituitarism. The mean period of onset of pituitary insufficiency was 4.5 years (0.5–35) for GH deficiency, 3 (1–16)years for TSH deficiency, HPA-axis after 6 years (0–24) and FSH/LH insufficiency after 7 years (0–16). Hyperprolactinemia occurred after approximately 2.5 years (0–21) years.
Conclusion
After long-term follow-up, hypopituitarism is highly prevalent in adult patients after cranial radiotherapy for non-pituitary tumors, but only 12% of patients at our center was subjected to endocrine testing. Periodical endocrine evaluation should be incorporated in the follow-up of all patients treated by cranial radiotherapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1454

**The treatment of results of endoscopic transsphenoidal surgery for GH-secreting pituitary adenomas**

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Objective
With the progress of neuroendoscopic surgery in recent years, the application of endoscopy to transnasal surgery for pituitary tumors is increasing. At our institution, endonasal surgery using an endoscope alone (eTSS) has been performed in 825 patients with pituitary adenomas since November 2001. Among them, 156 patients (18.9%) had GH-secreting pituitary adenoma (GHoma), so the outcome and complications of eTSS-treated GHomas were investigated in the present study.
Materials and methods
Rigid endoscopes with an outer diameter of 4 and 2.7 mm (visual field angle: 0, 30, and 70 degrees) manufactured by Machida Endoscope Co., Ltd. were used. These endoscopes were mounted on a new floor stand manufactured by Mitaka Kohki Co., Ltd. A CT navigation system set for bone conditions was used in all patients.
Results
The range of supracellar tumor resection was increased by employing a rigid endoscope with a visual field angle of 70 degrees. Resection of tumors invading the cavernous sinus was evaluated in patients with GHomas classified according to Knosp for which clear criteria are available. As a result, the curing rate improved to 84.2% in grades 0, 1, and 2, while it was a low 16.7% in grades 3 and 4. Also, Capsulectomy could even be performed by endoscopy in patients with GHomas of enclosed type. As complications, epistaxis, transient visual dysfunction due to postoperative hematoma, and abducens nerve paralysis were noted in 1.4, 0.7, and 0.7% of the patients, respectively.
Conclusion
Tumors could be approached accurately by eTSS with a wide visual field, and the incidence of complications could be minimized. Since the suprasellar excision rate was increased owing to a wide visual field, eTSS can also improve the QOL of patients. However, further studies will be necessary to improve the resection rate of tumors with extensive invading into the cavernous sinus.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1455

**Glucose metabolism in children with GH deficiency before and after GH therapy**

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Background
Adults with untreated GH deficiency (GHD) may have a cluster of cardiovascular risk factors. The effects of GH replacement therapy on insulin homeostasis in GHD subjects are still debated. Only a few studies investigated the effects of GHD and GH therapy on glucose metabolism in children.
Objective
To evaluate the effects of GHD and GH treatment on glucose metabolism in a large cohort of GHD children before and after GH replacement therapy.
Subjects and methods
Fasting glucose, insulin, HbA1c, and HOMA were assessed in 60 GHD children, aged 9.8±0.3 years, before and after 1, 2 and 4 years of GH therapy. 60 healthy, age-, sex- and BMI-matched healthy controls were enrolled.
Results
In GHD children at baseline, fasting glucose (77.8±1 vs 77.9±1.2 mg/dl), insulin (4.7±0.4 vs 4.6±0.4 μU/ml), HOMA (1.26±0.2 vs 1.12±0.1) and HbA1c (5.29±0.1 vs 5.29±0.04%) levels were comparable to healthy controls. One year of GH therapy (33 μg/kg per day) was associated with significant increase in insulin (7.69±0.6, $P<0.0001$) and HOMA (1.64±0.15, $P=0.009$), without significant changes in fasting glucose and HbA1c levels. Insulin and HOMA levels did not further increase after 2 (7.56±0.6 and 1.85±0.2 respectively) and 4 years (7.65±0.7 and 1.5±0.1 respectively) of treatment, remaining significantly elevated compared to pre-treatment levels. Fasting glucose and HbA1c did not change after 2 and 4 years of GH therapy.
Conclusions
Untreated GHD was not associated with significant alterations of insulin homeostasis. One year of GH treatment was associated with a slight impairment

in insulin sensitivity without further change during long-term GH therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1456

**Endocrine disturbances and quality of life in adult patients after multimodal treatment for brain tumors or leukemia**

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Objective
Brain tumour treatment with radio- and chemotherapy may lead to endocrine and other medical sequelae. Still, many countries lack tailored surveillance programs for such patients so that potential health problems may remain unrecognized. The present study was performed to investigate endocrine and psychosocial impairment in a single centre university setting in Germany in patients treated with cranial radiotherapy as a part of brain tumour or leukaemia treatment.
Patients and methods
38 (18 m, 20 f) brain tumour patients were investigated at least three year after completion of radiotherapy. 26 had been operated upon and 19 had received additional chemotherapy. 16 patients were childhood cancer survivors, 22 were diagnosed and treated as adults. In all patients basal 0800 h hormone levels (cortisol, ACTH, GH, IGF1, $fT_3$, $fT_4$, TSH, LH, FSH, testosterone or estrogen) were assessed. In case of abnormal basal hormone readings or clinical suspicion of hormonal abnormalities, functional endocrine assessment was additionally performed. An extensive self-rating battery pertaining to quality of life, sleep disturbances and depression was also completed by all patients.
Results
In one patient previously undetected deficiency of three pituitary hormone axes was noted, a total of eight patients had abnormally low IGF1 levels, three secondary and three primary hypogonadism. Primary hypothyroidism, probably associated with brain tumour treatment (radiotherapy of the neuroaxis in medulloblastoma patients) was noted in two patients. Patients presented with a wide range of psychosocial impairment with women and overweight patients being more impaired than others. Higher testosterone levels were related to better psychological quality of life as indicated by the psychological sum score of the SF-36 ($r=0.494$, $P<0.05$).
Conclusion
The results of the present study underscore the need for organized assessment and treatment of medical and psychosocial late sequelae even years after multimodal brain tumour treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1457

**Anterior pituitary autoantibodies in patients with diabetes mellitus**

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Objective
The presence of APA has been reported in patients with autoimmune polyendocrinopathies, but their prevalence in type 1 diabetes mellitus (T1D) is still unknown. The aim of this study was to assess APA prevalence in T1D patients as compared to patients with type 2 diabetes mellitus (T2D) and healthy subjects.
Research design and methods
The presence of APA was assessed by indirect immunofluorescence (IF) technique, with bovine adrenal gland and pituitary as substrates. To avoid non-specific interference only sera giving IF signal at 1:200 dilution were considered as positive. The study groups included 100 patients with T1D of varying duration (median 14 years, range 1 month to 43 years), 32 patients with T2D and 62 healthy controls.
Results
APA were detected in 7 of 100 (7%) patients with IDDM, 0 patients with NIDDM and 0 non diabetic subjects, corresponding to a significant increase ($P=0.033$ by $\chi^2$ test) in T1D patients vs healthy controls, while the difference vs T2D was slightly above the level of statistical significance ($P=0.124$). Evaluation of anterior pituitary function of the seven APA-positive T1D patients is presently under study.
Conclusions
This study indicates that the prevalence of APA is higher in patients with T1D than in the patients with T2D and in non diabetic controls. This observation, which confirms and extends previous reports, suggests that the spectrum of autoimmune disorders associated with T1D should be further expanded. The functional significance of autoimmunity against anterior pituitary requires further investigation, presently ongoing at our Institutions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1458

**Pitfalls in Cushing's disease: report of an ectopic ACTH-producing pituitary adenoma in the sphenoid sinus**

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Cushing's disease is caused by ACTH-secreting pituitary adenomas. Gold standard therapy is the resection of the adenoma by transsphenoidal surgery with high cure rates even in cases of negative MR imaging. Surgical failure despite clear endocrinological test results is possible and mostly explained by hidden minute adenomas within the gland.
We report on a 50-year-old woman suffering from ACTH-dependent Cushing's syndrome. Endocrinological work-up was most compatible with a pituitary origin. Although an MRI showed no pituitary tumor, CRH-stimulated petrosal sinus sampling revealed a clear central-peripheral ACTH gradient. The patient underwent transsphenoidal surgery with negative exploration of the pituitary gland. After intraoperative re-evaluation of the preoperative MRI, a 'polyp' at the bottom of the sphenoid sinus was identified. The intraoperative microscopic aspect as well as instantaneous sections and cytology of a biopsy confirmed an adenoma, which was removed. Histological analysis demonstrated an ACTH-producing pituitary adenoma adjacent to respiratory mucous membrane consisting of ciliated epithelium with submucous connective tissue. Histological evaluation of biopsies from the pituitary gland revealed normal pituitary tissue. Thus, an ACTH secreting adenoma was only detectable in the sphenoid sinus. Postoperatively, ACTH concentrations dropped and after 3-month of follow up, Cushing's stigmata were found to regress and a temporary hydrocortisone substitution could be reduced.
The adenohypophysis originates from the floor of the nasopharynx, that is partly located in the precursor of the sphenoid bone. Ectopic pituitary tissue is thought to arise from remnants of Rathke's pouch, and ectopic adenomas are believed to originate from remaining cells resting along the path of the embryological formation of the pituitary gland. Ectopic adenomas growing in the sphenoid sinus have been reported in only 10 cases so far[1]. Keeping in mind that ectopic pituitary tissue may exist in a patient with Cushing' disease can potentially prevent failure of surgery.
1. Suzuki J *et al. Endocr J* 2004; **51** 97–103.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1459

### The effect of Ki67 index on tumor behavior in proloctanimas

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Majority of prolactinomas consists of the small microadenomas that can be treated with dopamine agonists, but they can present different biological behavior features and not infrequently seen as macroadenomas or in giant adenoma size. There are different results on finding of studies which investigate the relationship between high levels of cell proliferation indicators with tumor behavior in pituitary adenomas. In this study, Ki 67 index to investigate the relationship between tumor behavior and treatment response rates.
Thirty-one patients with the diagnosis of operated prolactinoma were included the study. The patients were divided into two groups as having non-invasive tumor and invasive tumor. The patients with a Ki67 index $\geq$3/100 were considered as having a tumor with high proliferative property. The patients cured with surgical treatment those had a normal level of prolactin after the surgery or with the primary medical therapy with at least 50% reduction in the tumor diameter and without worsening of these responses after cessations of the medical therapy were grouped as the patients with good responses.
High proliferation values were found in 14 (45.2%) patients, while Ki67 indices $<$0.03 were defined in 17 (54.8%) patients. Rate of the patients with Ki67 $\geq$0.03 was 37.5% in the noninvasive group and 47.8% in the invasive group. The difference between two groups was not found statistically significant. Reduction in tumor size and prolactin level were found respectively 75.19 and 90.96% in low Ki67 group while 86.36 and 88.16% in high Ki67 group ($P=0.561$, $P$: 0.922). When the responses to the treatment were evaluated in terms of the Ki67 cell proliferation indices, no statistically different was found between the groups with high and low indices in terms of the change in tumor size or prolactin level.
Mean levels of Ki 67 index were determined quite close in non-invasive and invasive prolactinomas. Three patients (37.5%) in Invasive group and 11 patients (47.8%) in non-invasive group were high level of Ki67 index. In addition, Ki67 index does not change the results of treatment. According to these results, Ki67 index has not prognostic indicator in patients with prolactinoma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1460

### Cyclin D1 gene exon 4/intron 4 region A / G polymorphism and allele ratios in patients with prolactinoma

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Excessive expression of CyD1 in CCND1 gene increase G1-S phase transition and cellular proliferation. In various tumor types and of the few studies of pituitary adenomas, over expression of CyD1 or amplification in the locus of CCND1 gene, have been demonstrated. In this study, we aimed to investigate the effect of cyclin D1 gene polymorphism on tumor formation in prolactinoma patients.
A hundred and thirteen patients and 108 age and gender matched control subjects with the diagnosis of prolactinoma were included in the study.
Male/female ratio was 27/86 and mean age of diagnosis was 34.4$\pm$10.0 years in 113 patients included to the study. Mean age of diagnosis was found as 40.3 $\pm$12.6 years in the male and 32.6$\pm$8.3 in the female patients. A and G allele rates were found as 41.7 and 58.3% in the controls, while these rates were 61.1 and 39.9% in the patients; respectively. GA genotype was the the most common genotype subgroup in the control and patient groups by 40.7 and 51.3%; respectively, while GG genotype was found significantly lower in the patient group compared to the controls. Differences between the groups in terms of the allele rates and genotype distribution were found statistically significant.
Rate of AA genotype and A allele incidence were seen to be significantly higher in the prolactinoma patients than in the controls. Genotype distribution and allele incidences of 113 cases (68 noninvasive, 45 invasive) are given in Table 1 with the values from the previous studies. Accordingly, the rate of AA genotype and A allele incidence are seen higher compared to other studies. This high rate compared to other studies may be resulted from the differences in the measurement methods as well as in the time of the studies. From this point, the rate of increase in A alleles supports the concept of the increase rates may be related to the increase in the incidence of prolactinomas, and Cycline D1 protein may play a role in the occurrence of prolactinomas.
Declaration of interest
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**Table 1** CCND1 genotype distributions and allele frequencies in three studies involving prolactinomas.

| | *n* | AA *n* (%) | GA *n* (%) | GG *n* (%) | A allel % | G allel % |
|---|---|---|---|---|---|---|
| Simpson | 57[a] | 13 (23) | 26 (46) | 18 (32) | 46 | 54 |
| Gazioglu | 41[a] | 7 (17) | 30 (73) | 4 (10) | 54 | 46 |
| Cander | 113 | 40 (35) | 58 (51) | 15 (14) | 61 | 39 |

[a]Data for subgroups of prolactinomas.

## P1461

### Cancer detection in long-term follow-up of patients with acromegaly

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Objective
To evaluate the prevalence of cancer in patients with acromegaly followed in our institution.
Design and Patients
Retrospective analysis of clinical data from our Quebec pituitary tumor registry. A total of 107 patients with acromegaly (49% females) followed at the Centre de l'Université de Montréal (CHUM) from 1980 to 2011 for a median of 7 years (Q1–Q3: 3–12).
Results
Thirteen cancers were detected in 11 (10.3%) patients over a period of 12 years. Thyroid (papillary) carcinoma was found in four patients, urologic cancer in three, two different skin cancers (basocellular carcinoma and melanoma) in the same patient, breast cancer in one, ovary adenocarcinoma in one, oesophageal cancer in one and acute leukemia in one patient. Cancer was more common in male patients (73%). Cancer was diagnosed prior to, simultaneously and after diagnosis of acromegaly in four, one and eight cases, respectively. Age of patients at cancer diagnosis was 59 years (56–62). Age of patients at diagnosis of acromegaly was significantly higher in patients with cancer compared to patients without (56 (45–62) vs 42 (33–52) years, $P=0.02$). Time delay from reported acromegaly symptoms onset to diagnosis seemed to be longer, as well as diabetes seemed to be more frequent in acromegalic patients with cancer than in those without, but these differences did not reach statistical significance. There were no significant differences between patients with cancer and those without for initial GH and IGF1 levels, follow-up duration, as well as control of the disease defined by IGF1 normalization.
Conclusion
In our series, cancer seemed to be more frequent in aged patients with acromegaly. Thyroid cancer was the most common cancer associated with acromegaly. Therefore, routine medical follow-up of patients with acromegaly should include a careful assessment for thyroid nodules and cancers.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1462

**Study on IGF(CA)19 gene polymorphism in adults with GH deficiency**

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A highly polymorphic microsatellite in the IGF1 gene promoter, composed of variable cytosine-adenine (CA) repeats ($n=10$–24) has been linked to IGF1 serum concentrations in normal, acromegalic and GHD subjects with conflicting results. Aim of this study was to investigate whether this polymorphism may influence the clinical and biochemical characteristics of adult patients with GHD ($n=97$). Moreover, the response to 12-month rhGH replacement in terms of IGF1 levels, body composition (BF%), lipid profile and glucose homeostasis was evaluated. According to the most frequent 192 bp allele (equivalent to 19 CA-repeats) patients were divided in three genotype-groups: homozygous for 192 bp allele (192/192, $n=7$, 7.2%), heterozygous for the 192 bp allele (192/X, $n=68$, 70.1%) and non-carriers of the 192 bp allele (X/X, $n=22$, 22.7%). The IGF1 genotype did not influence the clinical and biochemical phenotype of GHD adults at baseline. However, when analyzing 12-month rhGH effects separately in the 3 groups, the increase in IGF1 levels and decrease in BF% were similar, while a worsening of insulin sensitivity, documented by a significant increase in glucose levels and HOMA-IR and by a significant decrease of QUICKI, was observed only in groups carrying at least a wild type allele (192/192 and 192/X). In conclusion, longitudinal follow-up of each genotype group showed that the absence of the wild type allele of the IGF1 gene promoter might protect GHD patients from the short-term worsening of insulin sensitivity induced by rhGH.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1463

**META-analysis on the effects of octreotide on tumor mass in acromegaly**

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The long-acting somatostatin analogue octreotide is used either as an adjuvant or primary therapy to lower GH levels in patients with acromegaly and may also induce pituitary tumor shrinkage. However, inconclusive evidences in this respect have been produced by either single-center research paper or pooled analyses. Therefore, we performed a meta-analysis to thoroughly assess the current literature on the effect of octreotide on pituitary tumor shrinkage. A computerized Medline and Embase search was undertaken to identify potentially eligible studies. Eligibility criteria included treatment with octreotide, availability of numerical metrics on tumor shrinkage and clear definition of a clinically relevant reduction in tumor size. Primary endpoints included the proportion of patients with tumor shrinkage and mean percentage reduction in tumor volume.

The electronic search identified 2202 articles. Of these, 41 studies fulfilling the eligibility criteria were selected for data extraction and analysis. In total, 1685 patients were included in the analysis, ranging from 6 to 189 patients per trial.

Octreotide was shown to induce tumor shrinkage in 52% (95% CI: 49–54%) of treated patients. In patients treated with the LAR formulation of octreotide, this increased to 70%, (95% CI: 67–74%). In the nine studies in which tumor shrinkage was quantified, the overall weighted mean percentage reduction in tumor size was 29.3% (95% CI: 24.6–34.0%), rising to 49.5% (95% CI: 39.0–59.9%) with octreotide LAR.

Declaration of interest

I fully declare a conflict of interest. Details below:

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## P1464

**Parasellar masses: experience in 47 patients**

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Introduction

The differential diagnosis of nonpituitary sellar masses is broad. Clinical presentation may be similar to that of pituitary adenomas. Sometimes certain findings are particular to some lesions and may help in their differentiation. Correct preoperative diagnosis is important to better guide therapeutic management. The aim of this work was to analyze patients with parasellar lesions (craniopharyngiomas excluded), attended in the Department of Endocrinology of our hospital.

Methods

We studied clinical presentation, laboratorial and imaging results of different parasellar lesions of 47 patients before and after treatment.

Result

Sixty-six percent female, mean age $45.2 \pm 17.4$ years old. Meningiomas (18) and Rathke's cleft cyst (16) were the most frequent lesions. We also found arachnoid cysts (5), chordomas (3), gangliogliomas (2), germinomas (2) and hamartoma (1). At presentation 80.9% of patients had visual complaints, 46.8% headaches. Preoperatory evaluation: 26.2% with hypogonadism, 21.4% hypothyroidism, 11.9% adrenal and 16.7% GH insufficiencies. 11.9% of patients had panhypopituitarism, 31.5% hyperprolactinemia. Lesion location: 37.8% intrasellar with extrasellar extention, 35.6% exclusively suprasellar, 26.7% intrasellar. 80.9% of patients were submitted to surgical treatment, 52.6% of those to transcranial approach. Nine patients submitted to radiotherapy and seven reoperated. Major surgical complications: CSF leakage (seven patients) and III pair injury (six patients). After treatment, headache improved in 66.7%. Visual complaints improved in 13%, persisted in 43.5% and worsened in 39.1%. Post-operatory analytical study showed worsening of hormonal deficits (panhypopituitarism in 26.3%, hypogonadism and hypothyroidism in 52.6%, GH and adrenal insufficiencies in 44.7 and 42.1% respectively). 47.4% maintained residual tumor, 21.1% relapsed. There were no deaths, 42 patients maintain follow-up.

Conclusion

Parasellar lesions represent a very heterogeneous group. In this series, where craniopharyngiomas were excluded, meningiomas and Rathke's cleft cyst were the most frequent lesions. They originate variable clinical presentation and some degree of hormonal dysfunction. Surgical treatment leads to clinical improvement but worsening of hormonal dysfunction.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1465

**Colonic neoplasms in acromegaly: are there serum risk factors?**

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Hyperinsulinemia has been associated to an increased risk of colorectal cancer and adenomas, while higher serum 25-hydroxy vitamin D3 and folate seem to reduce the development of colonic lesions in general population. Acromegalic patients have an increased risk of colonic tumors and an association between higher fasting insulin levels and risk of colonic adenomas has been previously demonstrated. No data are available about the influence of vitamin D and folate-homocysteine levels on the development of colorectal lesion in acromegaly.

To investigate the role of insulin levels, 25-hydroxy vitamin D3 and folate-homocysteine status on the occurrence of colonic lesions in acromegalic patients, 146 acromegalic patients were submitted to a first colonoscopy screening after diagnosis. At the same time they were evaluated for fasting insulin levels, serum 25-hydroxy-vitamin D (25(OH)D3), homocysteine and folid acid. Data were collected retrospectively.

Colonic lesions were detected in 46 patients (31.5%) and consisted in hyperplastic polyps in 32 cases (21.9%), and adenomatous polyps in 14 patients (9.6%). Acromegalic patients with ($n=14$) or without ($n=132$) colonic adenomas did not significantly differ as to gender, age, BMI, family history of colorectal cancer, smoking habits, estimated duration of disease and baseline IGF1 even after excluding patient with drugs interfering with insulin levels. Fasting insulin, 25(OH)D3, folate and homocysteine levels, had not significant differences in

patients with or without colonic adenomas (7.00±3.83 vs 9.93±7.3 $P=0.66$; 16.85±10.85 vs 18.18±9.92 $P=0.64$; 7.35±2.59 vs 7.49±2.94 $P=0.94$; 7.74±2.55 vs 8.38±2.58 $P=0.36$). All the parameters evaluated were not associated to colonic adenomas at univariate and multivariate analysis.
No relationship was identified between insulin, 25(OH)D3, folate and homocysteine serum levels and risk of developing colonic lesions in this subset of acromegalic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1466

**No long-term weight reduction after gastric banding in obese patients with craniopharyngioma involving hypothalamic structures: experiences from KRANIOPHARYNGEOM 2000**

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Background
Craniopharyngiomas are embryogenic malformations which lead to eating disorders and morbid obesity due to hypothalamic involvement. The experience with laparoscopic adjustable gastric banding (LAGB) in obese craniopharyngioma patients is limited especially in regard to long-term effects and tolerability.
Patients and methods
We are reporting on four patients with childhood craniopharyngioma diagnosed at age 2, 13, 12, and 20 years.
Results
Body mass index (BMI SDS) at diagnosis was −0.9, +4.5, +4.7 and +0.23 SD. All patients developed morbid obesity (BMI SDS: +10,87, +10.36, +11.4, +6,2) so that 11, 5, 9 and 3 years after diagnosis LAGB were performed. LAGB were well tolerated. During long-term follow-up, the nadir BMI SDS (+6.9, +9.5, +7.8, +4.9) were reached 2.0, 0.5, 1.0, 0.8 years after LAGB. At last evaluation 9.1, 5.3, 7.1, 7.1 years after LAGB, the patients BMI (BMI SDS at last evaluation: +10.2, +13.9, +10.2, +6.3) had increased again but remained at a constant level comparable with baseline BMI SDS at the time of LABG. Quality of life was not decreased due to LAGB and tolerability was sufficient.
Conclusions
We conclude that LAGB is feasible and could have clinical relevant effects on long-term weight stabilization of obese craniopharyngioma patients with hypothalamic syndrome. However, a significant weight reduction was not achieved after LAGB in patients with childhood craniopharyngioma. Non-reversible bariatric procedures such as gastric bypass are not recommended for treatment of obese children and adolescents with craniopharyngioma due to ethical considerations.
Declaration of interest
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## P1467

**Predictors of the acromegaly-associated mortality in the last decade**

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Introduction
Acromegaly is associated with an increased mortality in untreated patients. Recent papers suggest an improvement of survival in the last years.
Aims
To assess mortality ratio and to identify prognostic factors associated with mortality in acromegaly in the last decade.
Methods
297 patients (186 F/111 M, mean age 49.8±0.7 years) with acromegaly admitted in a single Neuroendocrinology Department between Jan 2001 and Dec 2010 were retrospectively studied. Serum GH levels were measured by IRMA (sensitivity 0.1 ng/ml). PAMCOMP computation program was used to calculate standardized mortality ratio (SMR). Cox regression analysis revealed independent factors associated with mortality.
Results
During follow-up (median 5.9 years −1689.85 person years), 21 patients died (7.07%), corresponding to a death rate of 12.5 deaths/1000 person years. All causes mortality was not statistically different from that of general population: SMR was 0.94 (95% confidence interval (CI) 0.58–1.45). Those dying (mean death age 62.7±2.8 years) were significantly older at diagnosis (age at diagnosis 50.3±2.6 years in dying patients vs 43.7±0.7 years in survivors, $P=0.02$) and had higher posttreatment serum GH levels (16.4±4.1 ng/ml in dying vs 8.1±0.9 ng/ml in surviving patients, $P=0.04$). Survivors were more likely to be treated by pituitary surgery (160/274 patients), and/or somatostatin analogues (79/274 patients) than dying patients (7/21 received operation, $P=0.02$ and 2/21 received somatostatin analogues, $P=0.04$); radiotherapy did not seem to influence overall mortality. When assessed by Cox-regression analysis, last serum GH levels (hazard ratio (HR) 1.06, 95% CI 1.03–1.09), acromegaly duration (HR 1.08, 95% CI 1.02–1.1) and age at diagnosis (HR 1.06, 95% CI 1.02–1.1) were independent predictors of mortality. Patients with posttreatment GH levels above 5.5 ng/ml had an increased SMR: 1.7 (95% CI 1.003–2.853).
Conclusions
Patients with acromegaly admitted in the last decade had a mortality rate close to the expected level, mainly due to modern therapy. Posttreatment serum GH levels and acromegaly duration were the main predictors of survival.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1468

**Endoscopic transsphenoidal surgery as the therapy of choice for acromegaly: a 13-year experience in a single centre**

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Aim of the study
To assess the efficacy of endoscopic transsphenoidal surgery (ETS) in acromegalic patients.
Methods
Over the past 13 years, 207 consecutive patients (117 females; median age, 46 years, range 14–78) affected with GH-secreting adenoma (79 microadenomas, 38%) were operated on in our centre by the same surgical team. Age, gender, tumor size, extension, invasiveness of surrounding structures, and histopathologic features (ki67, mitoses, hystotype) have been considered together with preoperative clinical history and postoperative follow-up. An early postoperative assessment was carried out 3–6 months after surgery. The follow-up period ranged from 3 to 160 months. A statistical analysis was performed to assess a correlation with poor outcome.
Results
In the early postoperative assessment, biochemical control rate with normalization of IGF1 was 72%. Control rate remained the same during the follow-up. After surgical debulking, pharmacological treatment (somatostatin analogues and/or pegvisomant) further increased the control rate, which achieved 97%. The remaining 3% of patients still remains uncontrolled after surgery and complementary treatments (pharmacological therapy and/or radiotherapy). No mortality was observed. Diabetes insipidus (DI) and pituitary insufficiency (three and four cases, respectively) accounted for permanent postoperative morbidity. Transient morbidity encompassed transient DI (13 cases), symptomatic SIADH (seven cases), epistaxis (two cases), and CSF leak requiring intervention (one case). Both invasiveness and previous surgery correlated with unfavorable outcomes ($P<0.01$, $\chi^2$ test). In the multi-variate analysis, only invasiveness of the cavernous sinus and/or the surrounding bone structures was related with worse results ($P<0.01$).
Conclusion
Our data confirm that ETS performed by dedicated and experienced surgeons is the treatment of choice for acromegaly. Invasiveness was the main factor responsible for poor outcome. Control rate further improved by pharmacological treatments in patients with surgical debulking of the tumor, while a low percentage (3%) did not respond satisfactorily to any treatment.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1469

**MALE macroprolactinomas: response to medical treatement**

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Introduction
Male macroprolactinomas are very rare and more invasive compared to female ones. So, they are few reports concerning their response to medical treatment. We aimed to appreciate their response to Bromocriptine: the only product available in our country.
Subjects and methods
55 men (mean age: 36.3 years) with mean height pituitary tumor $=38.3$ mm (12–118) and mean PRL: 2942 ng/ml (132–28 000) were analyzed. Bromocriptine was given twice/day, mean dose: 24.8 mg/day (3.75–80), and mean duration: 32 months (1–195). After taking Bromocriptine, our patients underwent clinical, biological, visual and radiological control based on cerebral MRI.
Results
The treatment was considered as irregular in 57%. Side effects were present in 45% (hypotension and/or gastro intestinal troubles). Mean PRL was significantly reduced (2942 vs 192 ng/ml, $P<0.001$). PRL was normalized in 70% and significantly reduced in 93%. Tumor height decreased significantly (38.3 mm vs 18, $P<0.001$). Pituitary process disappeared in 20%, was significantly reduced (26–91%) in 69%. A decrease $<20\%$ was seen in 9%. The tumour increased in 1.8%. Headaches and visual abnormalities improved in almost all cases.
Conclusion
Although the treatment was irregular in 57%, the response to Bromocriptine was very good in male macroprolactinomas, as PRL was normalized or had significantly decreased in 93%, and tumor shrinkage was observed in more than 89%.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1470

**Evaluation of pituitary gland with magnetic resonance imaging in hypogonadic patients**

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Aim
Hypogonadism is a result of testicular failure and/or insufficient pituitary stimulation. Various hypothalamo-pituitary abnormalities or lesions can contribute to hypogonadism. Benign or malign tumoural lesions of cellar or paracellar region, may lead to hypogonadism. Therefore, pituitary magnetic resonance imaging (MRI), is needed in hypogonadic patients. In our study, we aimed to investigate our hypogonadic patients' hypothalamo-pituitary MRI findings.
Method
We evaluated 49 hypogonadic patients followed in our clinic. These patients' pituitary MRI findings are evaluated retrospectively.
Results
79.4% of patients had hypogonadotrophic hypogonadism, whereas 20.6% had hypergonadotrophic hypogonadism. 80.9% of the hypogonadotrophic patients' were male. 19.1% were female. Pituitary MRI findings of hypogonadotrophic patients revealed that; 59.5% were normal, 16.7% pituitary microadenoma, 11.9% partial empty cella, 4.7% pituitary macroadenoma, 2.4% empty cella, 2.4% ectopic neuropituitary, and 2.4% empty cella and ectopic neuropituitary together. In the hypergonadotrophic group, all of the patients were male. In this group pituitary MR findings were respectively; 71.4% normal, 14.3% ectopic neuropituitary, 14.3% pituitary microadenoma.
Conclusion
In half of the patients with hypogonadotrophic hypogonadism, Pituitary MRI findings may be normal. In these patients, if clinic and labarotuary results are harmonious, to determine the diagnosis dynamic tests are required and appropriate therapy must be done, even if pituitary MRI is normal. In the hypergonadotrophic hypogonadic patients, although etiology is not associated with hypothalamus and/or pituitary gland, we can find pathologic pituitary MRI results. So, we suggest, carrying out hipothalamo-pituitary imagining with hypergonadotrophic patients.
Declaration of interest
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## P1471

**Is acromegaly associated with irritable bowel syndrome?**

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Introduction
Gastrointestinal system is under the influence of excessive GH and IGF1 in acromegaly. Increased bowel length and delayed transit time may cause functional disturbence of the bowel in acromegaly. The objective of the current study is to evaluate the frequency of irritable bowel syndrome (IBS) in cases with acromegaly.
Methods
Twenty-five active cases with acromegaly who were newly diagnosed between 2010–2011 and 16 inactive acromegaly patients followed at Endocrinology–Metabolism out-patient clinic of Cerrahpasa Medical Faculty between 1983 and 2011 were included in the study. Twenty gender and age matched healthy subjects (HS) composed the control group. All cases were questioned for presence of IBS using Rome III criteria. Abdominal ultrasonography (USG) and colonoscopy results of acromegalic patients were obtained. In addition, cases with acromegaly were evaluated for their quality of life and status of depression by using acromegaly quality of life questionnaire (AcroQoL) and Beck depression inventory (BDI) respectively.
Results
IBS was present in 4 of 25 (16%) newly diagnosed active cases with acromegaly whereas one of 16 (6%) active acromegaly patients and 2 of 29 HS had IBS (6.8%) ($P=0.28$). Four of 5 patients with IBS had no pathologic finding in abdominal USG or colonoscopy that could explain their symptoms. One patien refused to have USG or colonoscopy. The median BDI and Acroqol scores in active and inactive acromegaly groups were 16 (IQR: 10.00–22.50), 11.5 (IQR: 4.50–19.50) ($P=0.25$) and 56 (IQR: 42.50–68.00), 65.50 (IQR: 40.63–76.75) ($P=0.45$), respectively.
Conclusion
A higher percentage of IBS was observed in acromegaly patients with active disease compared to inactive patients although there was not a statistically significant difference.
Declaration of interest
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## P1472

**Acromegaly, does tumor size matters?**

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Introduction
Acromegaly is a rare disease which results from a GH producing adenoma. Around 70–80% are macroadenomas. The therapeutic options currently available are surgery, radiotherapy (RT) and medical treatment (MT). According to literature microadenomas have a higher remission rate, around 80%.

Methods
A retrospective chart review of the patients with acromegaly treated in our centre from 1976 to 2011 was performed. In terms of disease control at present it was considered: Controlled with therapy when GH and IGF1 were normal but under medical therapy, persistent disease when the disease was not controlled and remission. Remission was considered when a period longer than 6 months without therapy on the last visit day, with GH $<2.5$ ng/ml, normal IGF1 and GH nadir with PTGO $<1$ ng/ml. This were the safe criteria.
Results
Among 57 patients, there were 42 (73.7%) female. Mean age of diagnosis 49.3 $\pm$14.9 and a mean follow up duration of 10.8$\pm$7.9 years. The median GH values on the diagnosis were 18.35 ng/ml, and IGF1 761 ng/ml. There was 44 (77.2%) macroadenomas. Remission was observed in 5 (50%) of the microadenomas and 23 (52.3%) of the macroadenomas, 9 (39.1%) with extension out of sella turcica. Eighteen (31.6%) were controlled under therapy and 11 (19.3%) had persistence of disease. All of the cases in remission were first treated with transfenoidal surgery, 10 (35.7%) were also treated with MT (three microadenomas and seven macroadenomas), and 5 (17.8%) macroadenomas with MT plus RT.
Conclusions
The results of these study shows a similar remisson rate between micro and macroadenomas, (52.3 vs 50%) even though the macroadenomas less frequently have safe criteria just with surgery. This results also showed that the macrodenomas confined to the sella gets in remission more frequently than the invasive tumours.
Declaration of interest
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## P1473

**Clinical and histological characteristics of giant GH producing pituitary adenomas**

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Introduction
Surgical treatment of giant GH cell adenomas ($>4$ cm in maximum diameter; GHomas) has been considered difficult. However, clinical and histological characteristics of these adenomas are still enigmatic. We retrospectively analyzed data of 17 giant GHomas experienced at Toranomon hospital to clarify their characteristics.
Methods
These 17 patients, accounting for 3.1% of 549 acromegalic patients undergoing surgery between 2006 and 2011, who consisted of 7 females and 10 males ranging from 17 to 48 years of age.
Results
Visual disturbance was found in 6 of 17 patients and serum GH and IGF1 levels were ranging from 2.6 to 427.6 ng/ml and 418 to 1390 ng/ml, respectively. Most tumors showed invasion to the cavernous sinus (11 patients showed Knosp's Grade 4). Preoperative medication was applied in 12 patients and tumor shrinkage was found in 10 patients. TSS, TSS followed by craniotomy, and simultaneous combined transsphenoidal & transcranial approach was performed in 11, 3, and 3 patients, respectively. Total tumor removal was accomplished in ten patients (59%; endocrinological cure was in five patients). As surgical complications, anterior hormone deterioration occurred in three patients and new postoperative diabetes insipidus was in two patients. In addition, transient CSF leakage was found in one, carotid artery injury in one and transient ophthalmoplegia in four patients. Postoperative medication was used in six patients, whereas radiotherapy was administered in five patients. Histological findings were also various as follows; pure GH adenoma 11 patients, GH–PRL adenomas three, GH–TSH adenoma one, and GH–PRL–TSH adenomas two. Interestingly, Ki67 index $<1$% was in nine patients, suggesting lower biological tumor activity.
Conclusion
Our study confirmed that giant GHomas showed various endocrinological and histological findings. Moreover, they are generally very difficult to be treated and therefore multidisciplinary treatment including various surgical approaches is required to control giant GHomas.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1474

**Long-term outcomes after sterotactic radiosurgery for non-functioning pituitary adenomas**

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Introduction
Sterotactic radiosurgery (SR) has been used to treat recurrent adenomas and also as a primary treatment. The objective is to evaluate long-term tumor control, development of hypopituitarism and other side effects in the follow-up.
Patients and method
Retrospective analysis of 21 patients with non-functioning pituitary adenomas (NFA) treated with modified linear accelerator (LINAC) between 1998 and 2009 in three tertiary Spanish hospitals. Control of tumor size was defined as unchage or decrease of tumor size.
Results
The average follow-up period was 6.2 years. The cohort consisted of 61.9% of women and 30.1% of men with an average age of 45.3 years. LINAC was the primary treatment in 3 patients, while the remaining 18 patients had undergone one/two prior surgical resections. Inmunohistochemical data shows 9 silent pituitary tumors, 4 patients were catalogued as "null cell " and the remaining 8 as NFA in the strict sense. The mean dose of treatment was 16.2 Gy ($\pm$2.01 SD). Prior to radiosurgery, pituitary hormone deficits were observed in 66.7% patients. New endocrine deficiencies were described in 11 patients during the follow-up. Tumor volume decreased in 52.3% patients, increased in 38.1% patients, and was unchanged in 9.5% patients. Tumor size decrease was statistically significant 12 months after radiosurgery (14.5 mm vs 12.3; $P=0.03$). Side effects presented in half of the patients were transitory. Seven patients presented cognitive problems 12–18 months after radiosurgery, one patient suffered a stroke in the fourth year after SR and other patients developed a second tumor nine years after SR.
Conclusions
LINAC resulted in a tumour size control of 61.8% in patients with NFA. Response to SR was statistically significant 1 year after treatment. Precocious side effects are usually transitory. The follow-up allows to detect new endocrine deficits and other side effects that may appear in the long term.
Declaration of interest
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## P1475

**Long-term outcomes after sterotactic radiosurgery for functioning pituitary adenomas**

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Introduction
Sterotactic radiosurgery (SR) has been used to treat recurrent adenomas after failure of medical and surgical procedures and also as a primary treatment. The aim of this paper is to evaluate long-term control of functioning pituitary adenomas (FPA).
Patients and method
Thirty-eight patients with FPA treated with modified linear accelerator (LINAC) between 1998 and 2009 in three tertiary Spanish hospitals. Hormonal control was defined as hormonal normalization. Control of tumor size was defined as an unchange/decrease of tumor size.
Results
The average follow-up period was 7.3 years. The cohort consisted of 78.9% of women and 21.1% of men with an average age of 39.9 years. LINAC was the primary treatment in 16 patients, while the remaining 22 patients had undergone one/two prior surgical resections. There were 4 prolactinomas,

17 adrenocorticotropic hormone-secreting and 17 GH secreting tumors. Prior to radiosurgery, pituitary hormone deficits were observed in 23.6% of patients. New endocrine deficits occurred of 75–82.3% patients during the follow-up. Seven patients achieved remission after SR treatment. The mean dose of treatment was 17 Gy. Tumor volume decreased in 68.4% patients, increased in 5.2% of patients, and was unchanged in 26.3% of patients. Three patients presented cognitive deterioration after radiosurgery and two acromegaly patients suffered strokes two and three years after radiosurgery. The decrease in levels of IGF1 and 24 h urine cortisol after 3 months of SR was statistically significant ($P=0.011$ and $P=0.017$ respectively), but not in prolactine levels ($P=0.144$). There were no differences between radiosurgery as primary or adjuvant treatment.
Conclusions
LINAC resulted in a durable rate of tumor control in 94.7% of patients with FPA; nevertheless hormonal normalization without treatment is more difficult to reach. Follow-up is important to detect new hormonal deficits and other side effects that may appear in the long term.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1476

### Comparison of immunoassay and mass spectrometry measurement of cortisol during $ACTH_{1–24}$ stimulation tests

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Measurement of plasma cortisol by immunoassay after $ACTH_{1–24}$ stimulation is used to assess the hypothalamic–pituitary–adrenal (HPA) axis. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) has greater specificity and equilibrium dialysis allows measurement of free plasma cortisol. We investigated whether measuring cortisol by LC–MS/MS improves the sensitivity of $ACTH_{1–24}$ stimulation testing in pituitary patients. We studied 60 controls (34 female, age $61\pm12$ years, BMI $27.7\pm5.6$ kg/m$^2$) and 21 patients with pituitary disease in whom HPA sufficiency ($n=8$) or deficiency ($n=13$) had been defined by insulin tolerance test (peak cortisol cut-off 550 nmol/l) or morning cortisol (deficient $\leq$100 nmol/l, sufficient $\geq$500 nmol/l). Subjects received 1 μg $ACTH_{1–24}$ intravenously and 250 μg $ACTH_{1–24}$ intramuscularly about 7 days apart. Total and free (following equilibrium dialysis) plasma cortisol were measured by in-house LC–MS/MS assay and total plasma cortisol by immunoassay (Elecsys 2010, Roche Diagnostics). The lower limits of the 95% confidence intervals derived from normal subjects were used to define the pituitary patients HPA status. The 30 min cortisol during the 1 μg $ACTH_{1–24}$ stimulation test and the 30 and 60 min cortisols during the 250 μg $ACTH_{1–24}$ stimulation tests were equally concordant with previous HPA axis assessment. Measurements of total cortisol by immunoassay were concordant with previous HPA axis assessment in 19/21 and 20/21 patients using the 1 and 250 μg $ACTH_{1–24}$ tests respectively. The sensitivities of total and free cortisol by LC–MS/MS were similar to those derived from the immunoassay (Table). We conclude that measurement of total or free plasma cortisol by LC–MS/MS after $ACTH_{1–24}$ stimulation provides similar results to immunoassay in pituitary patients.

**Table 1**

| | 0 min | 30 min | 60 min |
|---|---|---|---|
| | 1 μg $ACTH_{1–24}$ stimulation test | | |
| Total cortisol (immunoassay, nmol/l)[a] | 199–763 | 441–919 | 317–864 |
| Concordance ($n$, (%))[b] | 17 (81) | 19 (90) | 17 (81) |
| Total cortisol (LC–MS/MS, nmol/l)[a] | 213–933 | 497–1125 | 327–1067 |
| Concordance ($n$, (%))[b] | 16 (76) | 20 (95) | 16 (76) |
| Free cortisol (LC–MS/MS, nmol/l)[a] | 4.0–45.8 | 31.6–94.6 | 15.3–62.5 |
| Concordance ($n$, (%))[b] | 16 (76) | 20 (21) | 18 (86) |
| | 250 μg $ACTH_{1–24}$ stimulation test | | |
| Total cortisol (immunoassay, nmol/l)[a] | 176–703 | 492–978 | 606–1025 |
| Concordance ($n$, (%))[b] | 16 (76) | 20 (95) | 20 (95) |
| Total cortisol (LC–MS/MS, nmol/l)[a] | 192–824 | 524–1153 | 630–1233 |
| Concordance ($n$, (%))[b] | 16 (76) | 19 (90) | 19 (90) |
| Free cortisol (LC–MS/MS, nmol/l) | 3.6–36.1 | 32.1–101.8 | 42.4–119.4 |
| Concordance ($n$, (%))[b] | 16 (76) | 20 (95) | 20 (95) |

[a]95% confidence intervals derived from normal subjects.
[b]With previous HPA axis assessment in patients with pituitary disease

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1477

### Cushing's disease: prospective assessment of factors influencing the efficacy of transsphenoidal surgery

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Introduction
Assessment of the efficacy of surgical treatment for Cushing's disease (CD) is one of the biggest challenges in contemporary endocrinology. The aim of this study was prospective evaluation of factors influencing the result of transsphenoidal surgery for CD.
Methods
The study population consisted of 36 consecutive patients with CD hospitalized in the Department of Endocrinology from 2005 to 2009 and operated on using the same surgical protocol in the Department of Neurosurgery. Preoperative hormonal assessment, results of MRI and histopathological examination were taken into account. Particular attention was paid to the early postoperative cortisol levels measured at 6.00 on the first day after surgery.
Results
Mean serum cortisol level on the 1st postoperative day was $6.0\pm9.02$ μg/dl (median: 1.98 μg/dl). Twenty three patients (63.9%) were regarded as surgically cured from CD using commonly adopted criteria. In all cured cases, the serum cortisol level on the 1st postoperative day was $\leq$ 2.5 μg/dl. In the cured group, there was a significantly greater number of patients with pituitary microadenoma clearly visualized in the preoperative MRI than in the non-cured group (73.9 vs 38.5%; $P=0.036$). A difference was also demonstrated with regard to results of immunohistochemical examination. The confirmation of corticotroph adenoma was more frequently observed in the cured group in comparison with the non-cured group (87 vs 53.8%; $P=0.028$). There was no difference in terms of preoperative ACTH ($P=0.88$) and cortisol levels ($P=0.71$).
Conclusion
The optimal cut off value suggesting remission of CD is serum cortisol level $\leq$ 2.5 μg/dl on the 1st postoperative day. Other factors influencing the remission of CD are: the distinct pituitary microadenoma visualized in the MRI and the histopathologically confirmed presence of corticotroph adenoma. However, any of preoperative hormone measurements affected the efficacy of surgical treatment.
Declaration of interest
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## P1478

### The results of surgical treatment on prolactinomas in females of child bearing ages

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Introduction
Medical therapy with dopaminergic drugs is the preferred initial treatment for symptomatic prolactinoma. However, some drawbacks of medical therapy are still of concern. Surgery is indicated in cases of resistance or intolerance to drugs or where patients prefer definitive cure to lifelong drug treatment. In addition, young women with small and enclosed type tumor may be good candidates for surgical treatment because of high curability and safety. We evaluated the

long-term effect of surgery in patients who had been followed up longer than 1 year postoperatively.
Methods
Subjects are 16 females of child bearing ages who underwent transsphenoidal resection of prolactinomas from 2006 through 2010.
Results
Mean age at surgery was 25.8±6.2 years. Preoperatively, 37.5% patients had received medical treatment. Mean preoperative PRL concentration was 224.2 ±138.4 ng/ml. Mean tumor diameter was 12.1±6.0 mm. At the time of diagnosis, 81.3% patients had amenorrhea, and 18.7% patients had oligomenorrhea. Mean postoperative PRL concentration was 6.2±4.5 ng/ml. Surgical cure was achieved in all patients, and they had regular menstruation. No operative severe morbidity occurred. Gonadotropin secretory function was also well preserved. At the latest follow-up, 42.9±17.4 months after surgery, four patients have given birth, but a patient whose PRL level increased again has received cabergoline therapy.
Conclusion
The surgical treatment by skillful hands on small and enclosed prolactinomas in young females is still one of reasonable choices for its safety and efficacy.
Declaration of interest
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## P1479

### Treatment outcomes in prolactinomas with and without cavernous sinus invasion

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In prolactinomas, today first choice of treatment is medical treatment with dopamine agonists. Cavernous sinus invasion is not uncommon in prolactinomas and stands out as a blocking factor for surgical removal of the tumor fully. In our study, the presence of cavernous sinus invasion was to investigate the effect on the results of surgical and medical treatment in prolactinomas.
Eighty-seven patients followed up with a diagnosis of prolactinoma were included in the study. Presence or absence of cavernous sinus invasion were defined by examining detailed the coronal images of pituitary magnetic resonance imaging scans. Adenomas that surrounding 67% (2/3) of the internal carotid artery in the cavernous sinus segment were defined as adenomas with cavernous sinus invasion. Respond to treatment determined consider with before and after treatment tumor sizes, prolactin levels and recurrence rates of patients. Accordingly this, remission (tumor shrinkage of at least 50%, normalization of prolactin level) or complete recovery provided that patients respond well to treatment was classified as a group.
In 23 patients with cavernous sinus invasion (CSI group), male/female (M/F) ratio was determined as 16/7 with 37.7 average age at diagnosis and 7/57 with 33.5 average age at diagnosis in 64 patients without invasion (nCSI group). 73.4 and 78.3% of patients respond well to treatment with 70.2 and 69.2% average percentage of reduction in tumor size had been established in CSI and nCSI groups respectively. The percentage of operated patients was found 87% in CSI group while 32.8% in nCSI group. Twenty patients in total of 41 operated patients was in CSI group and only of them was found cured surgically while 10 patients were found cured surgically in nCSI group ($P<0.01$). The remission rates with surgery were similar in both groups (75 and 81%).
According to our study results, response to medical therapy is not affected by cavernous sinus invasion state but invasion state affects the results of surgical treatment. Accordingly these results, patients with cavernous sinus invasion are not suitable candidates for surgical treatment. When surgical treatment is recommended to prolactinoma patients, cavernous sinus invasion should be considered.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1480

### Change in somatostatinergic tone of acromegalic patients according to the size of GH-producing pituitary tumors

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Objective
Change in somatostatinergic tone (SST) is one of the key features of acromegaly, but the effect of tumor size on SST is not clear.
Design
The aim of this study was to determine how SST changes depending upon the size of GH-producing pituitary tumors.
Method
GH levels of 29 patients with newly diagnosed acromegaly were measured using a 75 g oral glucose tolerance test (OGTT), an insulin tolerance test (ITT) and an octreotide suppression test (OST). GH/glucose ratio during the OGTT and the ITT were calculated, and GH levels during the OGTT were subtracted from those during the ITT and the OST at the same time point (ΔGHIO and ΔGHSO respectively). These values were compared according to the size of tumors and the response pattern to the OST.
Results
Twenty-two of 29 tumors were macroadenomas. The ratio between GH and glucose (GH/glucose) during the OGTT but not the ITT was also higher in macroadenomas than microadenomas with borderline significance. ΔGHIO and ΔGHSO during the entire test time also were different between macroadenomas and microadenomas with borderline significance. According to the further analyses with macroadenomas based upon the response pattern to the OST, GH/glucose for both the OGTT and the ITT were higher in non-responders with borderline significance while ΔGHIO nor ΔGHSO did produce significant differences between responders and non-responders.
Conclusion
Our study suggests that, as the size of the tumor becomes large, the effect of glucose on SST was attenuated. Macroadenomas with non-responders to the OST had the portion of GH secretion beyond the range of regulation by SST. This is the first study to examine changes in SST of GH-producing pituitary tumors through the additional manipulation of test results necessary to confirm the diagnosis of acromegaly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1481

### Two cases of sever congenital central hypothyroidism with TSH and prolactin deficiency-possible new syndrome

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Instruction
Most of cases reported before who were detected by $FT_4$ or $T_4$ newborn screening are, TSH and GH deficiency or CNS anomalies, and their hypothyroidism is mild. We report two cases of sever congenital hypothyroidism with TSH and Prolactin (PRL) deficiency, detected by TSH and $FT_4$ screening.
Case report
Case 1. K.W. boy, was born at 38 gestations, weighted 3.17 kg. Thyroid function at 5 days of age was TSH 5.6 mU/l, $FT_4$ 0.07 ng/dl. Epiphysis of distal femur was not detected. L-$T_4$ treatment was started followed by TRH loading test. Thyroid studies were performed at 6 9/12 years after discontinued L-$T_4$. TSH 4.6 mU/l, PRL 1.0 ng/ml, $FT_4$ 0.13 ng/dl at baseline and low delayed response was observed after TRH loading. IQ was 94. Growth and development, MRI is normal.
Case 2, H.F. boy, was born at 38 gestations, weighted 3.37 kg. Thyroid function at 5 days of age was TSH 5.9 mU/l, $FT_4$ 0.14 ng/dl. Epiphysis of distal femur was 1×3 mm. L-$T_4$ treatment was started followed by TRH loading test. Thyroid studies were performed at 5 10/12 years after discontinued L-$T_4$. TSH 2.8 mU/l, PRL 1.1 ng/ml, $FT_4$ 0.1 ng/dl at baseline and low delayed response was observed after TRH loading. IQ was 100. Growth and development, MRI is normal.

Methods and result
TRH, TRH-receptor, GATA2 and TSH-β genes were analyzed and not found any mutations. Metoclopramide, 10–20 mg, was given intravenously and serum PRL was determined. Although prompt response was observed in controls, no response was observed in two patients.
Conclusion
This is the first report of severe congenital central hypothyroidism with TSH and PRL deficiency. Etiologies of patients have to be resolved.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1482

**Treatment response and comorbilities in acromegalic patients**

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To evaluate the prevalence of comorbilities related to acromegaly and the grade of response to the different therapeutic alternatives.
Material-methods
A retrospective study of 25 acromegalic patients diagnosed between 1990–2010 was made. It included a descriptive analysis on sex, age, hypertension, impaired glucose metabolism when diagnosed acromegaly, screening for dygestive and cardiac pathology, tumoral size, and percentage of disease control and healing depending on the treatment modality.
Results
The mean age at diagnosis was 44.4 years. 64% women and 34% men. 44% of the patients had hypertension and 32% impaired glucose metabolism (7 patients type 2 diabetes mellitus and 1 patient impaired fasting glucose) at the moment of diagnosis of Acromegaly. 16% had echocardiographic alterations (2 left ventricle hypertrophy, 1 mild aortic insufficiency and 1 cardiac insufficiency). Colonoscopy was made to 64% of patients being normal 62.5% and pathologic 37.5% of the patients explored (3 hyperplasic polyps, 2 tubular-villous adenoma and 1 mild dysplasia). The pituitary MR showed 24% microadenoma (none extrasellar) and 76% macroadenoma (79% invasives). Surgery was elected in 88% with a curative rate of 27.7%. Conventional/fractioned stereotactic radiotherapy was adiministered to 32% of patients. Currently, 9 patients have healing criteria (5 with surgery and 4 receiving radiotherapy after surgery) and another 10 patients have disease control criteria (4 with medical treatment, 3 with surgery and medical treatment and 3 with surgery, radiotherapy and medical treatment).
Conclusions
The curative rate after surgery still compromises a wide range depending on several factors such the surgeon experience.
The different therapeutical alternatives aims to reduce morbimortality to acromegalic patients in order to have the same as general population. However, two or more treatment modalities are often needed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P1483

**Reproductive function in acromegalic women: a one-center experience**

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Background
It is well-known that gonadal function is impaired in acromegalic females, due to several mechanisms.
Aim and design
Retrospective survey of our series.
Patients
Our electronic database encompassing data of 180 acromegalic patients collected over 30 years was searched, extracting first data of women ($n=91$) and then of females aged less than 50 years at diagnosis of acromegaly ($n=34$).
Results
Among the 34 women of potentially fertile age, 18 patients had had 28 pregnancies (24 singleton at term) before the diagnosis of acromegaly (group 1) and 9 patients had no pregnancy at all (group 2). Seven patients had 11 spontaneous pregnancies (9 at term, 2 twin) after the diagnosis of acromegaly (group 3): two conceived after non-curative surgery, 4 while on GH-suppressive treatment (3 on dopamine-agonists and 2 on somatostatin analogs), and the last without any treatment. One patient had 3 pregnancies before and one after the diagnosis. On comparing patients of groups 1 and 3, the former group was older (36 vs 28 years), with lower levels of GH at diagnosis (31 vs 83 ng/ml) and lower prevalence of hyperprolactinemia (4/18 vs 4/7) but without difference in adenoma size distribution (microadenoma vs intrasellar macroadenoma vs extrasellar adenoma). On comparing patients of groups 2 and 3, unexpectedly the former had lower levels of GH at diagnosis (32 vs 83 ng/ml) and lower prevalence of hyperprolactinemia (2/7 vs 4/7) but no difference in age and adenoma size was observed.
Conclusions
In our experience 20% of acromegalic patients conceived spontaneously in spite of ongoing disease. On considering that the diagnosis of acromegaly is often delayed by several years, at least a few pregnancies in patients of group 1 might be added to the total. Furthermore, patients can be reassured that even very high GH levels and extrasellar macroadenoma at diagnosis are not an absolute barrier to conception.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1484

**Evaluation of bone mass and fracture in patients with prolactinoma**

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Patients with hyperprolactinemia have impaired bone metabolism and bone mineral density (BMD) partially represents bone's health.
To evaluate vitamin D levels, PTH, Ca and P, BMD and bone morphometry in patients with prolactin-secreting pituitary adenomas.
Forty-four patients (30 W, $40\pm11$ years, BMI $27.6\pm5$ kg/m$^2$) underwent to lumbar and femoral DEXA, to vertebral morphometry by X-ray and was calculated the SDI (spinal deformity index), a surrogate method for the estimation of microarchitecture and bone quality. Forty-two age and BMI matched served as controls.
Vitamin D were lower in patients than in controls ($24.1\pm10$ ng/ml vs $30.4\pm14$, $P=0.018$). Vitamin D deficiency was present in 39.5% (<20 ng/ml) vs 12% of controls ($P<0.01$), insufficiency in 32.6% (20–30 ng/ml) vs 32% ($P=$NS) and normal levels of vitamin D in 27.9 vs 56% (>30 ng/dl) in patients and controls ($P<0.01$). Lumbar spine $T$-score was $-0.2\pm1$ vs $0.11\pm1.15$ in patients and in controls ($P=$NS) and $-0.3\pm1.1$ vs $-0.16\pm0.5$ ($P=$NS) at femoral neck. At lumbar spine 37.5% of patients had osteopenia and 56.3% had normal BMD while in controls 7.1% had osteoporosis, 45.2% osteopenia and 47.6% normal BMD. At femoral neck 3.4% of patients had osteoporosis, 31% osteopenia and 65.5% had a normal BMD, and in controls 7.1% showed osteopenia and 92.9% a normal BMD. The SDI was higher in patients than in controls ($2\pm1$ vs $0.4\pm0.2$; $P<0.01$).
In hyperprolactinaemic patients vitamin D is lower than in controls; although BMD values are normal, our data seems to show that there is a higher prevalence of vertebral fractures and worse bone quality than expected by BMD values.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1485

**Prolactin levels are correlated with tumor size and invasiveness in prolactinomas**

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Prolactinomas are more common tumors by 60% among the hypophysis adenomas. These are often benign, but may present differences in terms of tumor size and treatment responses. Aim of this study was investigate the relationship of serum prolactin levels with tumor size and invasiveness in the prolactinomas.

113 patients with the diagnosis of prolactinoma were included to study. Pretreatment plasma levels of prolactin in the time of diagnosis and sella MR imaging findings. According to the sella MR imaging findings during the diagnosis, the tumors were defined as microadenoma (the longest diameter $<1$ cm), macroadenoma (the longer diameter between 1 and 4 cm), giant adenoma (the longest diameter $>4$ cm), invasive and non-invasive adenoma.

The mean age at the time of diagnosis was $34.4 \pm 10.0$ years in 113 patients. The mean age of diagnosis was found as $40.3 \pm 12.6$ in the male and $32.6 \pm 8.3$ in the female patients. The mean size of adenoma was found as $17.9 \pm 18.1$ mm in all the patients, $38.6 \pm 21.6$ mm in the male and $10.8 \pm 9.4$ mm in the female patients. Pre-treatment serum levels of prolactin were mean $1926 \pm 6662$ ng/ml. Positive correlation was defined in the correlation analysis between the pre-treatment serum levels of prolactin and tumor size. The correlation coefficient was found as 0.805 in the correlation analysis applied between log10 values of the prolactin levels (ng/ml) and tumor size (mm) (Figure ). When the case with a serum level of prolactin higher than 3300 ng/ml were examined (*n*:8), all are seen to fall into the invasive adenoma group. Of patients with a lower serum level of prolactin, 61 were seen to be in the noninvasive and 32 in the invasive group. When the cut-off value was taken as 400 ng/ml, sensitivity was calculated as 70% and specificity as 100%.

These findings show serum levels of prolactin to be an important factor in the definition of tumor behavior. As it is indicated in the previous studies, a strong correlation was found between the prolactin levels and the size and invasiveness of tumors in our study.

Figure 1 Correlation between pretreatment prolactin level and tumor size.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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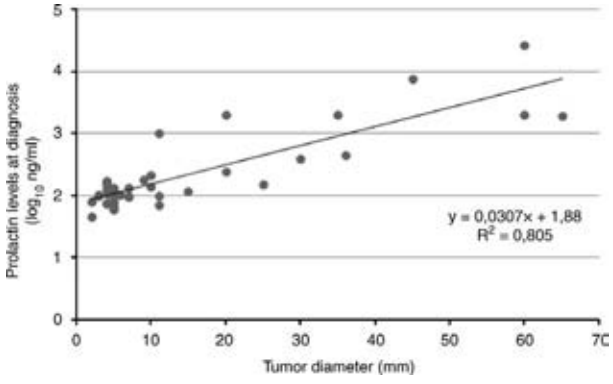


## P1486

**Is every joint symptom related to acromegaly?**

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Introduction

Acromegaly is a chronic endocrinopathy characterized by hypersecretion of GH and insulin-like growth factor 1 (IGF1). Musculoskeletal pain is a frequent problem encountered in acromegaly and is associated with a reduction in quality of life. In this study we investigated the presence of inflammatory, rheumatologic and degenerative disease retrospectively.

Methods

Fourty acromegaly patients who were in remission as laboratory findings but whose joint symptoms had not subsided and were referred to the rheumatology clinic with complaints of joint pain between 2006–2010 were evaluated in the study. Clinical, radiologic and laboratory data were examined.

Results

Mean age of the acromegalic patients were 47.1 (22–75). All patients were in remission as clinically and laboratory findings. When the radiologic data were evaluated, bilateral sacroilitiis was found at sacroiliac joint graphy and magnetic resonance imaging (MRI) in 1 patient and degenerative joint changes in 24 patients; however, there was no pathology at the radiologic data of 15 patients. Laboratory data revealed antinuclear antibody (ANA) positivity (3 nucleolar, 1 homogeneous) in 4 patients, rheumatoid factor (RF) positivity in 4 patients and cyclic citrullin peptid (CCP) positivity in 1 patient. After the evaluation of the patients with clinical, laboratory and radiologic methods, 3 patients were diagnosed as inflammatory rheumatologic disease (rheumatoid arthritis (RA), ankylosing spondylitis (AS), undifferentiated connective tissue diseases (UCTDs)) and 6 patients were diagnosed as Diffuse Idiopathic Skeletal Hyperostosis (DISH). Their symptoms subsided after the medical treatment for inflammatory joint disease.

Conclusion

While degenerative joint disease was observed frequently in our patient group similar to the literature, inflammatory rheumatologic disease was also discovered in three patients. Distinguishing these two joint disease is important, because response to medical treatment is dramatically good in inflammatory disease than the degenerative disease. A multidisciplinary approach involving endocrinology and rheumatology is imperative for appropriate management of these patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1487

**Acromegaly and pregnancy: case reports**

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Acromegaly is a rare clinical disorder characterized by progressive somatic disfigurement and a wide range of systemic complications which include gonadotrophic dysfunction, menstrual abnormalities and infertility. Thus, pregnancy in patients with acromegaly is a rare and challenging medical situation. Furthermore, the evidence about the use of somatostatin analogs (SSAs) during pregnancy in patients with acromegaly and its outcomes is still limited. Here we describe the clinical characteristics and outcomes of two patients with acromegaly who were treated with SSA during pregnancy, which add to the previous reports of similar cases. Case 1: 19 y.o. female, G1P0 with a GH- and prolactin-producing pituitary tumour (macroadenoma) previously treated with transesphenoidal surgery for tumor resection and cabergolide 0.5 mg 4x/w, octreotide LAR 30 mg IM every 28d. She continues on octreotide LAR 30 mg IM every 28d since week 9 of pregnancy achieving normal term pregnancy (38w3d gestation, 3200 g, 51 cm newborn) and 76, 34 and 30% reduction in GH and IGF1 levels and tumour size reduction, respectively. Case 2: 25 y.o. female, G1P0 with a GH- and prolactin-producing pituitary tumour (macroadenoma) previously treated with transesphenoidal surgery for tumor resection and bromocriptine 2.5 mg QD PO. She is started on lanreotide ATG 60 mg Q3 every 28 days since week 11 of pregnancy achieving normal term pregnancy (39w2d, 3120 g, 49 cm newborn) and a 66, 64 and 14.2% reduction in GH and IGF1 levels and tumour size reduction, respectively. These cases suggest that SSAs during pregnancy in patients with acromegaly reduce GH and IGF1 levels and tumor size with no effect on fetal growth or development.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1488

**Pegvisomant and cabergoline combination therapy in acromegaly**

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Objective
Combination with cabergoline may offer additional benefits to acromegalic patients on pegvisomant monotherapy. We evaluated the safety and efficacy profile of this combination and investigated the determinants of response.
Design
An observational, retrospective, cross-sectional, registry-based study.
Patients and methods
Fourteen acromegalic patients (9 females), who were partially resistant to somatostatin analogs and on pegvisomant monotherapy. Cabergoline was added because of the presence of persistent increased IGF1. The mean follow up time was $18.3 \pm 10.4$ months. The efficacy and safety profile was assessed. The influence of clinical, biochemical, and histological characteristics on treatment efficacy was studied.
Results
IGF1 levels returned to normal in 4 patients (28%) at the end of the study. In addition, some decline in IGF1 levels was observed in a further 5 patients. The % IGF1 decreased from $158 \pm 64\%$ to $124 \pm 44\%$ ($P=0.001$). The average change in IGF1 was $-18 \pm 27\%$ (range $-67$ to 24%). Lower baseline IGF1 ($P=0.007$), female gender ($P=0.013$), lower body weight ($P=0.031$), higher prolactin (PRL) levels ($P=0.007$), and positive tumour PRL immunostaining ($P=0.029$) were associated with a better response to combination therapy. There were no significant severe adverse events. Significant tumour shrinkage was observed in 1 patient.
Conclusions
Combination therapy with pegvisomant and cabergoline could provide better control of IGF1 in patients with acromegaly. Baseline IGF1 levels, female gender, body weight, PRL levels, and PRL immunostaining affect the response to this combination therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1489

**Influence of the family environment during the early years in life in the vulnerability to the development of pituitary adenomas**

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Introduction
Prolactinomas are the most common pituitary adenomas. Several authors hypothesized that there is an association between the development of pituitary adenomas and psychologically significant experiences, especially traumatic ones. This is particularly well-established for Cushing's disease. When it comes to prolactinomas, it was suggested that prolactin acts as an alternative to cortisol in the response to stress, especially in patients who present with passive coping strategies. We looked at the influence of traumatic experiences before the age of ten on the susceptibility to the subsequent development of prolactinomas.
Methods
We compared a group of 33 patients with the diagnosis of prolactinoma to the same number of patients of the same age and gender with non-functioning pituitary adenomas and Cushing's disease. We studied the difference between the patients with prolactinomas and the two other subtypes of adenomas in relation to the absence of a father before the age of ten, and to the presence of an alcoholic or violent father during the same period.
Results
We observed a higher frequency of traumatic childhood experiences, especially the presence of an alcoholic father, in patients with prolactinomas (33,3%) when compared to the other adenomas (9,8%, $P<0.05$). Contrary to what we expected, patients with Cushing's disease reported not living with their father during childhood the most. This result is similar to that found in previous studies, where there was also an association between traumatic events and the development of these neoplasms.
Conclusion
Traumatic experiences during childhood seem to increase the susceptibility to the development of prolactinomas, especially in patients who lived with an alcoholic father. On the contrary, the absence of a father seems to favor the development of Cushing's disease. Experiencing traumatic situations during childhood may interfere with epigenetic processes which silence tumor suppressor genes and promote the development of pituitary adenomas.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1490

**Hyperprolactinemia in polycystic ovary syndrome- diagnostic and therapeutic approach**

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Background
Prolactin (PRL) hypersecretion is the most common endocrine abnormality due to hypothalamic-pytuitary disorders. Hyperprolactinemia occurs in 20–30% of cases with polycystic ovary syndrome (POS). It is considered that hyperprolactinemia is not involved in POS etiopathogeny, but other factors are implied.
The aim of the study: was to retrospectively evaluate the diagnostic tools (Rotterdam criteria) and therapeuthic approach applied in 78 women with POS, evaluated in our clinic in the last 5 years. Out of the twenty six POS patients with hyperprolactinemia, half presented associated hyperprolactinemic conditions. A percentage of 15.3% presented an associated mycroprolactinoma (46.1% of the hyperprolactinemic cases). Plasma levels of PRL were significantly higher in the patients with mycroprolactinomas as compared to hyperprolactinemic women without a tumor ($P<0.001$). Other causes of hyperprolactinemia were represented by: combined contraceptive therapy (five cases), psychotropic medication (two patients), chronic methoclopramid treatment (one case), and severe hypothyroidism (three cases). The treatment of tumoral cases consisted of dopaminergic agonists (cabergoline, bromocriptine), in usual doses. The response was favorable, similar to the patients with microprolactinomas, but without POS. Mild hyperprolactinemic cases were regularly followed-up, noticing that the PRL values didn't increase significantly over time.
Conclusions
Due to the increased incidence of PRL secreting pituitary tumors in patients with POS, often hyperprolactinemia imposes also imagistic diagnosis (pituitary MRI). In hyperprolactinemic patients, factors which could further increase the PRL secretion should be avoided.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1491

**Hypothalamo-pituitary dysfunction in patients with chronic subdural hematoma**

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Introduction
Hypothalamo-pituitary dysfunction has been reported in patients after traumatic brain injury or subarachnoid hemorrhage with relatively high frequency according to published studies. Assessment of hypothalamo– pituitary functions in patients with chronic subdural hematomas has not been published yet, although dysfunction of hypothalamo- pituitary unit could be expected (head trauma, compression and oedema of the brain, shifting of the midline structures).

Aims
Evaluation of the pituitary functions in patients with chronic subdural hematoma immediately after surgical treatment and during one year follow-up.
Patients and methods
We have examined 59 patients (49 males, mean age 68.3 years, 36–88 years). Patients in the first days after evacuation of the hematoma and then 3 and 12 months after the operation were tested. Basal levels of pituitary hormones and hormones of dependent peripheral glands, plasma cortisol during 250 mcg ACTH test, GH after GHRH + arginine stimulation and TSH after TRH stimulation were assessed.
Results
Central hypogonadism was diagnosed in 13 patients (37%, $n=35$) in an acute phase, but in most of the cases had a transient character. GH deficiency was diagnosed according to the GHRH + arginine test in 25 patients (51%, $n=49$) in acute phase and according to the control tests 12 months after the operation was present in 18 patients
(37.5%, $n=32$). We did not find any serious case of hypocortisolism, hypothyroidism, nor diabetes insipidus centralis. Partial hypocortisolism was found in two cases, but has resolved according to the control tests. No relation between extension of SDH or clinical severity and development of hypopituitarism was observed.
Conclusions
There was GH deficiency or hypogonadism present in some patients in our cohort. No serious hypocortisolism, hypothyroidism, diabetes insipidus nor SIADH was observed. The possibility of hypothalamo-pituitary dysfunction should be considered in patients with subdural hematoma especially in those with history of trauma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1492

**Mood Disorders and Quality of Life in Patients with Acromegaly after Pituitary Adenomas**

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Objective
To evaluate frequencies of mood disorders in males and females with acromegaly and to compare their quality of life with that of control males and females of the same age.
Patients and methods
males (age 48.8 ± 10.3 yers) and 16 females (age 52.9 ± 13.7 years) with acromegaly after pituitary adenomas; 13 control males (age 46.3 ± 18.6 years) and 43 control females (age 54.8 ± 9.8 years).
Mood disorders diagnosed by Mini-International Neuropsychiatric Interview (MINI), quality of life evaluated by WHO Bref Quality of Life Questionnair.
Results
Current depression was diagnosed in 2 (18.2%) males and 1 (6.7%) female with acromegaly, depression episodes in the past - in 0 males and 1 (6.7%) females with acromegaly, social phobia - in 0 males and 2 (13.3%) females with acromegaly, general anxiety disorder - in 4 (36.4%) males and 3 (20.0%) females with acromegaly.
Comparing quality of life
Domins of physical health (13.9 ± 2.5 vs 16.1 ± 2.8) and social relations (14.5 ± 2.0 vs 16.3 ± 1.6) were significantly worse in acromegalic than in control males; environmental domain (15.2 ± 1.2 vs 12.8 ± 2.8) was significantly better in acromegalic than in control females.
In conclusion, Mood disorders were diagnosed in males and females with acromegaly after pituitary adenomas; quality of life (in domains of physical health and social relations) was found worse in acromegalic than in control males.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1493

**Although dentists are frequently visited by acromegaly patients they do not diagnose the disease**

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Objective
The aim of this study conducted in a German University Hospital was to systematically assess health care utilisation and delivery in patients with acromegaly and to thus identify possible starting points for the improvement of patient care.
Design/Methods
By means of a standardized anamnestic interview, 41 patients with biochemically proven acromegaly were questioned on the course of their disease. To investigate patient care the interview included questions on the time lapse of symptom onset, first seeking medical advice and time of diagnosis of acromegaly as well as questions on the medical speciality of the diagnosing doctor and other diagnoses made before. Patients were also asked to recall which symptoms they had noted first and to account which symptoms were still persisting.
Results
Time lapse between first symptoms and diagnosis was on average 3.8 years. 1.76 years elapsed while patients waited to see the doctor, a lapse of 2.05 years was due to delay in the diagnostic process. Among the most frequently mentioned symptoms experienced by the patients were teeth and jaw problems. 36.6% of the patients reported to have visited the dentist more than usual at any time during the disease. 9.8% even remembered this to be one of the first symptoms they experienced, 22% reported these symptoms to still persist. Nevertheless, none of the patients was diagnosed with acromegaly by a dentist. Most diagnoses were made by internists, neurologists, orthopedists or the family doctor.
Conclusion
Diagnostic delay is still considerable in patients with acromegaly. In part, this might be due to the fact, that although many patients with acromegaly visit dentists, the disease seems to be widely unknown to these. One starting point for the improvement of patient care might, therefore, be a better information on acromegaly for dentists.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1494

**The long term recurrence rates of cushing's disease in turkish patients after transsphenodal surgery: a single-center experience**

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Introduction
The treatment of choice in Cushing's disease (CD) is transsphenoidal adenomectomy. However, the long term recurrence rate of the CD after transsphenoidal surgery in Turkish patients has not been determined so far.
Purpose
We aimed to determine the long term recurrence rates of CD in Turkish patients after transsphenoidal surgery.
Methods
The computer records of the 23 patients, operated for CD in our center during the interval 1999–2010, were retrospectively analysed.
Results
Nineteen (82%) of the patients were female and 4 (18%) were male [ranging in age between 20–62 years old, (41,7 ± 11,2 years)]. Among the 23 patients, 14 (87%) had microadenoma, 2 (13%) had macroadenoma and 7 had normal pituitary MRI findings. Bilateral inferior petrosal sinus sampling (BIPSS) were performed in 9 (39%) patients. Four of the patients were operated for at least 2 times and 16 patients were operated on once. Two patients denied surgery. Two patients underwent gammaknife therapy (one as primary therapy). All of the patients were followed-up for 38,5 ± 31,9 months (between 3–96 months) at 3–6 month intervals by basal cortisol, ACTH, urinary free cortisol levels and 1 mg dexamethasone supression test. Persistent cure was achieved in 11 patients (48%) at first surgery. Two (8,6%) patients had persistent disease after first surgery. Recurrence was determined in 8 (34,7%) patients after first surgery.

Recurrence was determined after 9 months from surgery in one patient, between 12–24 months in 2, between 24–60 months in 3 and at 95th month in 2 patients, respectively.
Conclusion
All patients who undergo transsphenoidal surgery for CD should be followed properly for a long time, because recurrence could occur after a very long time period in patients who thought to be cured at first surgery.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1495

**Xanthoma disseminatum with cutaneous and pituitary stalk involvement**

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Introduction
Xanthoma disseminatum (XD) is a rare, non - familial disease characterised by lipid deposition in skin and internal organs due to histiocytic cell proliferation, classified as a non-Langerhans cell histiocytosis. The disease is characterised by symmetrically distributed, coalescing cutaneous papules, initially red–brown then yellow involving the face, trunk, flexural and intertriginous areas. Involvement of mucous membranes has been reported. The upper and lower respiratory tract may also be affected, along with the gastrointestinal tract and bone. In addition, involvement of the pituitary gland, with diabetes insipidus (DI) is reported in 30–50% of cases. Central nervous system (CNS) involvement outside the pituitary/hypothalamus carries a poor prognosis.
Case
A 36 year old Caucasian female presented with a 5 month history of a symmetrically distributed, red–brown papular rash affecting the eyelids, axilla and groin regions. The appearance of the rash was shortly followed by symptoms of polydipsia and polyuria. A water deprivation test with a vasopressin challenge was diagnostic of cranial DI. The patient responded well to vasopressin therapy. Cerebral MRI imaging demonstrated pituitary stalk thickening with no other cerebral involvement. Anterior pituitary function and prolactin levels were normal. Isotope bone scan was unremarkable. High resolution CT thorax did not demonstrate any granulomatous lesions. Histological examination of a skin biopsy showed a mixed spindled and epitheloid fibrohistiocytic lesion with scattered Tuoton - like giant cells compatible with a diagnosis of non - Langerhans cell histiocytosis. The lesions of Juvenile Xanthogranuloma (JX) histologically are indistinguishable from XD. Iron staining showed faint background positivity which has been described in XD.
Discussion
XD is a rare cause of cranial DI. Our case illustrates the challenge which may arise diagnosing XD and differentiating it from JX. In addition, it demonstrates the importance of correlating clinical features with histological findings in diagnosing the disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1496

**Acromegaly and charles bonnet syndrome**

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Introduction
Charles Bonnet syndrome is almost unknown by the endocrinologists. It is composed by visual hallucinations that do not react with the patient contrary to psychiatric hallucinations. The abnormal visions (generally hidden by patients) appear in subjects with an impaired vision of one of both eyes as in the following case:
Case report
A man aged 27, was referred for acromegaly secondary to a huge invasive somatolactotrope pituitary adenomas difficult to manage by surgery. He was very conscious, but almost blind because of bilateral optic atrophy. As the surgery failed, he was given somatostatin analogs plus a dopamine agonist (bromocriptine). One month later when his doctor was looking for bromocriptine intoxication (especially hallucinations) he complained about the fact that just after pituitary surgery he became totally blind and was seeing abnormal things. The medication was reduced then totally stopped, but after that the landscapes and cars visions remained. Psychological evaluation was absolutely normal and Brain MRI did not show anything apart from a consistent decrease of the huge adenoma.
Conclusion
Charles Bonnet syndrome, which is probably due to a reaction of the visual cortex to the rapid loss of the vision, should be known in endocrinology as it is a differential diagnosis with hallucinations due to dopamine agonist intoxication.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1497

**Planning for the future: preparing the endocrine specialist nurse of tomorrow**

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We have come a long way in the UK over the past ten years towards our goal of meeting the educational needs of nurses specialising in adult endocrinology. Endocrine nurses are now able to access annual training updates and attend specific nurse-led sessions at scientific meetings thanks to the ongoing work undertaken by the Society for Endocrinology's nurse committee.
It is therefore time to turn our attention to how we can encourage nurses to want to specialise in endocrinology and to start to prepare them for such a role before rather than after they find themselves working in this field. One way to do this is to ignite their interest in endocrinology while they are still students. With this aim in mind the first undergraduate course in adult endocrine nursing in the UK was developed and introduced as part of the Bachelor of Nursing (Hons) programme at Edinburgh University for the 2011/12 academic session. The course, which runs over ten weeks, is available as an Honours option to students in their third and fourth years and is delivered as a series of lectures and tutorials. The content covers specific endocrine conditions and alongside these critically explores issues such as compliance with prescribed treatment, quality of life, patient support and patient self-management, the role of the specialist nurse and nurse-led clinics. It is taught primarily by an endocrine nurse/lecturer with some specific input from visiting speakers. In addition to the formal taught aspects students are encouraged to attend endocrine out-patient clinics, observe pituitary surgery and attend local patient support group meetings as well as national patient conferences.
Fifteen students have now successfully completed the course. Course evaluation has been extremely positive. Of the seven final year students, two are now proactively seeking endocrine nursing positions to apply for on graduation.
In class.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1498

**Changes in body composition in patients with acromegaly**

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Introduction
With the advancement of surgical techniques and equipments, many patients with acromegaly have achieved complete remission after surgery. Normalization of growth hormone (GH) brings about improvement of vital prognosis, but recovery

from high GH hematologic disease induces various abnormalities. We focused on the body weight and body composition of the patients with acromegaly and examined the post-surgical changes.
Materials and Methods
19 patients with new-onset acromegaly who had surgery at our facility and achieved post-surgical complete remission were enrolled (Male: 6, Female: 13, average age: 52.4, range: 29–74). Body weight and body composition as well as lipid metabolism and glucose tolerance were examined at pre/post-operation and 3 months after operation.
Results
Pre-surgical mean BMI was 25.2 but decreased to 23.4 one week after surgery, then increased to 25.1 three months after surgery. Pre-surgical body fat percentage was 23.9% and remained at 23.3% one week after surgery, then increased to 27.0% at 3 months after surgery. Body fat amount decreased from pre-surgical 14.9 kg to 14.0 kg one week after surgery but increased to 17.2 kg at 3 months after surgery. On the other hand, body water content decreased from pre-surgical 33.7 kg to 32.2 kg one week after surgery, and further decreased to 31.7 kg at 3 months after surgery. Neutral fat decreased from pre-surgical 108.1 mg/day to 84.7 mg/day one week after surgery, and further decreased to 73.6 mg/day at 3 months after surgery. Glucose tolerance also has improved in many cases.
Discussion
Patients with acromegaly are observed with post-surgical transient weight loss, which is considered due to body water loss. Body weight then begins to increase due to body fat accumulation. Lipid metabolism and glucose tolerance improve in a short term. Finally, the effect of obesity on mind and body also needs examination in a long process.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1499

**Complications of pure endoscopic transsphenoidal surgery for pituitary adenoma**

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Object
The aim of the study was to evaluate the safety and efficacy of the pure endoscopic removal of the pituitary adenomas with special references to the complications.
Material and methods
The authors analyzed retrospectively a database of 52 consecutive patients (16 males and 36 females) with pituitary adenomas who underwent endoscopic transsphenoidal surgery. Preoperative examination was based on radiological visualization of the tumor and endocrinological evaluation. The extend of the resection was gauged on postoperative contrast-enhanced 1.5 T MR imaging. Endocrinological remission was defined as normalization of the pituitary function (acromegaly: basal serum GH level $<1$ ng/ml and a nadir GH level $<0.4$ ng/ml after oral glucose load, Cushing's disease: early morning cortisol level $<2$ ug/dl, PRL-oma: serum PRL level $<20$ ng/ml).
Results
There were 15 patients with NFPA, 24 patients with GH-secreting pituitary adenoma, seven patients with ACTH-secreting pituitary adenoma and five patients with PRL-secreting pituitary adenoma. The majority of patients had macroadenomas. Remission rate was 69%. A retrospective analysis of complications was conducted. There were no fatal complications and permanent morbidity was 7.7% (one permanent diabetes insipidus and three pituitary insufficiency). Other surgical complications included one syndrome of inappropriate secretion of antidiuretic hormone, one epistaxix and one sinusitis. There were no medical compications.
Conclusions
Endoscopic transsphenoidal removal of pituitary adenoma is safe and effective method. It leads to a high rate of tumor resection and endocrionological remission. A number of surgical complications is low and comparable to those found in patients undergoing microsurgical transsphenoidal operation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1500

**A case of nephrotic syndrome hidden by cushing's disease**

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We report a case of Cushing's disease associated to nephrotic syndrome. A 21-year old female was admitted to Cittadella-Hospital due to recent occurrence of weight gain, acne, hirsutism, amenorrhea and mild hypertension. Physical examination revealed truncal obesity, moon face, buffalo hump and a bilateral ankle edema. Suspected hypercortisolism was confirmed by elevated 24 h urinary cortisol (385 μg/24 h, normal range 32–250 μg/24 h) and failure to suppress plasma cortisol after 1 mg DST (42 μg/dl.). Plasma ACTH was high on two consecutive days (69.5–62.3 pg/ml, normal range 10–46 pg/ml) supporting the diagnosis of ACTH-dependent Cushing's syndrome. Brain MRI revealed in fact a tumor mass of 5 mm in the pituitary gland, and transsphenoidal pituitary adenomectomy was programmed. During pre-surgery hospitalization, biochemical tests revealed hypoalbuminemia (2 g/dl), hypercholesterolemia (345 mg/dl) and severe albuminuria (21.7 g/24 h) in the presence of normal creatinine levels (1.08 mg/dl), all consistent with a nephrotic syndrome. After surgery, ACTH and cortisol levels rapidly normalized and clinical features of Cushing's disease improved. Low serum albumin (2.1 g/dl) and proteinuria (6.89 g/24 h) persisted. Renal biopsy was then performed and microscopic examination showed a marked mesangial expansion, indicating a rather advanced stage of glomerulonephritis. Combined therapy with albumin, furosemide and exogenous glucocorticoids was of benefit on proteinuria but induced recurrence of Cushing's phenotype. Pituitary gland at MRI control was normal. The present case represents a rare association between endogenous hypercortisolism due to an ACTH-secreting pituitary adenoma and a nephrotic syndrome. We suggest that the renal damage was hidden by Cushing's disease and that normalization of cortisol levels due to pituitary adenomectomy triggered the full appearance of kidney disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1501

**Preliminary report of hypoglycemic response in obese metabolic syndrome males treated with metformin after weight loss intervention**

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We conducted this study to determine the degree of obesity influence on the hypoglycemic response of growth hormone and cortisol after weight loss of 5%. A total of 45 non-diabetic, male subjects followed in the outpatient endocrinological department were divided into three groups comprising 15 subjects in each group, based upon body mass index (BMI) to healthy, overweight and obese group. Metformin was administered in the dose of 500 mg daily to the overweight and obese participants. Cortisol was measured at 0, 60 and 120 min. Growth hormone (GH) was measured at $-15$, 0, 30, 60, 90 and 120 min. Values of cortisol and GH were compared upon changes in hypothalamo–pituitary–adrenal (HPA) response to insulin induced hypoglycemia initially and after weight loss of 5% for overweight and obese participants.
The BMI of healthy group ranged 20.0–24.5 kg/m$^2$ ($Z=3.83$; $P\leq0.001$); overweight group ranged 25.9–29.7 kg/m$^2$; and obese group ranged 30.9–34.6 kg/m$^2$.
There were no significant differences of cortisol values among groups at 0 ($\chi^2=2.0$; $P=0.365$); 60 ($\chi^2=0.754$; $P=0.686$) and at 120 min ($\chi^2=0.466$; $P=0.792$).
The comparison among groups were significant for differences of GH values at $-15$ ($\chi^2=25$; $P\leq0.001$); 0 ($\chi^2=16.2$; $P\leq0.001$); 30 ($\chi^2=16.2$; $P\leq0.001$); 60 ($\chi^2=32.8$; $P\leq0.001$); 90 ($\chi^2=30.2$; $P\leq0.001$); and at 120 min ($\chi^2=27.3$; $P\leq0.001$). Healthy and obese subjects significantly differed in growth hormone response at $-15$ ($Z=4.67$; $P\leq0.001$); 0 ($Z=3.83$; $P\leq0.001$); 60 ($Z=2.78$; $P\leq0.005$); 90 ($Z=4.67$; $P\leq0.001$) and at 120 min ($Z=4.23$; $P\leq0.001$.
Changes on the various levels of HPA axis, when it is activated by a stress as it is in the case in insulin-induced hypoglycemia correspond to the degree of obesity. Weight loss of 5% was not enough for restoration of a normal stimulated growth hormone release and did not influence on the level of cortisol.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1502

**A difficult case of hyponatraemia in a neurosurgical patient**
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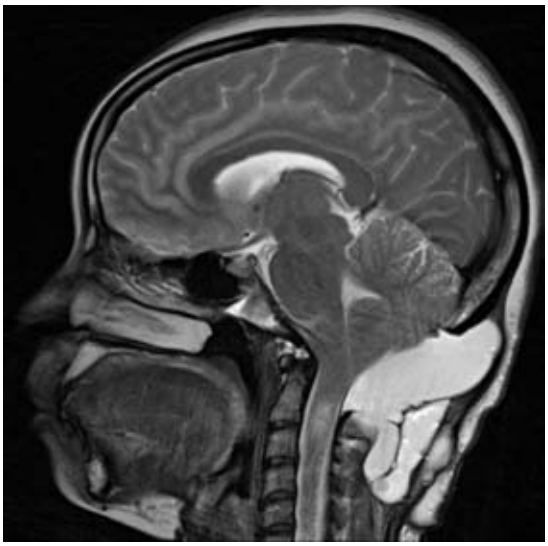
A 40-year-old female presented with a 6 week history of right-sided headaches and nausea. She was found to have an Arnold-Chiari malformation and an unusual C3 syrinx on MRI brain scanning. She underwent a foramen magnum decompression and C1 laminectomy. Three weeks later, she was admitted with headaches, nausea and vomiting, and blurring of her vision on left lateral gaze. Whilst in the emergency unit, the patient had a tonic-clonic seizure.
Following the seizure, she was noted to have a left 6th nerve palsy. She was hyponatraemic (119 mmol/l) with a blood osmolality of 253 mOsm/kg, urine osmolality of 640 mOsm/kg (specific gravity 1.030) and urinary sodium concentration of 97 mmol/l. Brain imaging demonstrated a large pseudomeningocele which had not been present on previous scans.
Administration of intravenous saline following the seizure further exacerbated her hyponatraemia (114 mmol/l). She was reviewed by the endocrinologists and a diagnosis of syndrome of inappropriate ADH secretion (SIADH) was made; strict fluid restriction maintained her serum sodium level at 119 mmol/l. Her urine osmolality remained inappropriately high (531 mOsmol/kg). Five days into her admission placement of a ventriculo-peritoneal shunt resulted in volume reduction of the pseudomeningocele. Following sugery, she began to pass large amounts of urine and her urine specific gravity fell to 1.005 within 12 h. Her sodium level gradually improved back towards the reference range (139 mmol/l) over the following four days. Both her headache and 6th nerve palsy resolved.
Hyponatraemia is the most common electrolyte disorder in neurosurgical patients. Here we describe a difficult case of hyponatraemia due to SIADH. Risks and benefits of administering hypertonic saline or Tolvaptan had to be weighed against the possibility of further hyponatraemic seizures. Early diagnosis, strict fluid restriction and concerted management by a multidisciplinary team were crucial for a beneficial outcome.



Pseudo-meningocele at the site of the Foramen Magnum decompression prior to supratentorial VP shunt placement.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1503

**Invasive and giant pituitary adenomas in the elderly**
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Clinical presentation of pituitary adenomas is influenced by age of patients and stage of the tumour at diagnosis. In this study we intended to analyse the features of pituitary adenomas in old patients.
Subjects and methods
It is a retro- and prospective study concerning 37 patients (19F/18M) aged 60 years and over harbouring a pituitary adenoma, these patients were divided into two groups:

Group 1 (G1): Patients with invasive and/or giant tumours which means tumours $\geq$40 mm or tumours invading the cavernous sinuses
Group 2 (G2): Patients with tumours $<$40 mm without cavernous sinuses involvement

Clinical presentation, hormonal, radiological and ophthalmological finding were recorded.
Results
Thirty-eight percent ($N=14$) of the tumours were giants and/or invasive, the adenoma size was $40.53 \pm 11.38$ mm in G1 (with involvement of cavernous sinuses in 6 cases) and $22.7 \pm 7.96$ mm in G2, patients of G1 were older than those of G2: $70.71 \pm 6.70$ years vs $66.17 \pm 5.41$ years. Neuro-ophthalmological manifestations were appealing in 77% in G1 and 61% in G2; the tumour was incidentally discovered in 21% in G2 and in 7% in G1. No secreting adenomas were the more prevalent in the two groups (57% in G1 and 65% in G2), GH secreting adenomas were recorded only in G2. Pituitary deficiency wasn't different between the two groups (61% in G1 vs 60% in G2). Severe visual loss was more prevalent in G1 than G2: 71% vs 33%.
Conclusion
Pituitary adenomas in the elderly are predominantly no secreting, frequently diagnosed lately at an advanced stage of visual loss and pituitary deficiency.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1504

**Hyperprolactinemia: prolactinoma or pregnancy?**
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Introduction
High prolactin levels may have several etiologies including the presence of a prolactin producing pituitary adenoma or the treatment with drugs of different classes. Certain physiological states, from which pregnancy stands out, are also characterized by elevated levels of prolactin.
Case
Woman, 28 years old, with no relevant medical history or chronic medication apart from oral contraceptives, begins with amenorrhea in May 2010, without galactorrhoea, headache or changes in visual acuity. She presented no history of menstrual irregularities. 'Home' pregnancy test was performed with a negative result. The patient was evaluated by a Gynecologist in June. An endovaginal ultrasound was performed with no evidence of pregnancy. Therapy with medroxyprogesterone, for 10 days, was attempted without success. Gynecological ultrasound was repeated, again without evidence of pregnancy. The patient was advised to consult an Endocrinologist (first visit in October, on the private sector). Analytical study was conducted with prolactin pool 219.9/182.8/156.8 ng/ml, FSH $<$0.05 mIU/ml and LH 0.42 mIU/ml. Pituitary MRI was performed later revealing an increased pituitary gland with homogeneous contrast enhancement, suggestive of pituitary hyperplasia. Treatment with bromocriptine was initiated (November) initially 1.25 mg/day with titration up to 5 mg/day. The patient was subsequently oriented to Neurosurgery appointments, from which she was sent to the Endocrinology department of the same institution. At that time, imaging and analytical study was found to be compatible with pregnancy ($\sim$33 weeks) confirmed with physical examination (fetal movement) and ultrasound. The analytical findings were normal 5 months after delivery.

Discussion
The onset of amenorrhea in women of childbearing age implies the exclusion of pregnancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1505

### DHEAS: a new marker in Cushing's disease? Preliminary results of 32 patients

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Introduction/objective
The objective was to determine if peri-operative levels of DHEAS correlate with levels of ACTH and cortisol and therefore are useful as a new marker for the definition of cure in patients suffering from Cushing's disease. DHEAS is an ACTH-dependent precursor of androgens and estrogens secreted from the adrenals. Numerous clinical trials have shown that DHEAS in humans and other mammals is a multi-functional steroid implicated in a broad range of biological effects, including obesity, diabetes, bone metabolism, neuroprotection and anti-tumorigenesis; it has not yet undergone research in the context of Cushing's disease.
Methods
Forty-three patients suffering from Cushing's disease were treated at our department from September 2009 to February 2011 and were perioperatively monitored for ACTH, cortisol and dehydroepiandrosterol-sulfate (DHEAS).
Preoperative and early postoperative ACTH, cortisol and DHEAS levels were correlated with each other to determine the usefulness of DHEAS as a parameter in patients suffering from Cushing's disease.
Results
Forty-three patients were included. All were treated for Cushing's disease via a transsphenoidal approach. Pre-operative blood samples were taken on the day before the operation and revealed high normal to elevated levels of ACTH (mean: 104 ng/l), cortisol (mean: 283 μg/l) and DHEAS (mean: 2.59mg/l) according to the pathology of Cushing's disease. Postoperative blood checks showed decreased levels of ACTH down to 15.5% (mean: 14.93 ng/l) of its preoperative figure. Cortisol levels were reduced down to 12.5% (mean: 35.39 μg/l) of its preoperative level and DHEA(S) levels decreased down to 19.61% (mean: 0.51 mg/l).
Two patients had decreased levels of all three hormons postoperatively (DHEAS 53.7% and 9%, Cortisol 11.2% and 5.3% and ACTH down to 28.4% and 11.8%), 1 showed elevated levels of DHEAS, ACTH and Cortisol on re-evaluation 5 months after the initial operation and had to undergo a second surgical treatment. The second patient underwent first surgical treatment in 2009 and is currently being re-evaluated as recurrent disease seems highly possible.
Statistical analysis showed a highly significant correlation between changes of ACTH and DHEAS levels and a significant correlation between changes of cortisol and DHEAS levels.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1506

### Clinical experience with acromegalic patients who have macroadenomas and long term follow-up results

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The increased morbidity and mortality of acromegaly makes early diagnosis and therapy critical. Long term follow-up and investigating comorbidities of this disease is also important. We present here retrospective results of 70 patients. There were 41 female, 29 male patient included in the study. Follow-up period was longer than 5 years. Fifty-nine of the patients were macroadenoma, 4 of them were empty sella, 7 of them were microadenoma. Seven patients refused surgical treatment and they were followed only with medical treatment. Sixty-three patients were treated with surgical and medical treatment. Transsphenoidal surgery was the treatment of choice. For the patients whose macroadenomas were not controlled by surgery alone (22 patients), radiation therapy was applied after surgical and medical treatment for the residual tumor (7 gamma-knife, 10 conventional, 5 cyber-knife). One patient was operated sucessfully when she was 75 years old and there was no complication. In 1 patient acromegaly was a clinical component of multiple endocrine neoplasia type 1, 2 patients had fibrous dysplasia. Renal transplantation was applied to one patient because of diabetic nephropathy. One patient had a successful pregnancy period after she was diagnosed as acromegaly and she has a healthy child. Octreotid treatment was stopped during pregnancy. Malignancy was diagnosed in 5 patients (1 pancreas adenocarcinoma, 2 thyroid follicular carcinoma, 1 lung adenocarcinoma, 1 colon adenocarcinoma and thyroid papillary carcinoma coexistent in the same patient). Colonic polyps were discovered in 7 patients. As a result it can be concluded that acromegalic patiens have to be followed lifelong. Usually remission is not achieved easily. The patients should also be investigated for other comorbidities.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1507

### Glucose metabolism and lipid disturbances in acromegalic patients before and during treatment with long-acting somatostatin analogues

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Pomeranian Medical University, Szczecin, Poland.

Introduction
Acromegaly causes hyperlipidemia and glucose metabolism disturbances what finally results in increased mortality. Correctin of those lipid and glucose disturbances during treatment with long-acting somatostatin analogues was examined in this study.
Material and methods
Eleven patients (9 women and 2 men) with active acromegaly and macroadenoma diagnosed at our Department in the years 2008–2011 underwent monitoring of lipids and glucose levels before and during one-year treatment.
Results
The therapy with long-acting somatostatin analogues leaded to statistically significant reduction of GH and IGF1 level ($P=0.00585$; $P=0.0094$ respectively). Normoglycaemia occured in 45.45% of patients, diabetes mellitus was found in 9.09% impaired fasting glucose in 27.27% and impaired glucose tolerance in 18.18% before treatment. There was not statistically significant reduction of glucose level during therapy. The mean fasting glucose concentration and the level in 120 min of glucose test tolerance before treatment were $99.1 \pm 20.67$ and $107.37 \pm 44.84$ respectively and after therapy the levels were $94.43 \pm 18.62$ and $87.31 \pm 22.41$ respectively. There was observed impaired glucose tolerance in 9.09% of patients and normoglycaemia existed in 54.56% after treatment. According to the problems with lipid metabolism the lipid profile shows higher levels of total cholesterol in 72.72% of the patients, triglycerides in 27.27% and LDL-cholesterol in 90.09% with no statistically significant reduction of these parameters ($P=0.33$, $P=0.25$, $P=0.624$ respectively). Before and after therapy there were found no correlations between patient's age, GH level and fasting plasma glucose, total cholesterol, triglycerides. There were only correlations between the age and HDL-cholesterol level ($P=0.022$) and LDL-cholesterol and GH level before the treatment ($P=0.0045$).
Conclusions
The treatment with somatostatin analogues does not lead to improvement of total cholesterol, LDL-cholesterol, triglycerides and does not help to regulate glucose level in acromegalic patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1508

**The therapy with cabergoline evaluation of men with macroprolactinoma**

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Introduction
Pituitary gland adenomas producing prolactin are one of the most common hormonally active tumours. A pharmacological treatment with the usage of dopamine receptors agonists is a therapy of choice in case of prolactinoma.
The aim of the study was evaluation of the therapy with cabergoline of men with macroprolactinoma basing on the clinical, hormonal and radiological examinations.
Material and methods
Ten men aged 18 to 65 (mean 41.9 ± 15.01) with the presence of the pathological mass in the pituitary gland sized 16.7 to 40.5 mm (mean 29.8 ± 9.38 mm) and elevated PRL level from 673 to 4700 ng/ml (mean 1608.2 ± 1771.6 ng/ml) were included into the study. The PRL and the remaining trophic hormones levels were evaluated after 1, 3, 6 and 12 months and the tumour size evaluation was performed in MRI examination after 12 months of the therapy with cabergoline.
Results
The therapy with cabergoline leaded in all patients to headaches remission, visual acuity correction and significant improvement within libido and erection. In 90% of patients the PRL normalization was achieved, just after first months of the therapy. The mean PRL concentrations were before and after 1, 3, 6 and 12 months of the therapy respectively 1608.2 ± 1771.6 and 263.4 ± 223.4, 136.1 ± 244.7, 91.31 ± 105.5 and 27.5 ± 57.7 ng/ml. The significant tumour size reduction was observed from 29.8 ± 9.4 mm, mean about 6 mm, that states 25.1% (from 4 to 48.5%). No significant correlation between the mean tumour size and PRL level was observed before and during the treatment. The decreased testosterone level before the therapy was proved and it's gradual increase during the treatment was observed.
Conclusions
The cabergoline administration in patients with macroprolactinoma is effective in reaching the PRL level normalization as well as in the tumour size reduction. The tumor size is not a prediction factor for the effectiveness of therapy with cabergoline.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1509

**Efficacy of testerone 2% gel replacement therapy on erectile function, muscle strenght and general wellbeing in men affected by normo-hypogonadotropic hypogonadism**

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Background
Hypogonadism is a clinical entity characterized by low serum testosterone (T) levels associated with several clinical signs and symptoms which can negatively affect the quality of life. T replacement therapy (TRT), restoring serum T concentrations, improves signs and symptoms related to hypogonadism. Many T formulations are presently available. The aim of the study was to assess effects of 2% gel TRT on serum T concentrations, erectile function, muscle strength and general wellbeing in men affected by normo-hypogonadotropic hypogonadism.
Materials and methods
Twenty-five patients (mean age 33 ± 5 years) affected by mild to moderate normo-hypogonadotropic hypogonadism with different etiology (20 functional, 3 post-pituitary surgery, 1 autoimmune, 1 post-traumatic) were enrolled into the study. T 2% gel was administered at a dose ranging between 20 and 90 mg/day. Serum total T, DHT, International Index of Erectile Function-5 (IIEF-5) and the Aging Male Symptom Scale (items 1 and 10) questionnaires were evaluated before and after 3–6 months of TRT. IIEF-5 evaluates erectile function, whereas item 1 and 10 of AMSS evaluate respectively general wellbeing and muscle strenght.
Results
Normal serum testosterone levels were restored in all patients after 3–6 months of treatment. TRT determined a significant increase in serum total T ($P<0.001$) and DHT ($P=0.008$) levels. T normalization resulted in an improvement of sexual function ($P<0.001$), muscle strength ($P=0.004$) and general wellbeing ($P=0.002$). A direct correlation was found between the increase of total testosterone and the improvement of IIEF-5 ($P=0.002$), muscle strenght ($P=0.012$) and general wellbeing ($P=0.039$). No significant correlation was discovered neither between dose of T administered and improvement in IIEF-5 score ($P=0.15$) nor between dose of T administered and improvement of muscle strenght ($P=0.093$).
Conclusions
Transdermal 2% gel TRT is able to restore physiological total T levels in men affected by normo-hypogonadotropic hypogonadism improving signs and symptoms related to hypogonadism. Therefore, it can be considered an effective alternative TRT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1510

**Follow up of pituitary incidentaloma: a study in 140 patients**

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Introduction
Pituitary incidentaloma is a relatively frequent imagistic finding. Since no therapy is necessary, the follow up protocol is the most important in patients' approach.
Aim
We present a study in patients diagnosed with pituitary incidentaloma, admitted in I.Parhon, Bucharest, between 1999 and 2011.
Patients and method
This is a retrospective study in 140 patients, diagnosed with pituitary incidentaloma based on CT or MRI. We included asymptomatic patients to whom an incidental imagistic scan found the pituitary tumor of less than 1 cm. The informed consent was obtained.
Results
One hundred and forty patients (p) were included. The female/male ratio was 130/10. The av. age was 40.84 ± 13.3 years, with ranges between 14 and 72 years. The av. values of pituitary hormones were normal according to patients' age: prolactine 14.18 ± 12.18 ng/ml, FSH 43.97 ± 55.08 mUI/ml, LH 16.93 ± 19.01 mUI/ml, TSH 2.52 μUI/ml, ACTH 35.52 ± 19.15 pg/ml. The secretor profile was found in one patient with prolactinoma that was ruled out. 3 p had bilateral incidentalomas. The av. transverse diameter was 0.57 ± 0.2 mm, and vertical diameter 0.39 ± 0.15 mm. 69 p had at least one evaluation in av. 35.33 ± 27.39 months (ranges from 6 to 108 months). For these patients, the initial av. transverse, respective vertical diameters were: 0.538 ± 0.17 mm, 0.374 ± 0.09 mm. The av. final values were: 0.545 ± 0.174, respective 0.368 ± 0.09 mm. The student ttest between initial and follow up evaluations was no statistically significant. In 25p increased the transverse diameter with av. 0.159 ± 0.119 mm, and in 32p the diameter decreased with 0.158 ± 0.132 mm. In 19 p the vertical diameter increased with 0.122 ± 0.182 mm, and in 37 p decreased with 0.115 ± 0.128 mm. In one case, the microadenoma increased to 110 mm.
Conclusions
Based on our study, once the non-secretor profile is established, the diagnosis of incidentaloma is sustained,and a follow up CT is not necessary sooner than 3 years.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1511

**Macroprolactin levels in healthy blood donors**

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The incidence of macroprolactinemia decreased notably because of wide use of prolactin (PRL) assays measuring macroprolactin at a lower rate. However, in case of high PRL levels, measurement of monomeric PRL (mPRL) levels after polyethylene glycol (PEG) treatment is still required. Our aim was to determine – by the use of a second generation PRL assay – how total PRL (tPRL) levels in native sera of healthy blood donors change after PEG treatment. In women, the use of oral contraceptives (OC) was taken into consideration as well.
Methods
PRL levels were determined in 131 healthy blood donors (50 men and 81 women; age 33.5±10.5 years). Forty-one women were taking OC. tPRL was measured before and after PEG treatment with a fully automated method (ECLIA, Cobas e411, Roche). PRL recovery (PRL-r%) was determined as the quotient of mPRL/tPRL.
Results
There were increased tPRL levels in 16 native sera (11%). PRL levels after PEG treatment decreased into the reference range only in two of these cases. The other 14 women had mild true hyperprolactinemia (558±238 mU/l), 12 of them took OC. tPRL levels were significantly ($P<0.05$) higher in women than in men (312±224 vs 235±140 mU/l, respectively), but there were no significant differences in mPRL levels (255±173 vs 223±126 mU/l). PRL-r% was higher ($P<0.001$) in men than in the two groups of women. There was a significant, albeit week positive ($r=0.24$; $P<0.01$) correlation between age and PRLr %. There was no significant difference in the PRL levels between women taking or not taking OC.
Conclusions
According to our results the incidence of macroprolactinemia among healthy blood donors is only 1.5%. In women taking OC only true hyperprolactinemia was present. The weak, but significant positive correlation between age and PRLr% proves the increased antibody production during ageing.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1512

**Sleep apnoea syndrome in acromegaly**

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Sleep apnoea syndrome (SAS) is common in acromegaly and both diseases are independently associated with hypertension and insulin resistance contributing to increased morbidity and mortality.
The aim of this study was to assess the prevalence and risk factors of SAS in acromegaly, to study clinical and polysomnographic particularities of SAS in acromegaly and to analyze the effects of acromegaly treatment on SAS.
It's a retrospective study on fifteen patients (nine women and six men). The mean age was 53.6 years (32–71). All patients had newly diagnosed acromegaly. All patients had biochemical assessment (IGF1, GH, fasting blood glucose). The overnight respiratory polygraphy was performed only before acromegaly treatment in three patients, in ten before and after treatment, and in two it was just performed after treatment. Echocardiographic parameters were measured before acromegaly treatment in twelve patients.
Among the thirteen patients tested before acromegaly treatment, ten had SAS (76.9%). In a multivariate analysis only age was associated with SAS. The values of GH and nadir of GH were associated to the severe cases of SAS. Diabetes, high blood pressure, goiter and left ventricular hypertrophy were more prevalent in patients with SAS, especially in severe cases. Among the patients from whom acromegaly was cured 40% recovered of SAS.
The prevalence of SAS in acromegaly is high especially in older patients. SAS can persist after recovery of acromegaly in several patients. Respiratory polygraphy or polysomnography should be included as routine procedure in the work up of acromegaly. Appropriate intensive treatment should be implemented to minimize the clinical impact of SAS in acromegaly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1513

**Therapeutic trends, long-term outcome and efficacy of different treatment modalities in acromegaly: a single center registry covering a 40 year period**

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The aim of the study was to examine the therapeutic trends and the long-term outcome of available therapeutic modalities for acromegaly in a single center over a 40 year period.
We retrospectively studied 300 acromegalic patients (131 men, 169 women, 153 macroadenomas and 147 microadenomas) from 1970 until 2010; 154 patients were diagnosed before 1990 (group A) and 146 after 1990 (group B). Outcome was evaluated by IGF1 and GH (random plus post OGTT) measurements.
The outcome of each treatment modality in the two groups of the cohort is shown in Table 1.
Surgery (TSS) and somatostatin analoges (SSA) were used more often in group B (TSS: in 62% of patients vs 40% in group A, $P=0.0001$, SSA: 52 vs 6.4% respectively, $P=0.0001$), while pituitary irradiation was used less often in group B (30 vs 56.4% $P=0.0001$). Overall the outcome at latest follow-up following all treatment interventions in groups A and B respectively was as follows: 40.6 vs 66.3% achieved GH<2.5 ng/ml, $P=0.0001$; 20 vs 42.8% achieved GH<1.0 ng/ml, ($P=0.0001$); 15.2 vs 34.9% achieved GH nadir post OGTT <0.4 ng/ml ($P=0.004$); and 50.4 vs 68.1% achieved normal IGF1 ($P=0.04$).
Conclusions
These results indicate that in our cohort the evolution of treatment modalities in favor of TSS and medical treatment resulted in a better overall outcome of acromegaly in patients treated during the last 20 years. However, the disease still remains uncontrolled in a substantial proportion of patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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| | TSS | | | XRT | | | SSA | | |
|---|---|---|---|---|---|---|---|---|---|
| | group A | group B | *P* | group A | group B | *P* | group A | group B | *P* |
| GH<2.5 | 45.5% | 50.6% | NS | 33.8% | 59.3% | 0.04 | 50% | 70% | NS |
| GH<1.0 | 18.2% | 31.6% | NS | 18.8% | 34.6% | 0.02 | 30% | 44.3% | NS |
| GHn<0.4 | 13.9% | 31.2% | NS | 11.7% | 28% | NS | 20% | 36.7% | NS |
| IGF1 | 32.2% | 47.9% | NS | 71.4% | 64% | NS | 44% | 75.8% | NS |

## P1514

**Prevalence of the metabolic syndrome in patients with adult growth hormone deficiency before GH treatment**

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Introduction
A increased prevalence of metabolic syndrome (MetS) has recently been described in patients with adult growth hormone deficiency (GHD). This fact could influence the increased risk of cardiovascular morbidity and mortality observed in these patients. Our objective was to investigate the characteristics and prevalence of MetS in adult GHD.
Design and methods
Forty-nine adult patients (30 women, 19 men; 14 childhood-onset), mean age of 36.2±13.8 years, with severe GHD (mean duration 9.1±9.4 years) were evaluate

before GH replacement. Complete information on all MetS components were collected. MetS was defined according to the National Cholesterol Education Program's Adult Treatment Panel III (NCEP) and the International Diabetes Foundation (IDF). The prevalence of MetS was calculated, and associations were assessed between baseline variables and MetS.
Results
MetS was present in 14.9% (NCEP) and 31.8% (IDF) of our patients, lower than data from KIMS analysis (43.1%) and similar from the normal population (20–30%). The prevalences of MetS components (NCEP) were: waist circumference 26.5%, HDL 45%, triglycerides 47.8%, hyperglycaemia 4.3% and hypertension (14%). No childhood-onset patient fulfilled the definition of MetS, while 23.3% of adult-onset patients were diagnosed. The prevalence was higher in women (25 vs 5.8%, non-statistically significant). Mean age and GHD duration before GH replacement were similar among MetS and no-MetS patients; MetS patients had higher glycaemia ($P=0.005$), triglycerides ($P<0.001$), waist circumference ($P<0.001$), lower HDL-cholesterol BMI ($P=0.001$), and were shorter ($P=0.034$) than no-MetS patients.
Conclusion
The prevalence of MetS in our GHD patients were lower than KIMS study. That could be influenced by a lower age in our patients (13 years younger). However, we should diagnose and treat MetS components as they could contribute to the increased risk of cardiovascular morbidity and mortality found in GHD patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1515

**Familial central diabetes insipidus with extremely high water intake**

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Water intake in central diabetes insipidus (CDI) usually falls in range of 3–20 l a day. Intake of more than 20 l is regarded as physiologically unnecessarily even in the absence of antidiuretic hormone. We describe a family with 8 members suffering from autosomal recessive form of CDI due to mutation C105Y (codon numeration is given for preprovasopressin) in AVP gene. In all family members disease had presented in neonatal period or early childhood. It is remarkable that without desmopressin the water intake/output ranges from 30 to 34 l a day and is up to 3–4 l on 0.8 mg of oral desmopressin.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1516

**Visceral adiposity index is associated with insulin sensitivity and adipocytokine levels in newly diagnosed acromegalic patients**

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Background
The Visceral Adiposity Index (VAI) has been suggested as a new gender-specific marker of visceral adipose dysfunction, strongly associated with insulin sensitivity in patients with cardio-metabolic risk.
Aim
To test VAI in active acromegaly for the assessment of disease-associated metabolic risk evaluating its association with hormonal data, adipocytokine levels, insulin sensitivity and secretion parameters in a cohort of 27 subjects (15 M, 12 F, mean age 54.9 years).
Methods
Glucose, HbA1c, nadir and AUC of GH during OGTT, AUC of C-peptide (CP) during a mixed-meal tolerance test (MMTT), M value during an euglycemic hyperinsulinemic clamp, leptin, adiponectin, TNF-α, IL-6 were evaluated in newly diagnosed patients grouped into those with normal (Group A, No 15; 55.5%) and high VAI (Group B, No 12; 44.5%).
Results
VAI value was positively correlated with age of patients ($P=0.048$), basal, nadir and AUC of GH ($P=0.001$, 0.007 and 0.002, respectively), IGF1 ($P=0.001$), TNF-α ($P=0.010$) and negatively with adiponectin ($P<0.001$). Group B showed 1) significantly higher levels of basal GH ($P=0.018$), AUCGH ($P=0.047$), IGF1 ($P=0.047$) and AUCCP ($P=0.018$); 2) significantly lower *M* value ($P<0.001$) and adiponectin levels ($P<0.001$); 3) higher prevalence of systolic blood pressure ($P=0.006$) and impaired glucose tolerance ($P=0.001$).
Conclusions
In active acromegaly, VAI appears to be independently associated with hormonal parameters, insulin sensitivity and secretion indexes, adiponectin and TNF-α levels. Therefore, VAI could be used as a new easy tool in daily clinical practice for the assessment of metabolic risk associated with active acromegaly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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| | **Acromegalic patients with normal VAI** (Group A) **No 15** (55.5%) | **Acromegalic patients with high VAI** (Group B) **No 12** (44.5%) | ***P*** |
|---|---|---|---|
| Basal GH (μg/l) | 3.3 (1.20–9.70) | 32 (3.10–36) | 0.018 |
| Nadir GH (μg/l) | 5.50 (2–8.80) | 19 (2.10–35) | 0.082 |
| AUCGH | 763 (345–997) | 3700 (525–4230) | 0.047 |
| IGF1 (ULN) | 1.61 (1.03–2.16) | 2.40 (1.40–3.50) | 0.047 |
| Fasting glucose (mmol/l) | 6.16 (5.58–6.49) | 5.94 (4.72–6.33) | 0.082 |
| M value (CLAMP) | 3.30 (3.14–4) | 1.65 (1.42–2.70) | <0.001 |
| AUCCP (MMTT) | 351 (279–421) | 769 (331–821) | 0.018 |
| HbA1c (%) | 5.8 (5.45–5.90) | 5.70 (5.10–6.70) | 0.392 |
| Leptin (ng/ml) | 4.80 (2.80–18.45) | 6.10 (2.40–9.60) | 0.865 |
| Adiponectin (μg/ml) | 10.50 (9.10–15.95) | 4 (3.40–7.20) | <0.001 |
| TNF-α (ng/ml) | 1.30 (1.05–3.05) | 3.30 (1.10–4) | 0.082 |
| IL-6 (pg/ml) | 1.72 (1.35–2.07) | 1.48 (1.06–1.80) | 0.252 |

## P1517

**Isolated diabetes insipidus happened during premenopause solution ten years after**

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Pathologies of pituitary gland are often revealed by diabetes insipidus. Etiologies are, in first, tumors, inflammatory and granulomatosis diseases after, but stay idiopathic in 24% of French study, 52% of Italian study and 33% of Tunisian study.
Case report
Mrs A., 53 years old, consults in 1998 because polyuria–polydipsia, without diabetes mellitus. She is hospitalized during 2 days and hydric restriction test shows the organic reality. The others neuro-endocrine explorations stay unproductive, without confirmation of appearance of menopause. The brain and hypophysis examination by CT scan is found normal. The MRI shows pituitary stalk "thick", but lightly…And, haematologic perturbation, or inflammatory syndrome, or osteo-arthritis pain, or cardiovascular or nephrologic perturbation don't exist, and treatment by MinirinR has permitted to get back stabilized health status. The next MRI controls, executed at 6 and 24 months later, are perfectly stable, and so is clinical status. During 2008, Mrs A., consults because undulant fever and asthenia. Physical examination permits to discover bilateral tibia pain, erythematous lesions on the neck and thorax. Cutaneous biopsy, myelogram and bone biopsy permit to obtain diagnosis of Erdheim Chester syndrome, it's to say non Langerhans histiocytosis which aims are cardiovascular system, central and peripherical nervous system and the bone.
The initial having few symptoms situation can explain the time for diagnosis establishment. Nevertheless, in the beginning of the story, it was not justified to propose chemotherapy and supervision stayed justified.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1518

**Metaplastic Rathke's cyst leading to hypopituitarism**

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A 22 years old woman, with secondary infertility, was found to have secondary hypothyroidism and referred to endocrinology. On review her symptoms consisted of headache for 6 months, amenorrhoea, weight gain and progressive loss of vision in the left eye for 6–9 months, which had been investigated by opticians and opthalmologists. Other history includes Von Willebrand disease type2. Clinical examination confirmed total loss of vision of the left eye and complete temporal defect in the right.

Investigation

Biochemistry confirmed secondary hypothyroidism with adrenal insuffieciency –serum $FT_4$ 5.1 pmol/l (12–22), TSH 0.72 mIU/l (0.27–4.2). A short synacthen test confirmed adrenal insufficiency with a plasma ACTH level of 8.6 ng/l, basal serum cortisol of 142 nmol/l rising to only 344 nmol/l at 30 min after 250 μg ACTH 1–24. Serum prolactin was normal, but serum oestrodiol, LH and FSH were low, consistent with secondary gonadal failure. Gadolinium-enhanced MRI confirmed a pituitary macroadenoma measuring 27 mm × 27 mm × 17 mm and extending to the suprasellar cistern and compressing the optic chiasm and left optic track with possible recent apoplexy. No sinus abnormality was seen.

Management

Appropriate replacement therapy was commenced. After discussion endoscopic transsphenoidal surgery was performed. Unexpectedly during surgery pus was encountered on first incision of the pituitary mass, resulting in decompression of the optic chiasm. Culture was negative. Histopathology revealed benign squamous epithelial metaplastic cystic lesion with acute on chronic inflammation, consistent with a Rathke's cleft cyst. Post-operatively she required desmopressin and full anterior pituitary replacement therapy. Remarkably her vision improved immediately after surgery and three months had returned to normal. Repeat MRI scan showed no evidence of recurrence of the cystic pituitary mass after 3 months of surgery.

Discussion

Complete recovery of vision after such an extended period of compression due to pituitary mass is unusual and fortunate in this case. Extensive squamous metaplasia in the Rathke's cleft cyst is unusual and it appears that this had undergone degenerative change with the formation of sterile pus, but the cause of this unusual presentation is unclear.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1519

**Pituitary macroadenomas: benefit from early GH substitution after surgery**

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Introduction

of non-functioning pituitary macroadenomas are associated with hyposecretion of the pituitary gland. In addition surgical therapy can lead to a partial or complete hypopituitarism. Data suggest that substitution of growth hormone can improve quality of life and reduce associated symptoms. However in many cases substitution is not started within the first 6–12 months after surgery. Therefore we intended to investigate if patients benefit from an early growth hormone treatment after surgery compared to control.

Patients and methods

Data was collected prospectively from 21 adult patients who were diagnosed with insufficiency of growth hormone axis using insulin hypoglycaemia test. Patients were divided into treatment and control group and followed 52 weeks after pituitary surgery. On each consultation laboratory testing was performed and body composition obtained. Also quality of life was assessed by a standardised questionnaire concerning health and life satisfaction.

Results

Whereas the control group showed a significant decrease of IGF1-levels after surgery the IGF1-levels of the treatment group stayed within the normal range.

Bioelectrical impedance analysis showed a significant increase of impedance as well as lean body mass in the treatment group compared to control ($P<0.05$). There was a significant difference in health status in the treatment compared to control.

Triglycerides, cholesterol and LDL-cholesterol increased to a greater extent in the control than in the treatment group without reaching statistical significance. Mean increase in HDL-level was 2.1 mg/dl in the control compared to a decrease of 0.2 mg/dl in the treatment group. Lpa-levels were reduced by 4.1 mg/dl in the treatment and by 2.7 mg/dl in the control group. Finally we saw a trend towards better improvement in pituitary function if GH was substituted.

Conclusion

Early supplementation of GH leads to a significant improvement in quality of life and body composition after pituitary surgery. It is possible that pituitary function recovers better with early GH substitution.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1520

**Pituitary insufficiency in the acute phase of traumatic brain injury or subarachnoid hemorrhage, prevalence and predictive factors: a prospective, population based study**

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Background

Traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH) can cause long-term morbidity and death. Studies indicate that this may partially be due to transient or chronic hypopituitarism (HP). Guidelines recommend screening for HP in moderate and severe TBI patients (TBIp), Glasgow coma score (GCS) 9–12 and <9 respectively. The aim of this study was to evaluate markers of severity and physiologic changes as predictive factors for HP following TBI and SAH.

Subjects and methods

During 12 months, patients admitted to the National University Hospital were included, 21 TBIp, (6 moderate, 15 severe, 17 males, 4 females, mean age $34 \pm 13$ years) and 19 patients with SAH (SAHp) (12 males, 7 females, mean age $54 \pm 14$ years). Baseline hormone levels were measured on admission and on day 6 when a synacthen test was also performed. Variables recorded were GCS, Hunt and Hess grade (SAHp), Injury severity score (TBIp), APACHEII, length of ICU stay and occurrence of systolic blood pressure (SBP) <90 mmHg and SpO2 <90% anytime during the first 24 h. Correlation was tested in SPSS using Spearmans rho (rho).

Results

On day 6, 9 of 21 TBIp, and 5 of 19 SAHp, had HP of one or more axis. Lost to follow up on day 6 were 4 TBIp and 3 SAHp. A significant correlation was found between length of ICU stay and any HP (rho 0.473, $P<0.01$) and occurrence of SBP below 90 mmHg during hours 2–24 (rho 0.337, $P<0.05$). Correlation between HP and other variables was non-significant.

Conclusion

The prevalence of HP in the acute phase following TBI and SAH was 42.9% and 26.3% respectively. The length of ICU stay and episodes of SBP below 90 mmHg during hours 2–24 may indicate HP. Further studies are needed on predictive factors making screening for HP following TBI and SAH effective.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1521

**Difference of response to TRH (thyrotropin releasing hormone) stimulation according to octreotide response and the size of GH-producing pituitary adenomas**

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Objective

TRH induces GH secretion presumably by expression of TRH receptor dedifferentiated in pitutiary tumors, but it is not clear how the TRH elegantly stimulates GH secretion in tumoral states.

Design
The aim of this study was to investigate how GH-producing pituitary adenomas respond to TRH with respect to GH secretion and how it differs according to octreotide responses and their sizes of tumor.
Method
GH levels of 45 patients with newly diagnosed acromegaly were measured using a TRH stimulation test (TST), insulin tolerance test (ITT), and an octreotide suppression test (OST) sequentially. These values were compared according to the size of tumors and the response pattern to the OST.
Results
Thirty-six of 45 tumors were macroadenomas, and 23/36 macroadenomas appeared to be non-responders during the OST. Stimulated GH levels during the TST were not significantly different according to the size of tumor ($P=0.212$), even though baseline and recovered GH after stimulation had significant difference during the TST ($P<0.024$). Also, microadenomas and macroadenomas with non-responders had significantly different GH during the TST, but not the ITT ($P=0.073$).
Conclusion
According to the TST, dedifferentiation of tumor was firstly influenced by tumor size and then, OST responsiveness. Regardless of the tumor size, the effect of hypoglycemia to stimulate GH secretion was equivalent, though the result of the OST played a significant role to determine the responsiveness to hypoglycemia in macroadenomas.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1522

**Ovarian hyperstimulation syndrome due to a functioning gonadotroph adenoma**

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Background
Gonadotroph adenomas usually present as non-functioning pituitary adenomas.We describe a patient with a functioning gonadotroph adenoma presenting with ovarian hyperstimulation syndrome: an exceptionally rare presentation of pituitary disease.
Case history
A 26-year-old female presented with a 10-year history of worsening lower abdominal pain, bloating, dysmenorrhea and irregular periods in 2006. She was found by ultrasound to have multiple ovarian cysts that were surgically drained. Her symptoms recurred with re-growth of 19 ovarian cysts that prompted removal in March 2008. Abdominal pain further recurred in June 2008 and the repeat USS showed enlarged 11 ovarian cysts. As part of evaluation for subfertility an elevated prolactin was identified (PRL: 1338 mU/l $n<530$) leading to a pituitary MRI that revealed a right-sided ($11\times8\times7$ mm) pituitary adenoma.
Investigations
After endocrine evaluation, baseline hormone profile revealed extremely high oestradiol levels (20 000 pmol/l). Clinical and biochemical features were considered consistent with ovarian hyperstimilation syndrome (OHSS) in the absence of exogenous gonadotrophins and therefore presumed secondary to a gonadotroph secreting adenoma.The patient underwent endoscopic transphenoidal surgery in December 2008 with subsequent intact anterior pituitary endocrine axes, although requiring DDAVP. Oestradiol fell rapidly from 20 000 pmol/l down to a normal level, her menstrual cycle returned and was followed by two successful pregnancies. The pelvic cysts resolved and pelvic anatomy returned to normal on ultrasound. Serial MRI Pituitary scans have shown no evidence of residual tumour.
Conclusion
Gonadotrophin-secreting pituitary tumours should be considered in patients with multiple recurrent ovarian cysts and extreme elevations of Oestradiol. Although positive immunohistochemistry for gonadotrophins is common amongst pituitary tumours, functioning gonadotrophinomas are very unusual with only a handful reported in the literature and with variable outcomes. In our patient, the recognition occurred after propitious diagnosis by MRI.

Biochemical results

| Test | **30.09.08** (pre-op) | **18.02.09** (post-op) | **28.10.11** |
|---|---|---|---|
| Oestradiol (pmol/l) | 20 000 | 371 | 219 |
| FSH(U/l) | 8.8 | 2.1 | 7.4 |
| LH(U/l) | 2.5 | <0.8 | 4.9 |
| Prolactin (mU/l) | 1560 | 281 | 249 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1523

**GH deficiency after treatment of medulloblastoma and thyroid cancer: is this the case for GH therapy?**

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The hypothalamic–pituitary unit is a particularly radiosensitive region in the central nervous system. As a consequence, hypopituitarism commonly develops after radiation treatments for sellar and parasellar neoplasms, extrasellar brain tumours. Increasing tumour-related survival rates provide an expanding population at risk of developing hypopituitarism. The severity of hypopituitarism is related to the radiation doses given while whole body irradiation regimens to a dose of 18 Gy result merely in isolated GH deficiency. Somatotrophs are reported to be more radiosensitive in children as compared with adults: 59% of childhood cancer survivors show a blunted response on GH stimulatory tests. GH has consistently shown to be the most radiosensitive pituitary axis, with series reporting a prevalence of GHD between 50 and 100% after radiotherapy. Radiation-induced GHD is also progressive with time, developing more frequently in the first 10 years after radiation delivery. GHD severity and speed of onset closely correlate with lower final height, decreased lean body mass (LBM) and higher fat mass in children. There are several data indicating the significance of radiotherapy as a risk factor for hypothyroidism.
Children with malignancy treated with chemotherapy and radiation to the head, neck and mediastinum are at risk of developing hypothyroidism and thus should be monitored with regular thyroid function test surveillance up to around 10 years post-diagnosis. A 20 years old female patient's case is presented: after successful treatment of medulloblastoma at the age of 6, she was operated and treated with radioiodine for papillary type of thyroid cancer in her 15 years of age. As a consequence of her previous diseases and treatments last year we detect a severe GH deficiency. We show how is she currently managed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1524

**Thyrotropinoma. A rare case report of hyperthyroidism**

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Case presentation
A 84 year-old male without family history of thyroid disease was referred to our clinic by his cardiologist, diagnosed as having primary hypothyroidism, characterized by persistent elevated TSH without response to L-thyroxine over the course of a year. Co-morbidities included arterial hypertension, auricular fibrillation (AF) and glucose intolerance, treated with propafenone, clopidogrel, enalapril, and digoxine. On physical examination, he presented AF with tachycardia, mild distal tremor, and no goiter.
Thyroid function tests (TFT) reported TSH: 6.87 mU/ml (normal 0.4–4.5), $T_4$: 2.31 ng/dl (normal 0.8–2.0). Thyroid auto-antibodies were negative. It was decided to lower L-thyroxine dose. Six months later, the patient remained symptomatic despite treatment discontinuation. TFT were repeated, showing high TSH and $T_4$ levels. More specific tests and a MRI were ordered (Fig. 1).
TSH response to the TRH test was flat. The TSH alpha subunit (α-SU) and the α-SU/TSH molar ratio were elevated. The MRI showed a pituitary macroadenoma ($1.2\times1.1\times1.6$ cm) with extension to the supraselar cistern, left

deviation of the pituitary stalk, without involvement of the optic chiasm. A thyrotropinoma(TSH-oma) was diagnosed.
First-line therapy is surgical. If this is contraindicated or declined, pituitary radiotherapy, dopamine agonists, somatostatin analogs, or thyroid ablative treatment should be considered. Because of old age and co-morbidities, our patient was treated with ablative thyroid treatment with radioactive iodine. Six months later, TFT showed normal $T_4$ and high TSH levels, requiring no further therapeutic modifications.
Conclusion
TSH-omas are rare tumors -0.5% of all pituitary tumors-, classically causing central hyperthyroidism. High levels of α-SU or an α-SU/TSH molar ratio $>1$ are indicative of TSH-oma in more than 90% of the cases. MRI is the preferred tool to the diagnosis. Ablative thyroid therapy is a good option for some cases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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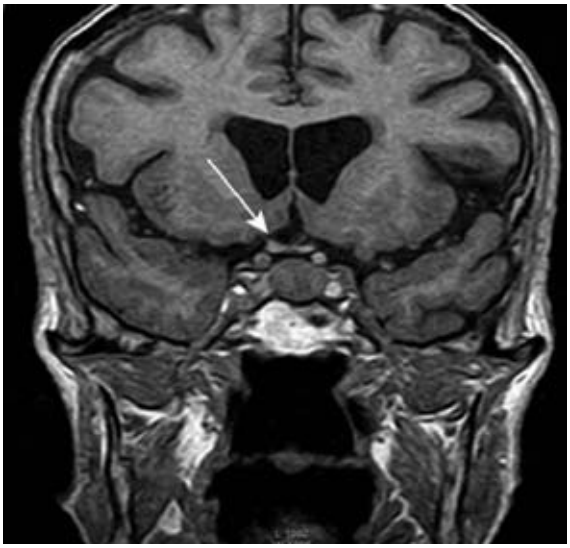

## P1525

**The dermatologic evaluation of acromegaly patients**
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Aim
To evaluate dermatologic findings in diabetic and nondiabetic acromegalic patients and compare with nonacromegalic diabetic patients. Also to investigate any possible correlation between skin lesions and colonic and thyroid neoplasms.
Materials and methods
Data from dermatologic examination of 32 acromegalic and diabetic non-acromegalic patients were compared. Colonoscopic, ultrasonographic and biochemical findings of acromegalic patients were evaluated for any correlation.
Results
Twenty nondiabetic and 12 diabetic acromegalic patients had similar number of skin tags compared to each other and to 21 nonacromegalic diabetic patients. Only IGF1 levels were correlated with the presence and number of skin tags and colonic polyps. Insulin levels were correlated with the presence of colonic polyps and thyroid nodules but not correlated with skin tags. The presence of skin tags was significantly correlated with colonic polyps but not correlated with thyroid nodules.
Conclusion
In our study group, IGF1 and not insulin levels affected the formation of skin tags. Skin tags could be a marker of colonic polyps, but are not related to thyroid nodules.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1526

**Growth hormone effects on lipid profile in patients with acromegaly**
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Introduction
Active acromegaly is associated with increased mortality, which has been attributed largely to cardiovascular disease.
Aims
To evaluate the effects of chronic excess of GH and IGF1 on lipid metabolism in patients with active acromegaly.
Subjects and methods
Ninety-seven patients (37 men and 60 women; aged 18–76 years) with macroadenoma of hypophysis (67 – somatotropinoma, 30 – somatomammotropinoma) were under investigation. Blood samples for GH, IGF1, total cholesterol (TC), triglycerides (T), HDL-C, Apo A-I and Apo B were taken in fasting state. LDL-C and VLDL-C were calculated using the Friedwald formula. Disease activity was evaluated by means of OGTT according to the Consensus Conference criteria. Data are given as $M \pm SE$ and multiple regression model equations.
Results
In 40.4% of patients with somatotropinoma (GH – $22.8 \pm 3.5$ ng/ml; IGF1 – $620.81 \pm 301.30$ ng/ml) and somatomamotropinoma (GH – $26.3 \pm 5.3$ ng/ml; IGF1 – $651.69 \pm 295.64$ ng/ml) different types of dyslipidemias (WHO of classification) were found out: IIa type in 18.9%; III type – in 15.8%, and IV type – in 5.3% of patients. Moderately elevated level of TC, T, LDL-C, and VLDL-C was appropriate to both groups of patients with macroadenoma. It was found out nonlinear regression between HDL-C, ApoA-I, ApoB and IGF1 approximated by equations: $\text{HDL-C} \approx 1/(0.7+30.5/\text{IGF1})$ ($R2=52.8\%$, $P=0.002$); Apo A-I $\approx 1/(0.6+16.3/\text{IGF1})$($R2=22.1\%$, $P=0.01$); $\text{ApoB}=2.07-0.17*\ln(\text{IGF1})$($R2=21.2\%$, $P=0.01$).
Conclusion
The GH hypersecretion is associated with altered lipid profile. IGF1 mainly predetermines the level of HDL-C, Apo-I and ApoB lipoproteins. Patients with acromegalia are at increased cardiovascular risk through the atherogenic dyslipidemias that may accompany the chronic excess of GH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1527

**Pituitary lesions: Pituitary adenomas and what else?**
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Introduction
Pituitary lesions are quite often in the general population and can be symptomatic or not. Most are attributed to pituitary adenomas. Differential diagnosis also includes other causes, such as granulomatous diseases (sarcoidosis, histiocyttosis). Neurosarcoidosis is rare. Sarcoidosis pituitary infiltration is even more rare and usually reported in multifocal forms of the disease.
Case report
We describe a 34-year old female with headache and oligomenorrhea.
MRI showed a pituitary lesion 1.5 cm and normal pituitary stalk.
Pituitary hormone profile was normal, except for mild hyperprolactinaemia, with no diabetes insipidus.
The lesion was considered to be a non functional pituitary adenoma and the patient underwent trans-sphenoidal excision of the lesion.
Histologic examination showed a granulomatous inflamatous mass, negative for tuberculosis. Three months later no mass was found in pituitary MRI.
The patient was referred to a rheumatologist, but was lost to follow up for personnal reasons.
One year later, the patient had an MRI showing a new pituitary lesion 1.3 cm with mild hyperprolactinaemia. She was investigated for sarcoidosis but no systematic

disease was found. Considering diagnosis of sarcoidosis, prednizolone was prescribed. Being on prednizolone for three months reduced size lesion to 0.9 cm. Six months later it disappeared. This confirmed diagnosis of sarcoidosis.
She received prednisolone for 18 months in total. Afterwards, she was lost to follow up again for 6 years, off corticosteroids, and recently came back with recurrence of the lesion.
She is again administered prednisolone.
Investigation for systematic disease is still negative.
Conclusion
To our knowledge, there is no other reported case of neurosarcoidosis with unique infiltration of the pituitary that persists for 6 years with no other systemic disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1528

**Progressive combined pituitary hormone deficiency produced by Prop1 gene mutation**

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Introduction
The appearance and normal development of the anterior pituitary gland requires several signalling molecules and specific transcription factors, such as PROP1, POUF1, HESX1/RPX, LHX3, LHX4, PITX2, T-PIT, SOX2 and SOX 3. Gene mutations of these pituitary transcription factors may lead to different degrees of combined pituitary hormone deficiency (CPHD) associated or not with morphological changes of the hypothalamic–pituitary region.
Material and methods
We present the first Romanian case of progressive CPHD in two brothers from a consanguineous family. Clinical, hormonal and MRI follow-up were performed during 20 years.
Results
Growth hormone deficiency was certified at the age of 5, respectively 3 years, followed by gonadotropin deficiency diagnosed at the age of 21, respectively 19 years, and by central hypothyroidism diagnosed at the age of 23, respectively 21 years. Substitutive treatment with recombinant human GH (rhGH) was commenced, followed by testosterone and later thyroxin, in adequate doses. Adrenal function was normal during the follow-up. Magnetic resonance imaging (MRI) revealed anterior pituitary hypoplasia in both siblings, with a partially empty sella in the younger brother. Surprisingly, we found a thick midline septum in the sphenoid sinus in both siblings, which was not described in previous reports. The progressive CPHD suggested a PROP1 deficiency, which was confirmed by genetic analysis. The 301-302delAG homozygous mutation in the PROP1 gene was identified, resulting in a complete loss of promoter binding and transcriptional activation of the mutant protein.
Conclusion
Mutations in PROP1 gene are a very rare cause of pituitary insufficiency causing progressive CPHD. The progressive decline in the anterior pituitary axis requires continuous monitoring of the patients, in order to adjust the doses of their complex substitutive hormonal therapy, to insure the adherence to treatment and to identify the occurrence of new hormonal deficits.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1529

**Neurohypohysis tumors: about two cases**

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Introduction
Neurohypophysis tumors also called pituicytomas are very rare low grade gliomas of the brain that developed in the posterior part of the pituitary gland. Our aim is to report two cases whose diagnosis was made by MRI in the first case and by histology in the second case.
Cases reports
Observation No. 1: a woman aged 33 consulted for polyuria and polydypsia due to central diabetes insipidus without pituitary insufficiency. The MRI discovered a 7 mm well limited tumor in the posterior part of the pituitary gland. According to different investigations a primary lesion was more likely than breast, thyroid, pulmonary or genital metastasis.
Observation 2: a man aged 55, consulted for headaches and decrease in visual acuity. Brain MRI showed an invasive pituitary tumor measuring $55 \times 32$ mm. Hormonal exploration argued for global pituitary insufficiency without any impairment of post hypophysis function, although the tumor was posterior. Histological exam argued for a pituicytoma.
Conclusion
Neurohyophysis tumors are very rare as less than 40 cases have been reported. They may be very small with diabetes insipidus as the only manifestation or be large or even huge with ophtalmological troubles and pituitary insufficiency without diabetes insipidus in spite of the posterior location as in our second observation. Surgery seems to be the best treatment for large tumors, but for small tumors wait and see attitude may also be another solution as these tumors grow slowly and are usually more vascularized than the anterior ones which makes the surgery more difficult and sometimes too risky.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1530

**Hypersomatotropism and glucose metabolism disorders**

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Introduction
Glucose metabolism disorder (GMD) is a classic complication of acromegaly, but its frequency varies from study to study.
Aim
We aimed to analyze GMD frequency in our population, and predictive factors such as: family history of diabetes mellitus (DM), age, gender, and growth hormone (GH) rates.
Subjects and methods
It is a prospective study where 75 hypersomatotropic subjects were analyzed. They all had fasting and postprandial blood glucose evaluation. When the last ones were normal glucose tolerance test was done and analyzed according to the WHO recommendations.
Results
In our group GMD were observed in 41 patients (22M/19F) = 55%. Diabetes mellitus was found in 27 subjects (65%), and impaired glucose tolerance (IGT) in 14 (35%). The diagnosis of DM was made before the hypersomatotropism's one in 37%, the delay was 5 years. Mean age at diagnosis for diabetic patients was $40 \pm 11$years, and $38 \pm 13$ years for non diabetic subjects. Glucose metabolism disorders were more frequent in women: 54% vs 46% for men, but the difference was not statistically significant. Mean plasma GH was = 75.25 ng/ml in DM subjects vs 46.1 ng/ml for non diabetic patients ($P < 0.05$). For family history of diabetes mellitus there was no difference between both groups.
Conclusion
In this study 55% hypersomatotopic subjects suffer from GMD. DM is more frequent than IGT (65% vs 35). On the statistical side, there is a positive correlation between GMD and GH concentrations, but not with gender, age and family history of DM.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1531

**Hyperprolactinemia: etiology, diagnosis and therapeutic aspects**

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The complex etiology of hyperprolactinemia was represented, in our study group (426 cases hospitalized during the period 2000 – 2010; F/M ratio = 372/54; age (years) = 40.29 ± 15.57), by the following entities: prolactin-secreting pituitary tumors (11.50%), growth hormone and prolactin-secreting pituitary tumors (1.64%), pituitary stalk compression (7.98%), primary myxedema (41.08%), polycystic ovary syndrome – PCOS (24.65%), iatrogenesis (8.69%) and chronic renal insufficiency (4.46%). The cases with prolactin-secreting pituitary adenomas (49 patients, F/M = 42/7, age = 32.53 ± 11.03) were divided in two groups (by clinical, functional and imaging criteria): macroprolactinomas (18 cases; F/M = 11/7; age = 32.39 ± 14.25); microprolactinomas (31 cases; F/M = 31/0; age = 32.61 ± 8.92).
The plasma prolactin levels were significantly higher ($P < 0.001$) in patients with macroprolactinomas comparing with microprolactinomas cases. It was also identified significant higher values of the plasma prolactin levels in prolactinomas in comparison with: iatrogenesis ($P < 0.001$), pituitary stalk compression ($P < 0.001$), primary myxedema ($P < 0.001$), PCOS ($P < 0.001$) and chronic renal insufficiency ($P < 0.05$). The patients with microprolactinomas showed a significant higher prolactin level versus the cases with PCOS.
All the cases with prolactinomas presented signs of hypogonadism and 8 patients with macroprolactinomas (F/M = 3/5) presented tumor mass effect. Excepting one case who imposed neurosurgical therapy, all the patients with prolactinomas were treated with dopamine agonist drugs with very good response regarding the lowering of prolactin level and tumor shrinkage (only one case didn't show a normal prolactin level despite the long term of medical therapy associated with tumor disappearance). Four women with macroprolactinomas became pregnant after one year of treatment with bromocriptine and all of them reached the parturition without any event (continuing during the pregnancy the therapy with bromocriptine).
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1532

**Clinical, biological and radiological confrontation in Cushing disease**

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Introduction
Cushing disease (CD) is a condition due to an ACTH-secreting pituitary adenoma that is usually a microadenoma. Seldom, the lesion may be atypical by his size or his radiological aspect.
The aim of this study is to analyze the clinical and biological data of CD with atypical radiological aspect (macroadenoma or other), to compare them to those of CD with a typical radiological aspect (microadenoma) and to determine the clinical or biological factors predictive of radiological aspect.
Methods
Retrospective study of 22 patients with CD comparing clinical and biological profiles of a group of patients with CD and a pituitary microadenoma on MRI or a normal pituitary MRI = (microadenoma) group, to a group of patients with atypical MRI aspect: macroadenoma or infiltrative lesion = (macroadenoma) group.
Results
Pituitary MRI showed a microadenoma in 59% of cases and a macroadenoma or an atypical aspect in 41% of cases. Clinical profile and non specific biological anomalies were comparable in the 2 groups. Basal cortisol and cortisol after low dose dexamethasone suppressing test were also comparable in the two groups. Free urinary cortisol of 24 h and cortisol after high dose dexamethasone test were higher in the (macroadenoma) group but with no significant difference. On the other hand, mean ACTH was significantly higher in the (macroadenoma) group (154 pg/ml vs 93 pg/ml; $P = 0.04$).
Conclusion
No clinical or biological factors can be predictive of tumoral size in CD. A particular attention should always be accorded to ACTH rate that may suggest a certain tumoral size.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1533

**Characteristics of giant and huge male prolactinomas**

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Introduction
Prolactinomas are more invasive in males. Giant (≥4 cm) and huge (≥6 cm) ones are relatively rare in literature, but in our population they are nearly common.
Aim
We have taken our 20 last cases to analyze their radiological aspects, and their endocrine, neurological and ophthalmological complications.
Methods
All of them had clinical exam, hormonal, ophthalmological, and radiological exploration based on cerebral MRI. Mixed adenomas were excluded. Positive diagnosis was based on clinical presentation, high prolactin concentration, positive response to dopamine agonists ± immunohistochemy study.
Results
Mean age = 42.45 years, mean tumor height = 50.12 (40–70) mm and mean volume = 48.6 $mm^3$ (15.5–184). Mean prolactin = 3199 ± 5437 ng/ml. Solid and kystic aspect, with or without calcifications, is observed in more than 50%. Cavernous sinuses are invaded in all except one. Other invasions are (parietal or frontal lobe: 9, posterior invasion = 9, anterior invasion = 9 (with orbital infiltration in 1case), ethmoidal invasion = 3. For endocrine complications we observed gonadic deficit in 78.9% cases, corticotrop and thyreotro insufficiencies were seen in respectively 35%, and 15.7%. Only 7 subjects had two or more pituitary deficits. Neurological and ophthalmological complications are as follows: the total loss of one or both eyes: 13, hydrocephaly: 2 cases, epilepsy: 2, conscious troubles or memory loss: 2, ptosis: 3, diplopia with or without strabismus: 2.
Conclusion
In this study concerning giant and huge male prolactinomas the total loss of one or both eyes is the most frequent abnormality (13/20). Severe neurological troubles are relatively frequent as they were observed in nearly one from 3 patients. But, multi pituitary deficits (7 cases) and active hydrocephaly (2 cases) are relatively rare, which argues for a low progression.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1534

**A case of acromegaly in the presence of coincidental liver cirrhosis**

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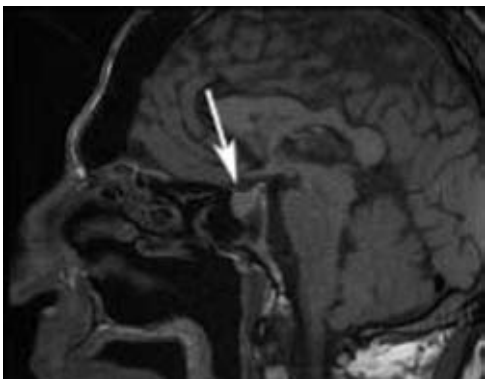
Acromegaly is a rare and serious syndrome and commonly associated with pituitary neoplasm. Classic cause of acromegaly in adults is the tumors of the somatotrophs that secrete growth hormone. Cirrhosis is the end stage of chronic liver disease and commonly cause of death. It is characterized by diffuse hepatic fibrosis resulting in altered construction of the lobular parenchyma with widespread connective tissue septae, circumscribed regenerative nodules of hepatocytes and anastomoses between vascular channels linking portal and central vessels. A 62-year old, male patient came to the hospital complaining of severe abdominal swelling. Laboratory and imaging findings were compatible with the presence hepatitis B virus related cirrhosis together with acromegaly. In this case, he had high GH level but lower IGF1 level because of hepatic failure which can impair IGF1 production by the liver. Definitive diagnosis was made by pituitary MR and 1 cm in diameter tumor was detected. This paper showed that cirrhosis can result in a low IGF1 level in patients with acromegaly. There is no previous report available of the in the presence of coincidental combination of acromegaly and cirrhosis in a patient.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Laboratory findings, hormon profiles and hepatitis markers of the acromegalic patient with cirrhosis.

| | At admission | Follow up (6 months later) | Reference |
|---|---|---|---|
| Glu (mg/dl) | 87 | 289 | 80–115 |
| Cr (mg/dl) | 0.6 | 1.05 | 0.7–1.2 |
| Na (mg/dl) | 141 | 136 | 136–145 |
| K (mg/dl) | 4 | 4.1 | 3.5–5.1 |
| Total bil (mg/dl) | 2.39 | 2.28 | 0.2–1.2 |
| Direct bil (mg/dl) | 1.06 | 1.25 | 0–0.5 |
| Prot (g/dl) | 6.3 | 5.8 | 6–8 |
| Alb (g/dl) | 2.8 | 2.3 | 3.5–5 |
| AST (IU/l) | 104 | 114 | 5–35 |
| ALT (IU/l) | 35 | 86 | 10–50 |
| WBC (K/μl) | 4.37 | 4.35 | 3.6–9.4 |
| Hgb (g/dl) | 11.9 | 10.2 | 13–17.5 |
| Plt (K/μl) | 47.9 | 49.8 | 142–424 |
| Protrombin time | %62 | %34 | 70–130 |
| HBsAg (S/CO) | >250.00 | >250.00 | <0.9 |
| Anti HBs (mIU//ml) | 2.06 | 2.06 | <7 |
| GH (ng/ml) | 15.4 | 51.4 | 0–1 |
| IGF1 (ng/ml) | 50.3 | 71.7 | 71–290 |



## P1535

### Adiponectin and cardiac structure in acromegaly

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Adiponectin, is an adipocyte derived hormone possess insulin-sensitizing, antiatherogenic, and antiinflammatory properties. The aim of this study to evaluate adiponectin levels at acromegalic patients according to healthy subjects in relation with ecocardiographic findings. We included 30 subjects (15 male, 15 female) were diagnosed as acromegaly and 30 healthy (10 male, 20 female) subjects. Mean ages of both group were similar. Serum glucose, insulin, GH, IGF1 (insulin like growth factor 1), adiponectin levels were obtained and insulin resistance were calculated by HOMA-IR. Echocardiography of subjects were performed. Adiponectin levels were significantly higher at acromegalic group than control group. At acromegalic group, there was no statistically meaningful relation between serum adiponectin and GH, IGF1 levels. IVST (thickness of interventricular septum in diastole), PWT (thickness of left ventricular posterior wall in diastole), LVMI (left ventricular mass index), E/A ratio, DT (deceleration time of E-wave), ET(ejection time), IVRT (Isovolumetric relaxation time), VPR (velocity of mitral flow propagation), LVESV (left ventricle end systolic volume) values were increased results were statistically meaningful. At acromegaly group adiponectin levels were positively related with LVMI. Adiponectin levels may be indicator of the cardiac involvement acromegaly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

Clinical and echocardiographic findings of groups.

| | Acromegaly ($n=30$) | Control ($n=30$) | *P* value |
|---|---|---|---|
| BMI (kg/m$^2$) | 29.06±6.08 | 25.06±2.78 | 0.001* |
| FPG (mg/dl) | 113.53±28.87 | 89.20±6.26 | 0.001* |
| Insulin (μIU/ml) | 8.38±9.43 | 6.51±2.45 | NS |
| GH (ng/l) | 15.39±14.13 | 0.68±1.29 | 0.001* |
| IGF1 (ng/l) | 649.53±373.45 | 124.54±44.86 | 0.001* |
| Adiponec tin (μg/ml) | 84.13±101.18 | 28.84±58.72 | 0.001* |
| IVST (mm) | 10.46±1.38 | 6.93±2.93 | 0.000* |
| PWT (mm) | 10.36±1.67 | 6.93±3.03 | 0.000* |
| LVMI (g/m$^2$) | 122.98±30.18 | 76.65±15.08 | 0.000* |
| E/A ratio | 0.98±0.31 | 1.19±0.36 | 0.044* |
| DT | 219.83±50.99 | 191.06±32.9 | 0.012* |
| ET | 314.03±40.04 | 276.93±26.72 | 0.000* |
| IVRT | 106.20±15.34 | 97.00±11.73 | 0.012* |
| VPR | 59.83±14.24 | 45.06±8.87 | 0.000* |
| LVESV | 39.13±11.24 | 31.90±11.06 | 0.001* |
| EF (%) | 63.00±6.55 | 64.06±5.63 | NS |

*$P$ value $<0.05$; statistically significant. NS: Non significant. EF: Ejection fraction.

## P1536

### A case of acromegaly diagnosed with diabetic ketoacidosis as a primary manifestation

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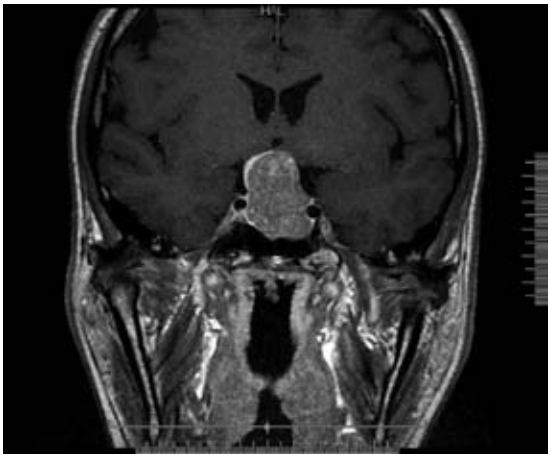
Although diabetic ketoacidosis (DKA) is recognized one of complications of acromegaly, it is very rare as only five cases are reported as a primary manifestation.
A 43-year-old Japanese woman, who had never pointed out impaired glucose tolerance, had emergency admitted by DKA. Her plasma glucose was 440 mg/dl, HbA1c 15.7%, urinary keton body (3+), BGA pH7.257, then she was cured by rehydration of saline and continues intravenous insulin injection. She was unlikely of type1 nor type2 diabetes because her endogenous insulin secretion was kept, as fasting serum c-peptide was 1.49 ng/ml, urinary c-peptide 85.5 μg/day, GAD antibody negative, and she was not obesity (BMI 20.4 kg/m$^2$). Because she had acromegalic features, existence of acromegaly was suggested. Her GH and IGF1 levels showed high (155 ng/ml and 476 ng/ml) under hyperglycemia. There were double floor and ballooning in sella turcica Xp, a cauliflower-like change of the distal phalanx in finger Xp. And there are expansion of sella turcica and mass lesion on pituitary MRI (Default 1).
From the above, we had a diagnosis of the acromegaly due to GH-producing pituitary tumor, and DKA had developed from secondary diabetes by it. She finally needed 30 units of insulin a day for diabetes and got good plasma glucose control, and then was transferred for the purpose of receiving pituitary operations.
We experienced a case of acromegaly diagnosed with DKA as a primary manifestation. It is thought that diabetes is complicated with acromegaly as a result of insulin resistance increased by overproducing of the GH, and DKA occurs because of absolute or relative lack of insulin and counter hormones participates in it.



Therefore, like this case, it is necessary to be careful about possibility to be complicated with DKA about acromegaly.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1537

### The role of long acting somatostatin analogues on glucose homeostasis in acromegaly

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Introduction

Impaired glucose tolerance and diabetes mellitus are frequently seen complications of acromegaly. Insulin resistance due to chronic growth hormone (GH) excess and inhibition of insulin and glucagon secretion by long-acting somatostatin analogues (SA) used in the treatment are the main reasons of the impaired glucose tolerance.

Objective

To determine the effect of long-acting SA treatment on glucose homeostasis regarding the insulin resistance and pancreatic β-cell function.

Method

Totally 30 patients (17 male, 13 female; mean age:44.63 ± 11.85 years) were enrolled in the study. Serum IGF1 concentrations and glucose, insulin, GH levels at 0, 30, 60 and 120 min during OGTT were measured. Patients were categorized into 3 groups according to their disease activity. Patients cured after operation, patients with active disease after operation but in remission after SA therapy and patients with active disease after operation and SA therapy were defined as cure, remission and active group, respectively. For the interpretation of glucose homeostasis, formulas for insulin resistance (HOMA-IR, QUICKI) and β-cell function (HOMA-β, Insulinogenic index) were used.

Results

There was no statistically significant difference between groups for HOMA-IR and QUICKI ($P=0.78$; $P=0.781$) and also between disease activity and insulin resistance. For the insulinogenic index, which was the marker of early phase of insulin secretion, there was a borderline difference in significance between groups ($P=0.076$). HOMA-β of cure group was higher than the other two groups independent of disease activity. In the cure group, serum insulin levels and peak insulin secretion was higher, time to reach the peak level was shorter than the other groups ($P=0.420$; $P=0.176$).

Conclusion

Treatment with long acting SA in acromegaly inhibits insulin secretion from pancreatic β-cells, so causes deterioration in glucose homeostasis. Due to the increase in cardiovascular morbidity and mortality, patients on SA therapy should be routinely followed-up for glucose intolerance.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Results of the insulin resistance and pancreatic β-cell function tests of the groups

| | Active | Remission | Cure | P |
|---|---|---|---|---|
| HOMA-IR | 2.21 ± 1.59 | 1.77 ± 1.34 | 2.12 ± 1.63 | 0.781 |
| QUICKI | 0.35 ± 0.03 | 0.37 ± 0.04 | 0.36 ± 0.04 | 0.781 |
| HOMA-β (%) | 72.48 ± 48.93 | 59.48 ± 44.86 | 104.79 ± 80.99 | 0.384 |
| Insulinogenic index | 0.33 ± 0.26 | 0.52 ± 0.45 | 0.97 ± 0.86 | 0.076 |

## P1538

### Clinical features of inferior and lateral invasive expansion of GH-producing adenoma

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The present study was undertaken to determine morphological and biochemical characteristics of GH-producing tumor. We collected consecutively 15 patients with acromegaly, and they were 6 males and 9 females with the ages of 58.5 ± 9.9 years. As for tumor size, 7 patients had macroadenoma, 5 had microadenoma and three had empty sella with some atypical expansions. Only 3 patients had intrasellar tumor and 2 patients had empty sella in MRI finding. Other 10 patients had atypical tumor growth and expansion. Invasive inferior expansion into sphenoid sinus with macroadenoma was found in 3 patients. Inferior and lateral expanded extrasellar tumor with empty sella was found in 2 patients. Unilateral or bilateral expansion along with microadenoma and macroadenoma was obtained in 3 patients. Other 2 patients had latero-inferior expansion, and both superior and inferior expansion of macroadenoma.

Endocrinological studies showed that serum GH and insulin growth factor (IGF1) levels were 33.3 ± 32.5 ng/ml and 699.3 ± 299.3 ng/ml in 7 patients with macroadenoma, 8.7 ± 7.4 ng/ml and 586.5 ± 35.2 ng/ml in 5 patients with microadenoma, 7.5 ± 2.9 ng/ml and 433.2 ± 91.0 ng/ml in 3 patients with empty sella and inferior expanded tumor. The increases in hormonal levels seemed to be dependent on tumor volumes, and did not relate with the direction of extrasellar expansion. Serum prolactin was mildly elevated to 17.49 to 90 ng/ml in 7 patients, that means simultaneous secretion of prolactin with GH in acromegaly. Hyperprolactinemia were not associated with compression of pituitary stalk and inhibition of prolactin-inhibiting factor. The present findings indicate that GH-producing tumors easily grow to macroadenoma, and the tumor growth may mainly develop to the inferior and/or lateral expansion by destroying bony tissues of sella turcica. The tumor expansion could be different from that of other functioning and non-functioning pituitary tumors in which tumors grow superiorly.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1539

### Predictive value of acute octreotide supression test in newly diagnosed acromegaly

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Background

It has been reported that primary treatment with somatostatin analogues (SSA) is effective in up to 60% of acromegaly patients, but predictive value of the acute octreotide suppression test (OST) for selection of patients with good response to depot SSA in long term treatment remains controversial.

Patients and methods

Twelve medical therapy naive patients (mean age 44 years, female 75%) with active acromegaly were included in prospective study. After administration of 100 μg octreotide the baseline and hourly GH measurements were taken for following 6 hours. GH response to the OST was defined as the percent reduction of GH from the baseline. Subsequently 6 patients received the long-acting SSA lanreotide (Somatuline Autogel), 120 mg every 28 days and 6 patients received long-acting SSA octreotide (Sandostatin LAR) 20–40 mg every 28 days for 3–24 month treatment period. GH and IGF1 were measured at baseline and during the treatment period.

Results

Fifty-eight percent of patients (7 out of 12) showed good response (>50% GH decrease) during the OST. 42% of them (3/7) reached target GH<2 ng/ml during the subsequent SSA therapy and only one patient (14%) achieved normalization of IGF1. 25% of patients (3 out of 12) showed moderate 30–50% GH decrease during the OST and only one reached normal IGF1 after administration of long-acting SSA. Despite that 17% of patients (2 out of 12) had only small <30% GH decrease during the OST, they reached the treatment target (GH<2 ng/ml) during the subsequent depot SSA therapy.

Conclusion

Acute OST has poor predictive value for effectiveness of subsequent long-acting SSA therapy and should not replace at least 3 month trial with depot SSA.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1540

**An unusual plurihormonal pituitary adenoma**

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Introduction
Plurihormonality of pituitary adenoma can be defined as the ability to express more than one hormone. 1–30% of pituitary adenoma are plurihormonal. We describe a case of plurihormonal adenoma with GH and ACTH secretion and triple immunostaining for GH, ACTH and prolactin.
Case report
A 40 years old patient presented with sudden weight gain central obesity, hypertension, menstrual disorders, acne, hirsutism suggesting hypercortisolism. Laboratory evaluation confirmed Cushing's disease (serum cortisol 0 h 234 nmol/l, cortisol after low-dose dexamethasone suppression test 613 nmol/l, ACTH 8 h 56 pg/ml). Furthermore, laboratory data showed elevated IGF1 levels (440 ng/ml, reference range 101–267 ng/ml) with elevated GH after HGPO (3.3 mUI/l). Prolactin, TSH, FSH and LH serum level were in normal range. The sellar MRI demonstrated microadenoma. Transphenoidal removal of the tumor was performed. Immunohistochemistry analyses showed staining for ACTH (40%), GH (80%) and prolactin (50%). Ki67 was less than 0.5%. Laboratory data realized after surgery were normal.
Conclusion
The majority of plurihormonal adenomas produce GH, PRL and TRH because lactotroph, somatotroph and thyreotroph cells have the same progenitor. Association of ACTH and GH secretion with positive immunostaining for GH, PRL and ACTH has not been described previously. These unusual plurihormonal adenomas exhibit aggressive behavior and poor prognosis. Plurihormonality of pituitary adenomas linked with ACTH co-expression seems to predict a higher risk of tumor recurrence. Furthermore, higher morbidity due to mixed ACTH and GH secretion needs a strict follow up.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1541

**Results of pegvisomant therapy in patients with acromegaly**

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Introduction
Acromegaly is chronic debilitating disease related with excess GH and insülin-like growth factor 1 (IGF1) secretion. Pegvisomant is a GH receptor antagonist that competes with endogenous GH for its receptor and is often used as a medical therapy in patients with inadequate response to somatostatin analogs.
Methods
Forty-six patients are followed with the diagnosis of acromegaly in our clinic. We present the results of six acromegalic patients (five female and one male patient) treated with pegvisomant.
Results
The mean age was 49.8 years and the mean disease duration was 12.5 years. All of them were treated with surgery. Five patients received external radiotherapy and/or gamma knife surgery. All of the patients received octreotide LAR 30 mg/month therapy. Despite these therapies, IGF1 levels were higher than the upper limit of sex and age matched levels. We evaluate the patients for relapse or residual lesions before pegvisomant treatment and found macroadenoma in two patient, micraoadenoma in one patient and no lesion in three patients. Mean IGF1 level was 558 ng/ml before pegvisomant therapy. After 40 mg loading dose, we started pegvisomant 10 mg/day. Mean therapy duration was 11.6 months. During follow-up, IGF1 levels decreased in four patients, however, remained high in two patients. Therefore, the dose is increased to 15 mg/day in these two patients. IGF1 levels decreased in one of them, but was still high in the other. In one patient with macroadenoma, pegvisomant was stopped at the second month due to lipodistrophy. No adverse effects were seen in other patients. Treatment is completed to one year only in two patients and we found no increase in adenoma sizes in these patients.
Conclusion
Four of six patients responded to pegvisomant treatment. Pegvisomant seems to be effective in patients in whom remission cannot be acheived with other modes of therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1542

**Panhypopituitarism and central diabetes insipidus: a case report**

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Patient in the case, an 18-year-old male, complained of constant tiredness, frequent malaise, day time sleepiness, epigastric pain, poor appetite and consumption of great quantity of water. These conditions had persisted for 3–4 years. He had gone to many different physicians, with different tests taken, but without diagnose. Upon physical examination, the pale skin, scanty of pubic hair, and no presence of beard and axillary hair raised the possibility of Panhypopituitarism. This suspicion was reinforced by the result of blood examination which showed normocytic anemia, hypokalemia (*K*: 3.27 mg/dl), secondary adrenal insufficiency, secondary hypothyroidism and secondary hypogonadism. With further examination, the Pituitary stimulation test showed poor response. Water deprivation test indicated severe central diabetes insipidus. Bone density was relatively low (*Z*-score: $-2.3$). The result of pituitary MRI showed a soft tissue lesion in the pituitary stalk with heterogeneous enhancement.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1542.1

**Evaluation of the efficacy and safety of pasireotide LAR in patients with mild-to-moderate cushing's disease: a randomized, double-blind, multicenter, phase III study design**

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Background
Cushing's disease is associated with high morbidity and mortality, and there are currently no approved medical therapies. Twice-daily pasireotide sc showed efficacy in patients with mostly moderate-to-severe (UFC $\geq 2\times$ ULN) Cushing's disease in a large, randomized, double-blind, 12-month trial. A monthly long-acting release (LAR) formulation of pasireotide has been developed to provide a smoother pharmacokinetic profile, potentially a better efficacy/safety ratio, and to enhance convenience. The current study is designed to evaluate the efficacy and safety of pasireotide LAR in patients with Cushing's disease.
Patients
Adult patients with persistent/recurrent or *de novo* (if not surgical candidates) Cushing's disease, with baseline mean UFC $\geq 1.5$ and $\leq 5\times$ ULN (mild-to-moderate Cushing's disease). Diagnosis of Cushing's disease will be made by: i) the mean of three 24 h urinary free cortisol (mUFC) samples; ii) morning plasma ACTH within or above the normal range; and iii) confirmation of pituitary adenoma by MRI, IPSS or histopathology. Patients will be pasireotide naïve.
Design
Global, multicenter, randomized, double-blind, Phase III trial. Target enrollment is ~148 patients; recruitment is under way. Patients will be stratified based on baseline mUFC values and randomized to receive pasireotide LAR 10 or 30 mg im every 28 days for 12 months. If mUFC $> 1.5\times$ ULN and patients tolerate pasireotide LAR at month 4, dose uptitration will be performed at month 4 to either 30 or 40 mg respectively. Dose uptitration to 30 or 40 mg will also be performed at months 7, 9 and 12 in patients with mUFC $>$ ULN and no tolerability issues. Patients achieving mUFC $\leq$ ULN or significant clinical benefit at month 12 may enter a 12-month extension.
Endpoints
Primary efficacy endpoint is the proportion of patients with UFC $\leq$ ULN at month seven, regardless of dose titration. Secondary endpoints include: proportion of

patients with mUFC ≤ ULN at month 7 in patients who did not uptitrate at month 4; changes in UFC, plasma ACTH, and serum cortisol; changes in quality of life, and signs and symptoms of Cushing's disease; tolerability and safety.
Conclusions
This phase III study will provide the basis for the evaluation of long-acting pasireotide as a medical therapy for patients with mild-to-moderate Cushing's disease.
Declaration of interest
The authors declare that there is a conflict of interest.
Funding
This work was supported, however funding details are unavailable.

## Steroid metabolism + action

### P1543

**Anti-inflammatory effect of a high dose of corticosteroids is associated with some paradoxical pro-inflammatory effects**

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We have previously demonstrated that a low dose of hydrocortisone (100 mg) given intravenously suppresses intranuclear NFκB and AP-1 binding and the expression of pro-inflammatory genes like MMPs. We have now investigated the effect of a high dose of hydrocortisone (300 mg = 60 mg prednisolone) on NFκB binding and the expression of TLRs, the mediators of TLR signal transduction, MyD88 and TRIF and HMG-B1. Ten normal subjects were injected intravenously with 300 mg of hydrocortisone or saline in 2 separate visits one week apart in a randomized crossover study. Blood samples were obtained at 0,2,4,8 and 24 h after the injection. Mononuclear cells (MNC) were prepared by standard techniques and were tested for NFκB binding and the expression of TLRs, MyD88, TRIF, chemokines and chemokine receptors and HMG-B1. Plasma concentrations of glucose, FFAs, NO metabolites, chemokines and HMG-B1 were also measured. Following the injection of this dose, there was a significant increase in glucose concentration from 92 ± 4 to 116 ± 6 mg/dl, a marked increase in FFA concentrations from 0.38 ± 0.1 to 0.804 ± 0.15 mM. While NFκB binding and the mRNA expression of MyD88, TRIF, chemokines and chemokine receptors was suppressed significantly in MNC, the mRNA expression of TLR 2, 5 and 9 and HMG-B1 was increased (by 103 ± 24%, 107 ± 19%, 56 ± 13% above the baseline, respectively) in the MNC as was the concentration of HMGB1 (by 37 ± 12%) and MMP-9 (125 ± 22%) in plasma. Thus, while this high dose of HC exerts a powerful anti-inflammatory effect as shown above, it also exerts certain paradoxical pro-inflammatory effects. Since both glucose and FFAs have been shown to be pro-inflammatory, it is possible that they contribute to these effects. These paradoxical pro-inflammatory effects may account for the inability of these drugs to show benefit in clinical trials of septicemia and other severe pro-inflammatory states.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### P1544

**Acute and chronic effects of low dose prednisolone on carbohydrate metabolism in subjects with inflammatory rheumatologic disease**

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High dose glucocorticoids reduce hepatic and peripheral insulin sensitivity and insulin secretion. However, the metabolic consequences of typical therapeutic glucocorticoid doses (e.g. prednisolone < 10 mg/day) are poorly characterised. The aim was to determine the acute effect of low dose prednisolone on carbohydrate metabolism and then assess whether subjects taking chronic prednisolone had increased adiposity that amplified carbohydrate metabolism perturbations.
Nine controls (4 female, age 58 ± 11 years, BMI 27.5 ± 5.8 kg/m$^2$) with inflammatory rheumatologic disease who were not taking oral glucocorticoids were studied before and after prednisolone 6 mg/day for 7 days. Baseline data were compared with 12 matched subjects (6 female, age 61 ± 8 years, BMI 27.4 ± 3.3 kg/m$^2$) taking long-term prednisolone (6.3 ± 2.2 mg/day). Peripheral insulin sensitivity was assessed by hyperinsulinaemic–euglycaemic clamp (80 mU/m$^2$ per min for 120 min) and insulin secretion by 60 min intravenous glucose tolerance test (IVGTT, 25mg/kg glucose). Total and visceral adiposity were quantified by DXA and abdominal CT. Quantification of hepatic glucose output (using 6,6-$^2H_2$ glucose) and insulin concentrations are underway (data to follow).
Glucose infusion rate during hyperinsulinaemic–euglycaemic clamp fell from 79.6 ± 5.9 to 68.9 ± 5.2 μmol/min per kg FFM ($P = 0.02$) after 7 days of prednisolone. Glucose AUC during IVGTT acutely increased after prednisolone (504 ± 14 to 579 ± 19 mmol/L*min, $P = 0.01$). There were no significant differences in total (27.8 ± 2.8 vs 26.5 ± 3.8 kg, $P = 0.78$) or visceral (97 ± 11 vs 108 ± 27 cm$^2$, $P = 1.00$) fat mass between chronic prednisolone users and controls. Glucose infusion rate during hyperinsulinaemic–euglycaemic clamp (68.7 ± 6.6 vs 68.9 ± 5.2 μmol/min per kg FFM, $P = 0.78$) and glucose AUC during IVGTT (564 ± 18 vs 579 ± 19 mmol/l*min, $P = 0.67$) were not significantly different in subjects taking chronic prednisolone and following acute prednisolone administration.
In conclusion, low dose prednisolone acutely reduces peripheral insulin sensitivity and may reduce insulin secretion. Perturbations of carbohydrate metabolism during chronic prednisolone therapy match those found acutely. These findings provide insight into targeting treatment of glucocorticoid-induced diabetes at the underlying metabolic abnormality.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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### P1545

**The influence of aromatizable and non-aromatizable steroids on anthropometric parameters**

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Objective
It is known sex steroids affect fat distribution with men and women. With men there is a tendency to deposit fat abdominally. Men are more likely to have more visceral fat than premenopausal women, with whom the preferential fat distribution is gluteofemoral and the percentage of body fat overall higher. Androgens may affect fat tissue with men either directly by androgen receptor stimulation, or indirectly by oestrogen receptor stimulation after aromatization. Interesting relationships between the parameters of metabolic syndrome and non-aromatizable metabolites of testosterone have been discussed in literature.
Aim of the study
The analysis of the relation between anthropometric parameters, lipid spectrum, glycemia, insulin resistance and the level of testosterone and dihydrotestosterone.
Methods
We examined a set of 195 men and determined their testosterone, dihydrotestosterone, SHGB, lipid spectrum, glucose metabolism parameters and the oral glucose tolerance test; also measured were their anthropometric parameters (weight, height, waist, hips, waist to hip ratio, 14 skin folds) and body composition was calculated.
Results
Comparing the hormone levels and anthropometric parameters, we found a negative correlation between weight, skin folds, waist, hips, waist to hip ratio, BMI, total cholesterol, LDL cholesterol and insulin resistance on one side and the level of both testosterone (T) and dihydrotestosterone (DHT5α) and SHBG on the other side. We found a positive correlation between HDL cholesterol and muscle mass on one side and the T, DHT levels and SHBG on the other side.
Conclusions
We found a negative relation between anthropometric parameters and both testosterone and dihydrotestosterone. We did not find any difference between aromatizable and non-aromatizable steroids with healthy, normosthenic men.
Acknowledgement
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## P1546

**Effect of smoking cessation on hormonal balance**

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Introduction
Cigarette smoking is one of the most serious substance abuse problems. It is generally accepted that nicotine and other chemicals in tobacco smoke alter endocrine functions in men and adverse reproductive outcomes, including deterioration of sperm. Studies on the effects of smoking and smoking cessation on male sex hormones are inconsistent. We studied changes of hormonal levels in men during smoking cessation and we looked for a possible predictive marker of success in smoking cessation.
Methods
We examined 76 men before initiating of smoking cessation and after 6 weeks and after a year of abstinence. Basic anthropometric data and testosterone, cortisol, dehydroepiandosterone, dehydroepiandosterone sulphate, LH, FSH and SHBG were measured using imunoanalysis. Kruskal-Wallis robust ANOVA model was used for evaluation of the data. The local Ethics Committee approved the study and all patients signed an informed consent form.
Results
Successful men in smoking cessation did not differ from unsuccessful ones in the levels of steroid hormones observed. There was a trend in levels of testosterone, men who failed to quit smoking even for a short time had lower levels of testosterone. After smoking cessation there were a statistically significant increase in BMI and decrease in levels of SHBG. Decrease in levels of testosterone and DHEA was statistically insignificant. There were no changes in levels of cortisol. Changes of SHBG and testosterone levels did not correlate with BMI.
Conclusion
One year of smoking cessation is associated with increase in BMI and decrease in SHBG, which are possible effects of smoking cessation. Long term study could clarify these founding. Because insult of smoking is long-term and one year is short period to reparation.
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## P1547

**Conversion of 7α-hydroxyDHEA into 7-ketoDHEA: Role of type 2 11β-hydroxysteroid dehydrogenase**

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It has been suggested that DHEA metabolites, especially 7-hydroxy and/or 7-keto derivatives could possess anti-inflammatory activity and enhance immune, cognitive and metabolic functions in rodents. It is thus important to elucidate the exact pathway (s) leading to the formation of these metabolites. While the role of cytochrome P450 CYP7B1 in the transformation of DHEA into 7α-OHDHEA is well recognized, the role of type 1 11β-hydroxysteroid dehydrogenase (11β-HSD1) in the transformation of 7α-OHDHEA into 7-ketoDHEA, as suggested by many authors, remains questionable. While 11β-HSD1 prefers NADPH as cofactor and is thus, essentially, a reductive enzyme, the transformation of 7β-OHDHEA into 7-ketoDHEA is an oxidative reaction. Since 11β-HSD2 prefers NAD+ as cofactor, and is the oxidative counterpart of 11β-HSD1, we hypothesized that 11β-HSD2 could rather be the enzyme responsible for the transformation of 7α-OHDHEA into 7-ketoDHEA.
To verify this hypothesis, we cloned the coding region of human CYP7B1, 11β-HSD1 and 11β-HSD2 genes into pCMV expression vectors and transfected them into transformed human embryonic kidney HEK-293 cells. Using transfected cells in culture, without addition of cofactor(s), thus better reproducing physiological conditions, and using [C14]DHEA as substrate, we have clearly observed that CYP7B1 catalyzes the conversion of DHEA into 7α-OHDHEA, while, upon the addition of 11β-HSD2, 7α-OHDHEA is oxidized readily into 7-ketoDHEA, a reaction which does not occur in the presence of 11β-HSD1. The present data indicate that 11β-HSD2, rather than 11β-HSD1, is the enzyme responsible for the conversion 7α-OHDHEA into 7-ketoDHEA (DHEA –CYP7B1–» 7α-OHDHEA —11β-HSD2 –» 7-ketoDHEA) in the biosynthetic pathway of 7-ketoDHEA formation.
Declaration of interest
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## P1548

**Steroid derivatives as pure antagonists of the androgen receptor**

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Introduction
While the androgens of testicular origin (representing about 50% of total androgens in men over 50 years) can be completely eliminated by surgical or medical castration with GnRH (gonadotropin-releasing hormone) agonists or antagonists, the antiandrogens currently available as blockers of androgen binding to the androgen receptor (AR), namely bicalutamide (BICA), flutamide (FLU) and nilutamide have too weak affinity to completely neutralize the 50% of androgens made locally from dehydroepiandrosterone (DHEA) in the prostate cancer tissue by the mechanisms of intracrinology.
Description of methods/design
Series of steroid derivatives having pure and potent antagonistic activity on the human and rodent AR were synthetized. Assays of AR binding and activity in carcinoma mouse Shionogi and human LNCaP cells as well as *in vivo* bioavailability measurements and in vivo prostate weight assays in the rat were used.
Results
The chosen lead steroidal compound, namely EM-5854, has a 3.7-fold higher affinity than BICA for the human AR while EM-5855, an important metabolite of EM-5854, has a 94-fold higher affinity compared to BICA. EM-5854 and EM-5855 are 14 times more potent than BICA in inhibiting androgen (R1881)-stimulated prostatic specific antigen (PSA) secretion in human prostatic carcinoma LNCaP cells *in vitro*. MDV3100 has a potency comparable to bicalutamide in these assays. Depending upon the oral formulation, EM-5854 is 5- to 10-times more potent than BICA to inhibit dihydrotestosterone (DHT)-stimulated ventral prostatic weight *in vivo* in the rat. These data indicating the high potency of EM-5854 and EM-5855 are supported by respective 40-fold and 105-fold higher potencies of these two compounds compared to BICA to inhibit cell proliferation in the androgen-sensitive Shionogi carcinoma cell model.
Conclusion
The present data suggest the possibility of a much higher potency of EM-5854 or EM 5855 compared to BICA (Casodex) for the treatment of prostate cancer in men.
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## P1549

**The cellular fate of CYP19A1 (aromatase) protein**

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Introduction
Aromatase cytochrome P450 is uniquely responsible for estrogen synthesis in vertebrates. The protein is expressed in the gonads and other extraglandular tissues. Deregulated expression of aromatase has pathological relevance and targeting the CYP19A1 product could be an effective strategy for treatment of affected tissues. Nevertheless, very little is known concerning the role of protein oligomerisation, glycosylation and degradation. Here we studied the aromatase cellular fate and the effects of different mutations in an *in vitro* setting.
Design
The coding region of CYP19A1 gene was cloned and mutations previously identified by us in patients (R365Q, E210K, M127R, R375H) were introduced by site-directed mutagenesis. Then HEK 293 cells, not expressing aromatase, were

selected for *in vitro* transient expression. Aromatase cellular localization and protein expression were analyzed. Glycosylation and degradation were investigated with specific inhibitors, and aromatase activity was evaluated by estradiol assay.
Results
No differences in wild type and mutated aromatase protein cellular localization were found. The time course of protein expression by Western blotting showed the highest degradation rate for the E210K mutation followed by R365Q then M127R and R375H mutation. The same trend was observed for aromatase activity. Proteasome inhibition by MG132 resulted in the appearance of a non-glycosylated form, confirmed by tunicamycin treatment. Only the E210K mutation showed a degree of instability due to proteasome-mediated degradation. Other pathways were investigated but neither autophagy nor lysosomal inhibitors affected degradation, except for chloroquine (specific for lysosomes).
Conclusions
The reduced functional activity of the protein caused by different mutations appears to be due both to partially or completely inactivating effects, and to the increased instability of the mutated forms. Glycosylation confers stability to the protein, confirmed by MG132 and tunicamycin studies. Different degradation pathways could be involved depending on the degree of stability of the protein.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1550

### Detection of BclI and A3669G polymorphisms in glucocorticoid receptor gene in patients with rheumatoid arthritis

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Introduction
Rheumatoid arthritis (RA) is a chronic inflammatory disease which is characterized with dysregulation of the immune system. Recent studies suggest that dysregulation of the HPA axis may as well play a contributory role in the pathogenesis of RA. Glucocorticoid hormones (GC) accomplish their effects through binding to glucocorticoid receptor (GR). Presence of GR gene polymorphisms can modulate GC effects. The A3669G polymorphism in human GR gene has been connected to decreased and BclI polymorphism to increased sensitivity to GC.
Study population/methods
The aim of this study was to detect frequency of BclI and A3669G polymorphisms and find possible association between these polymorphisms in 40 patients with RA (34 women, mean age $50.85 \pm 9.31$; 6 men, mean age $49.33 \pm 15.72$) and 160 healthy volunteers (92 women, mean age $46.29 \pm 9.28$; 68 men, mean age $45.47 \pm 7.28$) with presence of RA.
Healthy volunteers were recruited from the National Institute for Blood Transfusion. All subjects underwent medical, hormonal, genetic and biochemical testing. DNA was obtained from peripheral blood leucocytes. The BclI and A3669G polymorphisms were detected by using PCR, RFLP and DNA sequencing.
Results
The BclI polymorphism was detected in 26 (65%) patients and 47 (28.13%) healthy subjects, $P < 0.001$. A3669G polymorphism was found in 15 (37.5%) patients and 14 (8.75%) healty volunteers, $P < 0.001$.
Conclusion
The presented results show an increased frequency of BclI and A3669G polymorphisms carriers between RA patients, comparing to healthy volunteers. This may point to a contributory role of GC sensitivity in the predisposition to develop RA.
Declaration of interest
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## P1551

### Aromatase activity after a short-course of Letrozole administration in adult men at sea level and in men at high altitude (with or without excessive erythrocytosis)

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Men living at high altitudes in Peru compared to sea level counterparts have erythrocytosis (Hemoglobin: 16–21 g/dl) or excessive erythrocytosis (Hemoglobin > 21 g/dl). High testosterone (T) levels in men at high altitude (HA) were associated with excessive erythrocytosis. High androgen levels could be due to a low aromatase activity or to an elevated rate of conversion from precursors to testosterone. The aim of this study was to evaluate aromatase activity and rate of conversion from precursors to testosterone before and after administration of the aromatase enzyme inhibitor letrozole (5 mg/day) for a 5-day period to men at HA and at sea level (SL). The response to short term aromatase inhibition was assessed in 30 adult men living at sea level, 31 native men at HA with erythrocytosis (Hb 16–21 g/dl) and 35 men at HA with excessive erythrocytosis (Hb > 21 g/dl). Serum hormone levels, estradiol/testosterone, testosterone/androstenedione, and testosterone/dehydroepiandrosterone sulfate (DHEAS) ratios were measured. Men with erythrocytosis had lower basal serum T/androstenedione ratios than men with excessive erythrocytosis at HA and men at sea level. Men at HA with excessive erythrocytosis had higher T/DHEAS ratios than men with erythrocytosis and than those at sea level before and after letrozole administration. After letrozole administration, both groups of men at high altitude (with erythrocytosis or with excessive erythrocytosis) showed lower aromatase activities than those at sea level. In conclusion, higher serum testosterone levels in men with excessive erythrocytosis were associated with an increased rate of conversion from DHEAS to testosterone rather than to a lower aromatase activity.
Declaration of interest
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## P1552

### Myostatin and Follistatin expression in orchidectomized rats submitted to exercise

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Myostatin (MSTN) is a negative regulator of skeletal muscle growth, while androgens are strong positive regulators of muscle growth and strength. Follistatin (FS) is a binding protein which inhibits MSTN action. Evidences suggest that MSTN and testosterone actions in muscle may be associated. However, the mechanisms of androgens actions are not fully elucidated. The objective of this study was to evaluate the influence of exercise training in the expression of MSTN and FS in orchidectomized rats submitted to exercise. Adult male Wistar rats were housed under controlled conditions (20–220 C, 12 h light-dark cycle) and were allowed free access to standard rodent chow. After 3 days of acclimation, rats were orchidectomized (O) or sham-operated (S). One group of animals was maintained intact (I). After 1 week, animals were randomly assigned to a training group (O-Train, S-Train and I-Train) or a sedentary group (O-Sed, S-Sed and I-Sed). O-Train, S-Train and I-Train were submitted to resistance training for 8 weeks. In the training protocol, animals climbed a 1.1-m vertical ladder with weights attached to their tails. The sessions were performed three times a week, with 4–9 climbs and 8–12 dynamic movements per climb. After this period, rats were decapitated and white gastrocnemius muscle were dissected, immediately frozen in liquid nitrogen and stored at −700 C for subsequent analysis. MSTN and FS mRNA was quantified by real time RT-PCR. The animals were maintained according to the local University Committee guidelines for the care and use of laboratory animals. $P < 0.05$ was considered statistically significant. MSTN mRNA expression was significantly higher in gastrocnemius muscle of O-Sed group than in I-Sed group. FS mRNA expression did not vary among the groups. The results indicate that MSTN expression increases in skeletal muscle of orchidectomized rats and is not influenced by resistance training.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1553

**The role of long-acting parenteral testosterone undecanoate compound (Nebid 1000 mg) in the induction of secondary sexual characteristics (SSC) in males with hypogonadotropic hypogonadism (HH)**

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Testosterone (T) can induce SSC in young HH men. So far the T ester formulations have been used for inducing puberty in these patients. Moreover, it seems that the polymorphism CAG length of androgen receptor (AR-CAG) might co-regulate the effectiveness of testosterone therapy (Tth).
This study aims to evaluate the effectiveness of Nebid (1000 mg) for SSC induction in HH men and, secondly, whether these changes might be modulated by the AR-CAG.
Nine HH male (aged > 17), in particular, 6/9 suffered from idiopathic HH while 3/9 were affected by b-thalassemia were enrolled in our study. All patients underwent a determination of serum SHBG, total T, free T (FT) and the AR-CAG. For treatment, HH men received an oral testosterone undecanoate (Andriol, 120 mg/day) for 3 months, followed by Nebid every 14 weeks for 1 year, then every 12 weeks for a second year. Serum T and FT were assayed 3 months after the start of Andriol and also in the 10th week following the start of the second round of Nebid. Levels were also determined 12, 18, and 24 months after the start of Nebid.
Circulating T and FT increased in all HH men which allowed them to develop SSC progressively. Moreover, a slight delay in puberty development was observed in those patients with > 24 AR-CAG compared with those with < 24 AR-CAG, suggesting that AR-CAG might co-regulate the effectiveness of Tth. Nebid was able to induce the puberty in our HH patients, although additional studies are needed to elucidate the possible role of AR-CAG for SSC development in HH men.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1554

**IL-6, Cortisol and early morning stiffness in patients with rheumatoid arthritis: endocrine changes associated with clinical improvement in a chronic autoimmune disease**

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Joint stiffness in rheumatoid arthritis (RA) is worse in the morning and has been associated with increased secretion of the pro-inflammatory cytokine IL-6 and in decreased secretion of cortisol, suggesting that clinical symptoms may be related to hormonal and immune circadian variations. We measured 24 h plasma profiles of IL-6 and cortisol in RA patients to determine any changes in IL-6 and cortisol following a two week course of prednisone administered orally in a specially designed timed-release tablet (TRT).
Nine patients with active RA were clinically assessed and had 24 h blood sampling before and after a 2 wk course of TRT prednisone (5 mg per day). Patients took the TRT orally at 2200 h and the prednisone was released at 0200 h. Changes in circadian variation in cortisol and IL-6 and clinical measures were compared using random coefficient regression modeling and Wilcoxon matched-pairs signed-rank test.
Significant alterations in circadian profiles and concentrations of IL-6 and cortisol were observed following TRT prednisone. The peak value of IL-6 fell from 42.5 to 21.3 pg/ml, and occurred earlier (0134 h compared to 0827 h) ($P < 0.005$). Following TRT prednisone, the peak value of cortisol increased from 14.1 μg/dl to 19.3 μg/dl and the trough fell from 2.9 μg/dl to 2.1 μg/dl ($P < 0.001$). There was a close correlation between reduction of IL-6 and improvement in morning joint stiffness following TRT. We propose that these changes in IL-6 and cortisol, prior to the onset of morning joint stiffness, are functionally important in mediating the improvement in joint stiffness following prednisone in RA patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1555

Abstract withdrawn

## P1556

**Fetal liver may reinstate activity of GABAergic steroids inactivated by placental oxidoreductases**

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Recently we have completed steroid metabolomic study including unconjugated ($n = 39$) and conjugated steroids ($n = 30$) in maternal and fetal body fluids using GC-MS method with two separate multicomponent analyses (for unconjugated steroids and steroid polar conjugates). Our previous data indicate that primarily oxidative isoforms of pluripotent 17β-hydroxysteroid dehydrogenases and aldoketoreductases in placental tissue close to the fetal compartment convert 3α-,17β- and 20α-hydroxy-groups in progestogens, estrogens and GABAergic steroids to the corresponding ketones. Therefore, GABAergic steroids are mostly inactivated when entering the fetus, which may negatively influence neuroprotective potential of these substances in the fetal brain. In this study we investigated the role of fetal liver in the reinstatement of the steroid GABAergic activity. The trial was approved by the Ethics Committee of the Institute of Endocrinology of Endocrinology, Prague, Czech Republic. In the study participated twelve women giving uncomplicated birth after the 38th week of gestation and 38 women with preterm labors (28th–37th week) who were selected to provide maximum conformity of the steroid metabolome with the actual GA. The umbilical arteriovenous differences evaluated by Wilcoxon's robust paired test as well as by multivariate statistics indicated that the activity of GABAergic steroids inactivated by placental oxidoreductases may be reinstated by liver enzymes, most probably from the aldoketoreductase family.
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## P1557

**Unconjugated steroids and steroid sulfates in maternal blood show comparable predictivity for assessment of gestational age from the 28th to 41st week of pregnancy**

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Recently we demonstrated a close correlation between the gestational age (GA) and steroid metabolome in maternal and fetal body fluids using multivariate models including both unconjugated ($n = 39$) and conjugated steroids ($n = 30$) that were measured by GC-MS method including two separate multicomponent analyses (for unconjugated steroids and steroid polar conjugates). We focused on the comparison of the predictive value of the levels of unconjugated steroids

alone, conjugated steroids alone and conjugated and unconjugated steroids together in maternal blood (as obtaining a maternal blood is the least invasive procedure). The trial was approved by the Ethics Commitee of the Institute of Endocrinology, Pague, Cech Republic. In the study participated 12 women (giving an uncomplicated birth after the 38th week of gestation) and 38 women with preterm labors (28th–37th week). The predictive value of the free steroids alone (75.2% of variability in GA is explained by the unconjugated steroids) was slightly better than that for the steroid polar conjugates alone (74.3% of the variability). The results for unconjugated and conjugated steroids together showed 81.0% of the variability. These data demonstrate that analysis of both unconjugated and conjugated steroids can be replaced by less laborious analysis of the unconjugated steroids only. Free steroids (showing significant predictive value) shared the variability with the GA as follows: 16α-hydroxy-DHEA =72.9%, 16α-hydroxy-estrone =69.3%, 5-androstene-3β,7α,3β-triol =40.7%, 16α-hydroxy-progesterone =37.1%, DHEA =36.7%, 16α-hydroxy-pregnenolone =35.1%, estriol =33.3%, estrone =30.5%, 7β-hydroxy-pregnenolone =29.6%, 7α-hydroxy-DHEA =25.6%, 5-androstene-3β,7β,3β-triol =23.7%, estradiol =21.9%, epipregnanolone =21%, 17-hydroxy-progesterone =20.9%, pregnanolone =20.2%, androstenediol =13.6%, 20α-dihydroprogesterone =7.2%, 20α-dihydropregnenolone =6.4%.

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## P1558

### Anabolic hormones in acute exacerbation of chronic obstructive pulmonary disease: relation of hypogonadism with indexes of severity and phlogosis

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Nowadays Chronic Obstructive Pulmonary Disease (COPD) is not considered a lung specific disease: nutritional alteration, weight loss due to increased energetic expenditure, loss of muscle mass are systemic effects, correlated with the risk of hospitalization due to exacerbation (AECOPD). AECOPD, characterised by augmented indexes like C reactive protein, interleukin-6, procalcitonin and serum amyloid A (SAA), negatively influenced natural history of the illness and were found to be related to muscle dysfunction. Hypogonadism could play a pivotal role.

Our study was aimed to evaluate possible relationships among prognostic indexes of AECOPD (represented by Acute Physiology and Chronic Health Evaluation, APACHE II Score), phlogosis (SSA), evaluated by immuno-electro-chemiluminescence, and hormonal axes involved in metabolic balance, insulin like growth factor (IGF-1), testosterone (T) and its metabolites, assayed by RIA. 24 patients, aged 75±13 years, 17 males, were studied. Data were not normally distributed, thus nonparametric statistics was applied.

Descriptive analysis showed reduced values of testosterone (T) (1.85 ±2.28 ng/ml), free-T (0.028±0.030 ng/ml), calculated by Vermeulen's formula, dihydro-T (0.18±0.19 ng/ml) and IGF-1 (91.84±74.19 ng/ml). Frequency distributions of Apache II score and SSA were calculated and, using tertile as cut off point, three categories were defined (SSA: ≤8 mg/ml, 9–160 mg/ml, ≥160 mg/ml; APACHE II: ≤10, 11–12; ≥12). Using this classification, an inverse correlation between SAA and T ($P$: 0.01), f-T ($P$ :0.01), DHT ($P$ :0.001) and IGF1 ($P$ :0.05) was found. Data showed the same inverse correlation between APACHE II tertiles on one hand and T ($P$ :0.01) and f-T ($P$ :0.02) on the other hand. No relationship was found between hormones and arterial blood gas analysis parameters.

Even if we cannot establish a causal relationship between hypogonadism and severity of the disease, our data suggest systemic effects of AECOPD and a possible mechanism explaining wasting syndrome in our patients

Declaration of interest

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## P1559

### Long-term evaluation of cross-sex hormone treatment in transsexual persons

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Objective

To describe the effects and side effects of cross-sex hormone therapy in both transsexual men and women.

Design

Single center cross-sectional study in one hundred transsexual persons post SRS and on average 10 years on cross-sex hormone therapy.

Methods

Hormone levels were measured by immunoassays. Physical health was assessed by physical examination and questionnaires on general health and specific side effects, areal bone parameters and body composition by Dual energy X ray absorptiometry (DXA).

Results

Transsexual men did not experience important side effects as cardiovascular events, hormone related cancers or osteoporosis. In contrast, a quarter of the transsexual women had osteoporosis at the lumbar spine and radius. Moreover, 6% of transsexual women experienced a thromboembolic event and another 8% experienced other cardiovascular problems after on average 11.3 hormone treatment years. None of the transsexual women experienced a hormone-related cancer during the treatment. Many transsexual men and women had several cardiovascular risk factors which could have affected future cardiovascular health.

Conclusion

Cross-sex hormone treatment appears to be safe in transsexual men. Transsexual women experience more tromboembolic and other cardiovascular events possibly related to life style factors, older age and estrogen treatment.

Declaration of interest

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## P1560

### Physiological relevance of peripherally produced gabaergic and glycinergic steroids in human late pregnancy and around parturition

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Although neuroactive steroids are well characterized, their physiological relevance in human pregnancy remains indecisive. Hence, we attempted to derive this information from the combination of our metabolomic data, pharmacological data found in the literature and knowledge contained in gene expression databases. These results indicate that GABAergic steroids influence maternal CNS but have limited effect in the fetal brain and maternal and fetal periphery, partly because of the low expression of steroid-sensitive GABAA-R subunits and partly due to lower GABAergic steroid levels in the circulation compared to the brain. Placental overexpression of steroid low-sensitive GABAA-R ε subunits may be ascribed to compensation effect providing a space for modulatory effects of non-steroidal GABAergic modulators in the tissue with excessive amounts of GABAergic steroids. A specific role of GABAergic steroids may be expected for the GABAA-R π subunit, which is preferentially expressed in the uterus and which decreases at the onset of labor. Metabolomic, tissue expression and pharmacological data also indicate that the mechanism suggested by some authors(who demonstrated inhibition of HPA axis by GABAergic steroids in the rat pregnancy), may be also effective in human. Rapid withdrawal from the excessive concentrations of GABAergic steroids postpartum indicate that the increased expression of steroid-less sensitive GABAA-R subunits is linked to the mechanism participating in the pathophysiology of postpartal depressions. In contrast to specific expression of steroid-sensitive GABAA-R subunits, the uniform expression of steroid-sensitive glycinergic subunits points to the neuro-inhibitory effect of pregnenolone in the CNS but neuro-excitatory effect of its sulfate in the pregnancy-related peripheral tissues like uterus, cervix and placenta.

The trial was approved by the Ethic Committee. Grants IGA NT/11513 and NT/12211 and advanced education of own staff in clinical and molecular endocrinology (CZ.2.17/1.1.00/32386) supported the study.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1561

### Influence of endogenous hormones and testosterone in the prostate cancer

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Introduction
Androgen stimulation of prostate cancer (PCa) cells has been the basis for extensive studies evaluating the role of androgen in PCa but the diagnostic measurement of androgen as well as androgen values that potentially influence prognosis are unclear in patients with PCa. The aim of this studies is to analyze the hormone behavior in the prostate cancer (PCa), as well as the relationship between PSA-testosterone and testosterone-aggressiveness tumor and compare these results with those obtained in patients with benign prostatic hypertrophy (BPH).
Methods/design
We performed an observational study of cases and controls, between January and October 2011. We selected 30 cases diagnosed with PC and 15 controls diagnosed with BPH. The concentrations of total testosterone, SHBG-testosterone, FSH, LH, Prolactin, Progesterone and Estradiol were measured in both groups. For statistical analysis we calculated the Pearson correlation test and Student t-test between variables in both groups, using the statistical package 17.0 SPPS.
Results
In the cases group it was found a negative correlation between PSA and testosterone levels ($P=0.03$), positive PSA with estradiol and progesterone ($P<0.01$), and positive relationship between LH and FSH ($P<0.01$). We did not found significant differences in hormone levels.
Conclusions
No significant differences were found between groups or between patients with PCa based on their aggressiveness, as well as significant correlations between the values within each group.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Thyroid (non-cancer)

## P1562

### Hypothyroidism is associated with increased intestinally derived lipoprotein particles and postprandial endothelial dysfunction

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Atheroma is accelerated in overt hypothyroidism (OH) and possibly in subclinical-hypothyroidism (SCH), and is not completely explained by increased LDL-cholesterol. There is little data available regarding post-prandial metabolic changes in OH or SCH.
Subjects with OH ($n=21$), SCH ($n=28$) and age, sex and BMI matched controls ($n=50$) were studied fasting and for up to 8-h following a mixed-meal. Investigations included apolipoprotein(apo)B48, a marker of intestinally-derived lipoproteins, and flow-mediated-dilatation (FMD) of the brachial artery, a measure of endothelial dysfunction.
Under fasting conditions, lipid variables and FMD did not differ between groups. Data from SCH subjects were similar to normal subjects and are not shown. Triglyceride concentrations increased similarly in all groups postprandially, while apoB48 levels increased more markedly and significantly in overtly hypothyroid subjects compared to fasting conditions. FMD decreased significantly postprandially in the OH group only. At the 4-h time-point, apoB48 and FMD differed significantly between OH and normal subjects.
Intestinally-derived lipoprotein particles are increased and endothelial function impaired post-prandially in overt but not sub-clinically hypothyroid patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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| | Controls fasting | Controls 4-h postprandial | Overt hypothyroid fasting | Overt hypothyroid 4-h postprandial |
|---|---|---|---|---|
| Triglyceride (mmol/l) | 1.39 | 2.27* | 1.25 | 2.23* |
| ApoB48 (mg/ml) | 11.9 | 17.6 | 15.4 | 25.6*,† |
| FMD (%change) | 5.31 | 5.28 | 4.46 | 3.64† |

*$P<0.05$ compared to fasting, †$P<0.05$ compared to controls.

## P1563

### IgSF1 gene defects in children with central congenital hypothyroidism and macroorchidism

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Central Congenital Hypothyroidism (CCH) is caused by an innate TSH deficiency. Known molecular bases of human CCH only involve mutations in TSHB and TRHR genes. Activins-Inhibins are a complex system of endo- and autocrine factors with opposing effects and important roles in pituitary and reproductive organs, but so far unreported actions on the thyroid axis. IgSF1 has been proposed as a membrane receptor for Inhibin-B.
Aim
To investigate the molecular cause of CCH-macroorchidism using genome-wide genetic techniques.
Patients and methods
Three patients from two pedigrees with X-linked CCH-macroorchidism were studied with complete hormonal profiles and clinical follow-up till completion of puberty. Comparative Genomic Hybridization (CGH)-arrays and Next Generation Sequencing were performed on patients DNA.
Results
CCH was diagnosed by T4-based neonatal screening or clinical hypothyroidism (sTSH:1.4-3.9 mU/L; FT4:7.2-8 pmol/L) and readily treated. Poor TSH response at TRH tests indicated pituitary hypothyroidism. Excessive testicular growth was detected from 3-6 years of age (3-5 ml Prader). GnRH test showed stimulation of FSH and LH, but discrepantly low testosterone levels. Normal puberty started at 12.5 years with initial testicular volume of 8 ml (N: 2), reaching 35-40 ml at the end of puberty (N: 20-25). Inhibin-B (425-500 ng/L; N:200-400) and Antimullerian hormone (19-48 μg/L;N:5-9) were markedly elevated, suggesting increased Sertoli cell mass. In Patient1, CGH-array showed a 200 Kb deletion of the entire IGSF1 gene. In Patients 2 + 3, massive sequencing of Chr.X revealed a missense mutation (p.C942R) in IgSF1 cosegregating with the phenotype in a large pedigree. Cys942 is structurally essential for the IX Immunoglobulin(Ig)-like extracellular loop of IgSF1.
Conclusions
IgSF1 is a novel candidate gene for central hypothyroidism. We propose IgSF1 defects may disturb pituitary and testicular functions through disruption of the counterbalancing effect of Inhibin-B over the Activin A-derived signaling pathways in thyrotropes, gonadotropes and/or Sertoli target cells.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1564

**Hypothalamic mTOR pathway mediates thyroid hormone-induced hyperphagia in hyperthyroidism**

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Hyperthyroid rats and humans exhibit increased energy expenditure and marked hyperphagia. Recent evidence has pointed that alterations of thermogenesis linked to hyperthyroidism are associated to dysregulation of hypothalamic AMPK and fatty acid metabolism; however, the central mechanisms mediating hyperthyroidism-induced hyperphagia remain largely unclear.
Objective
The aim of our study was to assess if alterations in feeding in hyperthyroidism are associated with impairment of hypothalamic mammalian target of rapamycin (mTOR) signalling.
Methods
We used adult male Sprague–Dawley rats. Hyperthyroidism was induced by chronic subcutaneous administration of L-thyroxine. Intracerebroventricular treatments and stereotaxic microinjection of $T_3$ and adenoviral expression vectors. Analysis by blood biochemistry, *in situ* hybridization, Real-time quantitative PCR, Immunohistochemistry and western blotting.
Results
Here, we demonstrate that hyperthyroid rats exhibit marked upregulation of the hypothalamic mammalian target of rapamycin (mTOR) signalling pathway associated with increased mRNA levels of agouti-related protein (AgRP) and neuropeptide Y (NPY), and decreased mRNA levels of proopiomelanocortin (POMC) in the arcuate nucleus of the hypothalamus (ARC), an area where mTOR colocalizes with thyroid hormone receptor alpha (TRα). Central administration of thyroid hormone ($T_3$) or genetic activation of thyroid hormone signalling in the ARC recapitulated hyperthyroidism-effects on feeding and mTOR pathway. In turn, central inhibition of mTOR signalling with rapamycin in hyperthyroid rats reversed hyperphagia and normalized the expression of ARC-derived neuropeptides, resulting in substantial body weight loss.
Conclusion
Overall, these data indicate that in the hyperthyroid state, increased feeding is associated with thyroid hormone-induced upregulation of mTOR signalling. Furthermore our finding that different neuronal modulations influence food intake and energy expenditure in hyperthyroidism pave the way for a more rationale design of specific and selective therapeutic compounds aimed at reversing the metabolic consequence of this disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1565

**Potassium iodide, but not potassium iodate, as a potential protective agent against oxidative damage to membrane lipids in porcine thyroid**

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Introduction
Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\prime}OH + OH^-$) is of special significance in the thyroid gland, as both its substrates, i.e. $H_2O_2$ and $Fe^{2+}$, are required for thyroid hormone synthesis. Also iodine, an essential element supplied by the diet, is indispensable for thyroid hormone synthesis. It is well known that iodine affects red-ox balance. One of the most frequently examined oxidative processes is lipid peroxidation (LPO), which results from oxidative damage to membrane lipids.
The aim of the study was to evaluate effects of iodine, used as potassium iodide (KI) or potassium iodate ($KIO_3$), on LPO in porcine thyroid homogenates under basal conditions and in the presence of Fenton reaction substrates.
Methods
Porcine thyroid homogenates were incubated in the presence of KI (0.00005–500 mM) or $KIO_3$ (0.00005–200 mM), without or with addition of $FeSO_4$ (30 μM) + $H_2O_2$ (0.5 mM). Concentration of malondialdehyde + 4-hydroxyalkenals (MDA + 4-HDA) was measured spectrophotometrically, as an index of LPO.
Results
Potassium iodide, used in the lowest concentrations ($\leq$0.05 mM), did not affect LPO; KI in concentrations of 0.1–1.0 mM, resulting in thyroid level of inorganic iodine close to that observed under normal iodine supply, clearly decreased the basal LPO; in the highest concentrations of 10–500 mM, KI increased LPO. At the same time, KI used in the middle range of concentrations (10–50 mM) reduced Fenton reaction-induced LPO. In opposite, $KIO_3$ revealed, depending on the concentration, either no protective effect at all or even strong prooxidative action.
Conclusion
Potassium iodide, used in doses generally recommended in iodide prophylaxis, may prevent oxidative damage to membrane lipids in porcine thyroid. Toxic effects of iodide overload may result from its prooxidative action. Potassium iodate does not possess any direct beneficial effects on red-ox balance in the thyroid, which constitutes an additional argument against its utility in iodine prophylaxis.
Declaration of interest
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## P1566

**Response to high dose glucocorticoid therapy in patients with dysthyroid optic neuropathy (DON)**

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Dysthyroid optic neuropathy (DON) is a sight threatening complication occurring in 3–5% patients with Graves' orbitopathy. The medical treatment consists in the infusion of high dose of methylprednisolone (MP), while surgical orbital decompression is mandatory in patients non responding to the medical treatment. We aimed at studying the response to high dose steroids in DON and parameters for predicting therapy effectiveness. Twenty-three patients with DON were studied by complete ophthalmological evaluation. Visual acuity, Hardy Rand Ritter (HRR) for colour vision defects, visual field and fundoscopy were carried out in all patients at baseline and 1, 2 and 4 weeks after therapy. Twelve patients were treated with 500 mg while 11 with 1000 mg MP for 3 consecutive days over 2 weeks. Fourteen non responders patients were eventually decompressed. A complete DON recovery was observed in 5 and 11 patients who received 500 mg and 1000 mg MP, respectively. No response to treatment was observed in all 5 patients (21%) who at baseline showed optic disk swelling, 3 of whom treated with the lower and 2 with the higher steroid dose. At one month of follow-up a significant increase of the visual acuity ($P < 0.004$), an improvement of the colour perception ($P<0.001$) and of the visual field (defect $P< 0.03$ and pattern standard deviation $P<0.01$) were observed in responders. Non responders showed a significant decrease of pattern standard deviation ($P<0.004$), but only initial improvement with subsequent worsening was observed in all other parameters studied. Our data show that high dose MP may be effective in restoring visual function in about 40% of patients with DON. The efficacy of treatment was not dependent on MP dose but on the severity of baseline optic nerve function impairment. Severe colour vision impairment and visual field defects are parameters predictive of medical treatment failure.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1567

**Effect of environmental nitrates on the thyroid and salivary glands in the population affected by the chernobyl fall-out**

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Introduction
An increase in thyroid diseases after the Chernobyl, mostly due to radioactive iodine, has been scientifically recognized. It is also known the effect of nitrate on iodine metabolism: being a competitive inhibitor of sodium-iodine symporter nitrate prevents iodide uptake by the thyroid and compromises thyroid hormone synthesis.
The aim of the study was to investigate the incidence of thyroid and salivary glands' abnormalities by ultrasound (US) screening according to drinking water nitrate concentration.
Methods
Thyroid and salivary glands US with the volume estimation was performed in 264 Belarusian subjects living in the areas of Brest region polluted by radionuclides after the Chernobyl accident. The participants average age was $21.0 \pm 4.5$ yr. The urine iodine concentrations and nitrate drinking water levels were measured.
Results
Median urinary iodine varied from 160 to 175 μg/l indicating no iodine deficiency. According to nitrate concentration (Median) in drinking water, the examined subjects were divided in three groups: 1 – Stolin-city (20 mg/l), 2 – Olmany village (334 mg/l), 3 – Olshany village (880 mg/l). Thyroid hyperplasia was detected in 3,8% of 157 subjects in the group 1; in 11,4% – in the group 2 (out of 79 persons) and in 17.9% – in the group 3 (out of 28 persons) ($P<0.05$). The salivary gland ultrasonic abnormalities were found more frequently in people in Olshany village (abnormal echogenicity with hyperechoic threads in 53.6% of cases vs 17.7% in the group 2 and 22.3% in the group 1, ($P<0.01$).
Conclusion
The villages Olmany and Olshany of Brest region may be considered the areas with high nitrate drinking water levels, according to WHO criteria. Unfavorable ecological conditions such as high drinking water nitrate levels may contribute to the thyroid and salivary glands abnormalities' development and potentiate relative iodine deficiency even in the areas with normal iodine supply.
Declaration of interest
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## P1568

**Quantitative comparison of deiodinase I and II mRNA expression in ovine tissues**

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Iodothyronine deiodinases are important enzymes for metabolism of thyroid hormones. These enzymes which are expressed in a variety of tissues are able to catalyze removal of iodine from thyroid hormones. Iodothyronine deiodinases I and II (DIO1 and DIO2) remove iodine from T4 to convert it to a more biologically active T3. The relative contribution of different tissue deiodinases to the establishment of a euthyroid state (proper production and concentration of T3) is not known. The objective of this study was to quantitate the amounts of transcription of DIO1 and DIO2 deiodinases in different ovine tissues. Using real-time reverse transcription polymerase chain reaction (RT-qPCR) and a comparative cycle threshold analysis, we found that DIO1 deiodinase is transcribed in skeletal muscle, kidney, and heart, more than thyroid, in diaphragm in quantities very similar to thyroid, and in liver, spleen, lung, and mammary gland lower than thyroid. We also found that the level of DIO2 transcription in all other tissues was lower than that in thyroid. Skeletal muscle, heart, and diaphragm transcribed between 84% to 87%; liver, kidney and lung transcribed between 62% to 72%; and spleen and mammary gland transcribed around 40% of DIO2 amount which is transcribed by thyroid. The biological significance of the relative expression of DIO1 and DIO2 enzymes in different ovine tissues awaits further studies. However in clinical settings, measurement of DIO1 and DIO2 expression in a given tissue may provide important clues on the intensity of selenium deficiency and its effects on the metabolism of thyroid hormones.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1569

**Correlation between thyroid volume and body mass index 2 and 6 years later**

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Background
Thyroid volume correlates positively with body mass index (BMI). This correlation holds true for both iodine-sufficient and mild/moderate iodine-deficient areas. We examined the association between thyroid volume and BMI and change in BMI over 4 years in middle-age adults recruited from the general population.
Methods
A total of 2495 subjects, for whom thyroid volume, $FT_4$ and TSH were available (women aged 35–60 years and men aged 45–60 years), were derived from the Supplementation en Vitamines et Mineraux Antioxydants (SU.VI. MAX) cohort study conducted in France since baseline (1994). Weight and height were measured 2 and 6 years after inclusion. Linear univariate and multiple regression analyses were performed to evaluate correlations between thyroid volume and BMI at 2 and 6 years and BMI change from year 2 to 6.
Results
Baseline thyroid volume was positively correlated with BMI at 2 years (men: $\beta=0.09$, $P<0.01$; women: $\beta=0.09$, $P<0.01$) and 6 years after inclusion (men: $\beta=0.10$, $P<0.01$; women: $\beta=0.09$, $P<0.01$). The correlation between thyroid volume and BMI at 2 and 6 years remained significant after adjusting for free $T_4$, TSH, gender, age, smoking, alcohol consumption and TSH-thyroid volume interaction factor ($\beta=0.11$, $P<0.01$). Baseline thyroid volume was not correlated with BMI change from year 2 to 6 in linear regression analysis.
Conclusion
In French adults, thyroid volume predicted BMI at 2 and 6 years. This association may be explained by the observation that leptin stimulates biosynthesis of TRH *in vitro*.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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Linear regression analysis of BMI 6 years after inclusion with baseline characteristics ($n=2413$)

| | Men | | Women | |
|---|---|---|---|---|
| **6 years** | $\beta$ | *P* value | $\beta$ | *P* value |
| Age (years) | 0.04 | 0.05 | 0.10 | <0.001 |
| TSH (mU/l) | 0.001 | 0.99 | −0.02 | 0.73 |
| Free $T_4$ (pmol/l) | −0.07 | 0.17 | 0.004 | 0.94 |
| Thyroid volume (ml) | 0.10 | <0.001 | 0.09 | <0.001 |
| Alcohol consumption (%) | 0.08 | 0.74 | 0.21 | 0.26 |
| Tobacco consumption (%) | 0.44 | 0.03 | 0.36 | 0.09 |

## P1570

**The relation between severity of obstructive sleep apnea and diagnosis of Hashimoto's thyroiditis**

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Background
We investigated the frequency for diagnosis of Hashimoto's thyroiditis (HT) in obstructive sleep apnea (OSA) patients, based on rising interest in potential links of OSA's pathogenesis with impaired auto-immune mechanisms.

Design and methods
Polysomnography was performed to 245 euthyroid eligible subjects consecutively. Study population divided according to apnea-hypopnea index (AHI) as control ($n=59$, AHI$<5$), mild OSA ($n=59$, $5\leq$AHI$<15$), moderate OSA ($n=61$, $15\leq$AHI$<30$) and severe OSA ($n=66$, AHI$\geq30$) groups. All subjects undergone thyroid ultrasound; serum anti-thyroglobulin (anti-TG) and anti-thyroid peroxidase (Anti-TPO) levels were measured for diagnosis of HT.
Results
Diagnosis of HT was significantly lower in control subjects (32%) compared to severe OSA patients (51%) ($P=0.02$). Among OSA patients the HT frequency was %42 and %46 for mild and moderate groups respectively ($P=0.05$). HT was detected in 62% of females, 29% of males and 43% of overall study population ($P<0.001$). While 73.3% of severe female OSA patients were found to have HT, the ratio was %18.7 in male control subjects ($P<0.001$). OSA patients had the highest antibody levels compared to controls; as 1000 U/ml versus 400 U/ml for Anti-TG, and 4490 IU/ml versus 650 IU/ml for Anti-TPO respectively ($P<0.001$). There was no significant correlation between thyroid volume and severity of OSA but isthmus thickness was significantly correlated to AHI ($P<0.01$, $r=0.22$).
Conclusions
OSA patients were found to have higher HT prevalence parallel to severity, especially among women. These results may lead to further investigations on potential casual relation between OSA and auto-immune thyroid diseases, besides may lead to development of screening schemas for OSA patients for early diagnosis of HT before development of hypothyroidism, which are clinically overlapping.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1571

**An audit and patient satisfaction survey of the telephone endocrine clinic at University Hospital Lewisham, London**

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Introduction
The telephone endocrine clinic (TEC) was established as an adjunct to the face-to-face endocrine clinic to follow and monitor the progress in a selected group of patients after they were seen in main endocrine clinics. This model of care has been tried successfully in other specialities but not as widespread in endocrinology. We audited the effectiveness of TEC and patient satisfaction. Methods: A retrospective analysis of records of all patients contacted in TEC in 2010 ($n=209$, 79% female). Six patients were excluded due to incomplete data. Mean age was 46.26years (range 20–85). Patient satisfaction was assessed through a postal questionnaire. Results: The 203 patients identified had a total of 735 telephone encounters (March 2005-July 2011). The mean telephone encounters were $3.62\pm2.22$ per patient (range 1–11). There were 203 failed telephone encounters ($1\pm1.09$ per patient, range 0–5). Conditions managed include: Graves' disease including during pregnancy (43%), other thyrotoxicosis (14%), primary hypothyroidism including during pregnancy (10%), post-radioiodine hypothyroidism (6%), prolactinoma (11%), post-thyroid/parathyroid surgery (4%), hypocalcaemia/hypercalcaemia/hypovitaminosis D (2%), testosterone replacement (2%). Fifty-five percent ($n=111$) of patients were discharged to GP at the end of follow-up and 10% ($n=21$) were referred back to the main endocrine clinics because of patients' choice or changes to their clinical status that could not be dealt within the TEC. Two patients (1%) were lost to follow-up. Eighty percent of patients surveyed were satisfied most or all of the time and 75% said that the TEC was convenient for them. Eighteen percent would have preferred a face-to-face consultation instead. Conclusions: This model of telephone consultation service is effective and convenient for patients. Our data shows that this model is a useful adjunct to the main endocrine clinic in selected cases, which would free up clinic slots to for new patients, decrease waiting time and manage more complicated cases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1572

**Gestational hyperthyroidism in women from mild to moderate iodine deficiency (ID) areas**

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Graves' disease (GD) and gestational transient thyrotoxicosis (GTT) are the main causes of hyperthyroidism in pregnancy, their prevalence ranging between 0.1–0.4% and 0.3–11%, respectively.
Aim of the study
To evaluate both prevalence and outcome of gestational hyperthyroidism in mild to moderate ID areas.
Subjects and methods
482 consecutive pregnant women who had never undergone thyroid function evaluation before and enrolled within 13 wks of gestation. Serum FT3, FT4, TSH levels were measured every 6 wks up to pregnancy term, while anti-TPO antibodies at enrollment only. Anti-TSH receptor antibodies (TRAb) were tested when TSH was below the lower limit of the trimester-specific reference range (1st 0.03 mU/L; 2nd and 3rd 0.3 mU/L).
Results
Thirty-two/482 (6.6%) pregnant women had serum TSH values $<0.03$ mU/L at first sampling. Of these, 17/32 (53.1%) were overtly hyperthyroid, and 15/32 (46.9%) had subclinical hyperthyroidism. TRAb assay revealed the autoimmune origin of hyperthyroidism in 6/32 (18.7%) women. Of the remaining 26/32 (81.2%) TRAb-negative women, 12/26 (46.1%) were diagnosed with GTT and 14/26 (53.8%) showed isolated hypothyrotropinemia. Of the 6 GD women, 1/6 spontaneously recovered from hyperthyroidism at early 2nd trimester, whereas 2/6 were given anti-thyroid drugs, and 3/6 showed FT4 levels that were consistently at the upper normal limit for general population. TRAb titer decreased of 33–82% over gestation in 4/6, and became undetectable in the remaining 2/6 GD women. Of the 12 GTT women, 1/12 miscarried at early gestation. In the remaining 11/12, spontaneous remission of hyperthyroidism occurred, mostly within the 20th week.
Conclusion
The high prevalence of autoimmune hyperthyroidism (6/482, 1.2%), occasionally found in pregnancy, represents a further reason for recommending systematic and early thyroid function testing in pregnant women.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1573

**The association between thyroid volume, L-thyroxine therapy and hepatocyte growth factor levels in euthyroid and hypothyroid goitrous and non-goitrous Hashimoto's thyroiditis**

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Background and aim
Hashimoto's Thyroiditis pathogenesis remains unclear in spite of many factors currently suggested. Aim of the study was to explore the relationship between hepatocyte growth factor (HGF) and Hashimoto's Thyroiditis (HT), goiter formation, and to see if HGF levels are adjusted by TSH suppression.
Materials and methods
Sixty-one premenopausal women who admitted our department between November 2010-September 2011 were enrolled in the study. Three groups were determined according to their thyroid function tests (TFTs). (Euthyroid Hashimoto's, control and subclinical Hashimoto's groups; *n*: 26, 23, 12 respectively). Basal TFTs and lipit profiles, Anti-TPO, Anti-Tg, thyroid USG and HGF were performed. Subclinical hypothyroid group received levothyroxine sodium replacement therapy and was re-assessed for the same laboratory and radiologic features after a median 3,5 months of follow-up.
Results
The main age of patients were $26.39\pm5.7$. Statistical analysis showed no relationship between HGF and other parameters. In the subclinical hypothyroidism group pre- and post-treatment HGF change was statistically insignificant (*P*:0.496). Basal HGF did not differ between groups (*P*: 0.504).
Conclusion
In contrast with the existing literature, HGF level was not found different between patients diagnosed with Hashimoto's Thyroiditis and control group, also no

difference was shown whether nodular goiter is present or not. L-$T_4$ replacement therapy did not alter HGF levels.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector

## P1574

**Adaptation of the hypothalamo-pituitary-thyroid axis to pubertal development and exposure to acute and chronic physical stresses**

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The hypothalamo-pituitary-thyroid axis undergoes adaptive changes in response to increased energy expenditure at puberty, a process called thyroidarche. Furthermore, the secretion of thyroid hormones (THs) is influenced by various kinds of acute and chronic stresses. Amongst others, persistent and severe physical stress may affect the secretion of THs. The present study investigates changes in the secretion of thyrotropin releasing hormone (TSH), trioiodothyronine (T3) and tetraiodothyronine (T4) during puberty and the effect of acute and chronic physical stresses on the secretion of TSH, T3 and T4. Blood samples were obtained from non-working school/college going and working boys between the age of 10 and 20 years ($n=594$), plasma concentrations of TSH, T3, T4 and cortisol were determined using specific ELISA. Data were analyzed using Student's *t*-test, ANOVA and Pearson correlation. The secretion of TSH peaked at 14th year and was maintained until 18th year in non-working boys. In working boys, TSH peaked at 11 years and was maintained by 18th year. The concentrations of T3 increased progressively until 17 years. In working boys, T3 peaked at 10, 16 and 20 years. T4 concentrations increased significantly at 11, 15 and 17 years. In working boys, T4 peaked at 10, 13 and 18 years. In non-working boys, the concentrations of TSH and T3 were lower at early puberty and higher at mid and late puberty, whereas concentrations of T4 were higher at early and mid puberty. In both groups, a positive correlation was seen between plasma TSH and T3 concentrations and linear growth velocity during early and mid puberty. The concentrations of cortisol were significantly higher in working boys of all age groups. In conclusion, the present study demonstrates that the secretion of TSH and T3 increases at puberty and that acute stress increases TSH and T3 concentrations whereas chronic stress reduces them.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1575

**Thyroid nodules treated with percutaneous radiofrequency thermal ablation: a comparative study**

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Introduction
Percutaneous radiofrequency thermal ablation (RTA) is a new promising therapeutic approach to manage compressive thyroid nodules (TNs).
Aim
To investigate effectiveness and safety of RTA in the treatment of compressive TNs in patients not receiving surgery or radioiodine.
Study design
Forty patients (31–86 years) with compressive TNs were enrolled. Twenty-two patients had non-toxic TNs and 18 had toxic TNs and were treated with methimazole. Patients were randomized in two groups: group A (20 patients: 12 non-toxic, 8 toxic TNs) were treated with RTA; group B (20 patients: 10 non-toxic, 10 toxic TNs) did not receive any treatment. There was no significantly different characteristics between groups. RTA was performed by using a RITA© Starburst needle under ultrasonographic guidance. All patients were clinically, biochemically and morphologically evaluated 1, 3 and 6 months after baseline.
Results
At baseline, TN volume was $13.3\pm8.0$ ml in group A and $11.3\pm6.9$ ml in group B ($P=$NS). After treatment, TN volume significantly decreased in group A ($P<0.0001$), both in patients with non-toxic TNs and in those with toxic TNs, while remained stable in all patients of group B ($P=$NS). At 3 and 6 month evaluation, TN volume was significantly lower in group A than in group B ($P<0.0001$). At the end of the follow-up, pressure symptoms were improved in all patients of group A while persisted unchanged in group B. In the subgroup of patients with toxic TNs, hyperthyroidism completely recovered in 40% and improved in 40% of group A, while it persisted unchanged in all patients of group B. RTA was safe and well tolerated in all patients.
Conclusions
RTA significantly decreases toxic and non-toxic TN volume, resulting in parallel improvement of pressure symptoms and hyperthyroidism. RTA represents a valid therapeutic approach in patients with TNs not receiving conventional treatments.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector

## P1576

**Predictors of long-term remission in patients with Graves' disease-a single centre experience**

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Introduction
Antithyroid drugs (ATD) remain the first-line therapy in patients with Graves' disease (GD), despite high relapse rate. The purpose of the present study was to identify predictors of long term remission of GD in patients who received ATD as primary treatment.
Description of methods/design
We restrospectively identified patients with Graves' disease initially treated with ATD between 1984 and 2011 and extracted relevant data from the electronic records database. Patients with follow-up data of $>6$ months after ATD discontinuation were included.
Results
Two-hundred and eleven patients (mean age $47.2\pm13.8$ years, 44 males) were studied. One-hundred and thirty-five (64%) were treated with ATD only, 82 of which (61%) maintained remission during a follow-up of $56.6\pm57.5$ months. Mean duration of first ATD therapy was $38.6\pm26.3$ months.
Females ($P=0.049$), non-smokers ($P=0.017$), patients without orbitopathy ($P=0.033$) and those who developed hypothyroidism during therapy ($P=0.018$) experienced longer remission.
Duration of remission was positively associated with ATD therapy duration (rs$=0.151$, $P=0.03$), maximum TSH levels during ATD therapy ($rs=0.241$, $P=0.001$), TSH levels at the end of treatment ($rs=0.280$, $P<0.0001$) and at 3 months after ATD discontinuation ($rs=0.341$, $P=0.003$). There was a negative association with FT4 ($rs=-0.426$, $P<0.0001$) and FT3 levels at 6 months after ATD discontinuation ($rs=-0.467$, $P=0.038$). Age, block-and-replace therapy, type or dose of ATD were not associated with longer remission.
Conclusions
Female gender, non-smoking status, absence of orbitopathy and occurence of hypothyroidism during therapy were favorable predictors of remission in patients with GD treated with ATD. Longer duration of therapy, higher TSH levels during, at the end and at 3 months after ATD discontinuation, lower FT4 and FT3 levels at 6 months after ATD discontinuation were associated with longer remission.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1577

**The effect of L-thyroxine substitution on lipid profile, glucose homeostasis and coagulation in patients with subclinical hypothyroidism**

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Introduction
Subclinical hypothyroidism (SH) is associated with increased risk for atherosclerosis, mainly attributable to dyslipidemia, diastolic hypertension, altered coagulability and increased levels of inflammation markers. However, conflicting data exist regarding the effect of L-thyroxine substitution on these parameters.
The purpose of the present study was to quantify the effect of L-thyroxine therapy on lipid profile, coagulation markers, high-sensitivity C-reactive protein (hsCRP) and glucose homeostasis in patients with SH.
Description of methods/design
It was a comparative, prospective study conducted in the department of Endocrinology at a tertiary medical centre of northern Greece from November 2009 to November 2011. Patients with diabetes mellitus, cancer, renal, liver failure or receiving drugs affecting lipid metabolism or coagulation, were excluded.
The following parameters were measured before and 6 months after restoration of euthyroidism with increasing doses of L-thyroxine: anthropometric data, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), apolipoprotein B (apoB) and A1 (apoA1), lipoprotein (a) [Lp(a)], fasting plasma glucose and insulin, homeostasis model assessment-insulin resistance (HOMA-IR), hsCRP, antithrombin III (AT-III), protein C (PC), protein S (PS), fibrinogen and homocysteine.
Results
Thirty-two patients (30 women) aged $52.1 \pm 13.9$ years with SH completed the study. Mean TSH levels at baseline were $6.79 \pm 2.58$ mIU/ml. L-thyroxine and achievement of euthyroidism significantly reduced systolic blood pressure (BP) in patients with SH (from $135.2 \pm 18.5$ to $129.7 \pm 15.8$ mmHg, $P = 0.03$) and diastolic BP only in those with baseline TSH levels $>7$ mIU/ml (from $79.5 \pm 9.8$ to $72.1 \pm 7.3$ mmHg, $P = 0.03$).
No significant changes in body weight, waist or hip circumference, TC, LDL-C, HDL-C, TG, apoB, apoA1, Lp(a), glucose, insulin, HOMA-IR, hsCRP, AT-III, PC, PS, fibrinogen or homocysteine levels after restoration of euthyroidism.
Conclusions
Except for reduction in systolic and diastolic BP, thyroid substitution therapy does not affect lipidemic profile, systematic inflammation, glucose homeostasis or coagulation in patients with SH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1578

**A novel familial variation of the thyroid hormone receptor beta gene (I276N) associated with resistance to thyroid hormone**

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Introduction
Resistance to thyroid hormone (RTH) is a dominantly inherited syndrome, first identified in 1967 by Refetoff, characterized by reduced organ responsiveness to thyroid hormone. The most typical pattern being an elevated serum free T4 (fT4) concentration in association with non-suppressed TSH, usually accompanied by high serum levels of free T3 (fT3), with a considerable inter-individual variation and heterogeneous phenotype. The most frequent defect which causes RTH involves the thyroid hormone receptor beta gene (THRB) located on chromosome 3.
Methods
Genomic DNA was isolated from peripheral blood. Molecular analysis of THRB gene was performed. Exons 7 through 10 and their flanking intronic regions were amplified by PCR and the products were analyzed by direct sequencing.
Results
Molecular analysis of THRB gene identified a novel missense variation in exon 8 in the heterozygous state (I276N). The patient was a 3-year-old girl referred to our Pediatric Endocrinology Unit for statural and ponderal growth retardation. She presented elevated thyroid hormone levels with unsuppressed TSH, in presence of an unusual positivity to anti-thyroperoxidase antibodies for age. The child's mother, a 42-year-old women, reported from 25-years of age a history of tachycardia, high stool frequency and goiter, elevated fT3 and fT4 with normal TSH, increased levels of anti-thyroperoxidase and anti-thyroglobulin antibodies and a thyroid ultrasound and cytological pattern suggestive for lymphocytic thyroiditis. The same THRB variation was identified also in the mother in the heterozygous state.
Conclusion
We described the case of a child and her mother affected by RTH presenting with different clinical phenotype even if they carried an identical novel THRB missense variation. In both of them RTH was associated with the presence of anti-thyroid antibodies. RTH should be suspected every time elevated thyroid hormones and unsuppressed TSH are found, independently of the clinical phenotype, even in association with an autoimmune thyroiditis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1579

**Short-term combined treatment with L-thyroxine plus L-triiodothyronine in drops (LT4 plus LT3) in patients with persistent hypothyroidism and malabsorption during replacement therapy with L-T4.**

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The objective of our study was to evaluate the potential benefit of short-term combined treatment with LT4plusLT3 in patients with persistent hypothyroidism and malabsorption. We report ten cases of patients with hypothyroidism secondary to total thyroidectomy, in which high doses of L-T4 (2,7-3.5 mcg/kg/die) were not adequate to normalize thyroid function. TSH was persistently high (from 10 to 100 mU/L) with low serum levels of thyroid hormones.
All of the patients presented severe symptoms of hypothyroidism and did not have history of recent intake of drugs interfering with gastric function or history of previous gastric surgery, other disorders or alcohol abuse.
Anti-endomysium and anti-tissue transglutaminase tests, lactose hydrogen and 13C-Urea breath test, parasitic search in the stool were performed in all of the patients. Gastric endoscopy was necessary in few patients. An Helicobacter pylori infection was found in 4 of the patients. One patient presented a gastroesophageal reflux associated with congestive gastropathy, whereas lactose intolerance was found to be responsible for the malabsorption in 2 patients. In 3 of the patients the cause of malabsorption was not identified.
Combined therapy with L-T4 and L-T3 (45 drops of LT4 and 30 drops of LT3 mean dose) was started in all of the patients to improve the severity of hypothyroidism. This combined treatment was able to normalize serum TSH, improving the symptoms of hypothyroidism in all of the cases in about two weeks. TSH values progressively normalized, and L-T3 administration was withdrawal. Specific treatment for the causes responsible for malabsorption was contemporary performed and we were able to shift treatment to L-T4 in tablets.
Our results support the potential benefit of combined treatment with LT4plusLT3 in patients with persistent hypothyroidism due to malabsorption.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1580

**The role of surgery in the treatment of amiodarone-induced thyrotoxicosis (AIT)**

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Introduction
AIT develops in 15–20% of patients receiving amiodarone. Patients with persistent thyrotoxicosis or those with instable cardiac function, may be candidate

for thyroidectomy; however, thyrotoxicosis and underlying cardiovascular disease may increased surgical risk.
Matherials and methods
We retrospectively selected 23 AIT patients treated with a total thyroidectomy, from January 2000 to December 2010 [19 men, 4 women; mean (+SD), age 61.4±9.4 years, range, 46–79 years]. Nineteen patients have been operated, during hyperthyroidism, after a short course of iopanoic acid (1 g/die for 15.2 ±7.7 days).
Results
No death occurred during surgery; in addition no excessive bleeding or intraoperative arrhythmic event occurred. One subject presented atrial fibrillation 24 hours after surgery, requiring a short course of sotalol, with reinstitution of sinus rhythm. One patient experienced hypoparathyroidism and one experienced laryngeal nerve injuries. No deceased after a 12-month follow-up. Impaired cardiac conditions, with ejection fraction (EF) < 35% (mean 27±6.7%), was observed in 8 patients before surgery. In these patients a significant EF improvement (mean 37.2±8.38%, $P=0.05$) was observed 60 days after surgery and reinstitution of euthyroidism
Conclusions
Total thyroidectomy is a valid option for treating AIT patients that require a rapid restoration of euthyroidism, which is associated with improvement of cardiac function.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1581

### Carbimazole induced ANCA associated renal vasculitis

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A 22-year old female diagnosed with thyrotoxicosis with raised free thryroxine (16.8 pmol/l) and suppressed thyroid-stimulating-hormone (TSH < 0.03 mIU/l). She was started on Carbimazole 20 mg during end of August 2011. A fortnight later she presented with nausea, vomiting, fever and rigors. Her renal functions were deranged with a creatinine of 704 μmol/l, which worsened over the next 48 h. She subsequently needed renal replacement therapy with haemodialysis. She was cytpolasmic anti-neutrophil cytpolasmic antibody (c-ANCA) positive with raised proteinase-3 (PR3) titers (28AU/ml) (see Table). She was negative for anti-thyroid stimulating hormone (TSH) receptor antibodies, anti-glomerular basement membrane (GBM) antibody and for anti-nuclear antibodies with normal complement levels.
It was proposed, perhaps this could be drug-(carbimazole)-induced renal vasculitis with positive c-ANCA; for which carbimazole was stopped and a renal biopsy revealed concentric necrotizing-glomerulonephritis consistent with c-ANCA-positive-vasculitis. She was further treated with high dose prednisolone and cyclophosphamide following which her renal functions improved and she was weaned-off haemodialysis; subsequently her thyroid function tests also normalized without any further anti-thyroid therapy.
Conclusion
Our case report re-iterates that anti-neutrophil-cytoplasmic-antibody-(ANCA)-associated-vasculitis is a potentially life-threatening adverse effect of anti-thyroid drugs like carbimazole. Altered immune environment associated with auto-immune thyroid disease is not sufficient to develop ANCA but treatment with thionamides (which accumulate in neutrophils) is important in promoting ANCA

Investigations

| **Test** (units) | **Pre-treatment** | **Post-treatment** |
|---|---|---|
| Urea (mmol/l) | 20 | 13.5 |
| Creat (umol/l) | 704 | 173 |
| c-ANCA (Au/ml) | Positive | |
| PR3 (Au/ml) | 28 | 2 |
| TSH (mIU/l) | <3.03 | 0.87 |
| Free $T_4$ (pmol/l) | 16.8 | 7.1 |
| MPO (Au/ml) | <7 | |
| TSH receptor antibody (IU/ml) | <1 | |
| TPO antibody (IU/ml) | <60 | |

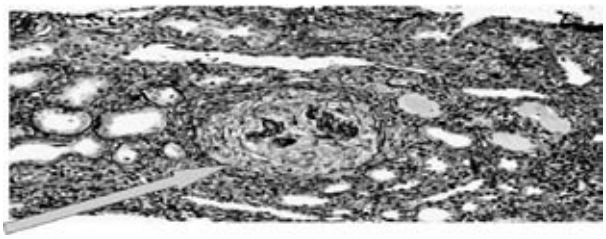

**Figure 1** Renal biopsy (with silver stain) with glomerulus showing fibrinoid necrosis of the glomerular tuft surrounded by a cellular crescent.

development. There is only limited literature available describing carbimazole-induced-ANCA-positive renal vasculitis; and as in other case reports, renal function improved after stopping carbimazole and immunosuppressive therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1582

### Thyroid dysfunction following cranial and craniospinal radiation in young adults

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Purpose
Radiation-induced anterior pituitary hormone deficiencie is the most common long-term complications of successful cancer treatment. The aim of our study was to analyze thyroid status in young adults after radiotherapy in childhood and their relationship with dose of radiotherapy.
Materials and methods
Thyroid function (TSH, free T4, free T3) and thyroid ultrasound were evaluated in 29 young adults with a history of radiotherapy for brain tumors (BT) and acute lymphoblastic leukemia (ALL) in childhood:
Group 1(lower irradiation dose cranial irradiation 18 Gy): 2 men and 10 women (median age 22.5 yrs (range 16–30)), 5.9±3.4 years after treatment for ALL. All patients received chemotherapy BFM-90.
Group 2(higher irradiation dose, craniospinal irradiation 55 Gy): 7 men and 10 women (median age 21.1 yrs (range 15–26), 4.4±2.1 years after treatment for brain tumors (BT). All patient received chemotherapy M-2000.
Results
One patient from group 1 had one thyroid nodule (FNA – follicular neoplasm, postoperative hystology of follicular adenoma). No patient had signs of hypothyroidism (mean TSH was 1.28 mME/ml (0.5–1.88), fT4 – 12.82 pmol/l (11.1–15.4), and fT3 – 10.03 pmol/l (7.1–15.0)).
Three patients (20%) from group 2 presented signs and symptoms of central hypothyroidism (fT4 5.2 (4.1–6.3)). They received substitution with L-thyroxine. Thyroid nodules were found in 2 patients (both colloid goiter after FNA). Mean TSH value (exept for three cases of central hypo) was 2.53 mME/ml (1.32–4.53), mean fT4 – 10.17 pmol/l (5.9–19.8), and mean fT3 – 6.22 pmol/l (4.1–13.0). The study is in progress.
Conclusions
These data indicate that treatment of cancer in childhood is associated with development of thyroid abnormality later during the life, and there is a possible link between exposure to higher total radiation dose and incidence of hypothyroidism.
Declaration of interest
I fully declare a conflict of interest. Details below:
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## P1583

**Multi nodular goitre: when is it time for the surgeon?**

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Introduction
Multi Nodular Goitre(MNG) has a slow, progressive growth. An apparently asymptomatic MNG can already have caused retrosternal upper airway obstruction. Yet MNG can also cause compressive manifestations without retrosternal extension. Timing of surgery can be critical for patients with a large goitre. The evidence is poor to guide management of large MNG with absolute indications for surgery. The aim of this uni-centre study is to analyze patients with large MNG and their surgery results.
Methods
We retrospectively searched the surgical database of the Academic Medical Centre for patients who underwent a Thyroid operation, between September 2009 and September 2011, because of large MNG. We analyzed patient characteristics, surgical aspects, and complications post-operative.
Results
120 patients (age $\geq$ 16 years) underwent thyroid operation during Sept. 2009 to Sept. 2011.
Forty-five (38%) had MNG with a preoperative contents of $\geq$80cc or post-operative specimen weight of $\geq$80 gram. Patients were 80% female and 20% men, mean age 52 years (range 16–81 years). Duration of the MNG was between 4 months to 30 years. All patients had complaints of their MNG, 24 (53%) experienced dyspnoea. Sixteen (36%) of the patients had retrosternal MNG. Twenty-one (47%) patients had tracheal deviation and obstruction. Seven patients underwent awake fiberoptic intubation. In one case tracheostomy was necessary. Complications occurred in 13(29%) of the MNG group, compared to 10(15%) in the smaller thyroid group. Mortality was 4% (2/45). One patient had an anaplastic carcinoma (MNG 150 cc) and one patient (MNG 200 cc) had an inhospital cardiac arrest 15 days postoperatively. In the smaller thyroid group 1 patient, with an Amiodarone-induced Thyrotoxicosis, died after an inhospital cardiac arrest.
Conclusion
Resection of large goiters is associated with a higher complication rate. Therefore we conclude that earlier referral to a thyroid surgeon might influence the operative results.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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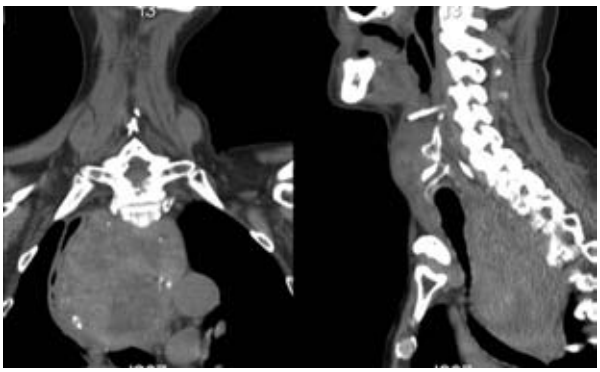

## P1584

**Amiodarone- induced thyroid dysfunction in an iodine- deficient region**

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Objective
To determine the incidence of thyroid dysfunction in a cohort of patients started on amiodarone therapy at a cardiac clinic in Tashkent, Uzbekistan, which is believed to be an iodine- deficient area.
Methods
Pharmacy records were used to obtain the names of patients who received amiodarone between 2006 and 2009.
Results
The original sample size was 96; 3 patients were excluded to incomplete data and data analysis was completed for 93 patients (21 women and 72 men).
The mean age was $59 \pm 15$ years (range, 22–89). The indication for amiodarone therapy were supraventricular tachycardia ($N=64$, 68.8%), ventricular tachycardia ($N=22$, 23.6%), and prophylaxis against tachycardia ($N=7$, 7.5%). The median duration of amiodarone treatment was 679 days (range, 3–6425 days) in the entire cohort. The median duration of amiodarone therapy in euthyroid patients was 547 days. The median duration of amiodarone therapy until thyroid disorder developed was 943 days. This was a significantly longer time period compared with euthyroid patients ($P=0.05$).
Discussion
There were 24 (25.8%) new thyroid dysfunction cases; 18 patients (19.3%) hadthyrotoxicosis, 1 (0.6%) had subclinical hyperthyroidism, 4 (4.3%) had hypothyroidism, and 1 (1.1%) had minor changes in thyroid function.
Conclusions
We found a high incidence of new-onset thyroid dysfunction, similar to the highest rates reported by other investigators. Any additional etiological factors responsible for this in Uzbekistan need to be investigated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1585

**Hashimoto thyroiditis and carbohydrate metabolism disorders in patients hospitalized in Department of Endocrinology and Diabetology of Ludwik Rydygier Collegium Medicum in Bydgoszcz between 2001 and 2010**

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Chronic lymphocytic thyroiditis, also known as Hashimoto thyroiditis, is the most frequent type of thyroiditis. An average 2% of the population suffer from the disease. The incidence of the disease is estimated as 0.3–1.5/1000 people a year. The main cause of the disease includes autoimmune disorders, that result in increased risk of diabetes type 1. Furthermore, during the course of Hashimoto thyroiditis, hypothyroidism may originate carbohydrate metabolism disorders, which lead to the increase of insulin resistance.
We examined 54 patients (45 females and 9 males at the age of 17–87) with the diagnosis of Hashimoto thyroiditis based on clinical picture and examination (autoantibodies anti-TPO and anti-Tg). The patients have been hospitalized in the Department of Endocrinology and Diabetology Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz between 2001 and 2010.
In the tested group with Hashimoto thyroiditis, diabetes was confirmed in 27.8% of the patients; impaired fasting glycaemia (IFG) or impaired glucose tolerance (IGT) occurred in 16.6%, whereas normoglycaemia was confirmed in 55.6% of the patients.
It has been proved there is a connection between the incidence of either hypothyroidism or hyperthyroidism and carbohydrate metabolism disorders. The abnormalities of glycaemia levels are directly proportional to the hormone disorders of the thyroid gland.
Carbohydrate metabolism disorders in the form of type 1 diabetes connected with an autoimmune process, as well as type 2 diabetes connected with the increase of the insulin resistance, occur in average of half of the patients with Hashimoto thyroiditis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1586

**Endocrine bioengineering: reconstruction of a bioartificial thyroid lobe using its natural, three-dimensional (3D) stromal / vascular matrix as a scaffold**

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To test the feasibility of reconstructing *ex situ* an entire bioartificial thyroid gland suitable for transplantation we have bioengineered a rat thyroid lobe using its decellularized stromal / vascular matrix, eventually 3D recellularized with thyroid stem / precursor cells. Sprague-Dawley male rats (220–240 g) were used as thyroid donors, and lobe matrixes obtained by freezing / detergent / enzyme processing. Test matrixes were made electrondense and analyzed by microtomography (microTC). Primary thyroid cells and ABCG2-positive, thyroid stem/precursor elements were expanded and isolated either in primary monolayer or 3D matrigel cultures for 72 h, using low-glucose DMEM and high vs low serum media. Following trypsinization, 250–450.000 cells were harvested to coat the empty follicular and vascular cavities of the inner matrix surface, and grown up to 21 days in static conditions. The colonized matrixes were either fixed in aldheydes for processing by light (LM), transmission (TEM) and scanning electron (SEM) microscopy or denaturated to get total proteins, and run for ABCG2 westen blotting. Culture supernatants were collected every 48 h, and free thyroid hormone levels assessed with chemiluminescent immunoassays. Complete decellularization and maintenance of the 3D native architecture of the thyroid SVS were achieved. Thyroid-derived cell, including differentiated thyrocytes, elements showing epithelial-mesenchymal transitions, and stem/precursor cells were found both to heterotopically migrate inside matrix septa and to orthotopically aggregate, link and give rise to intracytoplasmic cavities, up to recellularize the decellularized follicular spaces. Thyroid hormone secretion occurred for at least 7 days. These results show that the natural 3D matrix of the rat thyroid acts as a scaffold to bioengineer *ex situ* a functional thyroid lobe with progenitor-like elements (Fig.), suggesting that a biocompatible construct can be realized for eventual transplantation replacement. Grants FIL09, PRIN082008ZCCJX4, FIRB2010R-BAP10MLK7

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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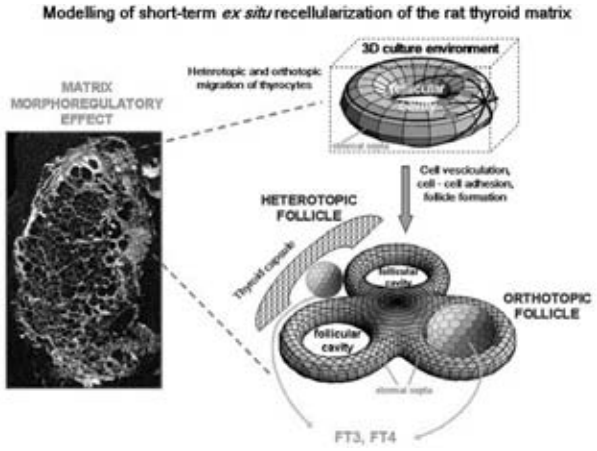


## P1587

**Thyroid hormone stimulates hepatic fatty acid metabolism via lipophagy**

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Autophagy is a catabolic process that promotes hepatic cell survival during starvation and metabolic stress. Currently, little is known about the endocrine regulation of autophagy. Recent studies by Singh et al. (Nature 458, 1131–1135 (2009)) showed a critical link between autophagy and beta oxidation in hepatocytes. Accordingly, we examined whether hormones known to promote beta oxidation of fatty acids also stimulated autophagy in hepatic cells. Surprisingly, we found that T3 stimulated autophagy in human hepatic cells *in vitro* and in mouse liver in vivo. In particular, T3 increased LC3 II and decreased p62 protein expression levels in HepG2TRalpha cells and hepatocytes. Moreover, studies using chloroquine treatment to block autophagsome/lysosome fusion and microscopic localization of RFP-GFP flurorescent tagged LC3 both demonstrated that T3 increased overall autophagic flux. Bodipy-staining and electron microscopy studies of hepatic cells showed that T3-induced autophagy remarkably involved formation of lipophagosomes that ingest lipids from fat droplets and fuse with lysosomes before fatty acid delivery to mitochondria. This T3-mediated lipophagy was also associated with increased beta-oxidation as T3 increased Cpt-1$\alpha$ expression. In this connection, knockdown studies using ATG5 siRNA demonstrated that T3-dependent beta oxidation of fatty acids was dependent upon autophagy. Our findings show that T3 plays a critical role in the regulation of fatty acid metabolism by co-ordinately inducing lipophagy and beta-oxidation. They also suggest that T3 or its analogs may be useful for the treatment of NAFLD, a common condition with high morbidity caused by excess fat accumulation in the livers of patients with obesity and diabetes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1588

**Linking thyroid and central nervous system functions of cathepsin K: Implications for non-classical thyroid regulation**

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Cathepsin K is a cysteine protease known for its importance in bone remodelling. Inhibitors of cathepsin K are in clinical trials for osteoporosis treatment, but side effects included altered levels of related cathepsins in peripheral organs and in the central nervous system (CNS). Importantly, cathepsin K has been identified not only in osteoclasts but also in most epithelia and, recently, in brain parenchyma. Lately, we have also shown that cathepsin K deficiency in mice induces structural and functional alterations in the CNS which are associated with learning and memory deficits. In addition, we demonstrated that cathepsin K contributes to thyroid hormone (TH) liberation from the thyroid gland, because cathepsin K- and L-double deficient mice are considered hypothyroid due to reduced serum T4 levels and enlarged thyroids. These findings led us to hypothesize that brain and thyroid functions of cathepsin K are linked. Ctsk-/- mice exhibit enlarged thyroids but no alterations in serum or brain T4, T3 or TSH levels nor in brain deiodinase 2 activity were observed. Cerebellum development is TH dependent and altered upon hypothyroidism. However, we show here that neither the overall structure nor single cells, like Purkinje cells or Bergmann glia in the cerebellum, are altered in Ctsk-/- mice. Thus, Ctsk-/- mice do not suffer from classical hypothyroidism. Interestingly, trace amine associated receptors (TAAR) as well as TH transporters appeared deregulated in the CNS of Ctsk-/- animals compared to WT mice. Since all markers of classical TH action were comparable in WT and Ctsk-/- mice, but markers of non-classical TH action like the TH transporters and TAARs differed significantly, we believe to have identified an animal model suited to study non-classical TH actions on the CNS as a target organ.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1589

**SECISBP2 syndrome: Mouse models for an atypical form of resistance to thyroid hormone**

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An atypical form of resistance to thyroid hormone (RTH) leads to retarded growth and bone age in humans. Aberrant thyroid hormone levels (high T4, low T3, elevated rT3 and high/ normal TSH) indicate a defect in deiodinase-dependent thyroid hormone metabolism. Findings of reduced concentrations of plasma selenoproteins (SePP, GPx3) suggested a generalized defect of selenoprotein biosynthesis and led to the identification of mutations in the SECISBP2 gene. SECISBP2 is thought to play an essential role for selenoprotein biosynthesis. Mutations in the SECISBP2 gene lead to reduced expression of selenoproteins and cause a syndrome with relatively mild to more severe phenotypes. Our group set out to create mouse models to test whether Secisbp2 is essential for selenoprotein biosynthesis and to study the changes in the thyroid axis of mutant mice.

First results showed the essentiality of Secisbp2: A homozygous null mutation leads to early embryonic death. In contrast, Secisbp2 heterozygotes have no obvious phenotype. They are fertile and their thyroid function tests are normal. Biochemical examination revealed only minimal changes in selenoprotein expression. Hepatocyte-specific Secisbp2 knock-out mice appear normal too, but show a dramatic reduction of hepatic selenoprotein expression.

Neuron-specific Secisbp2 knock-out mice have a more severe phenotype. They are smaller and weight less than their wildtype littermates. Their neurological phenotype involves a movement disorder and histological stainings demonstrated a specific loss of parvalbumin positive interneurons. It will be interesting to analyze Secisbp2-mutant mice carrying point mutations found in human patients.

Declaration of interest
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## P1590

**Polymorphism of the PPAR-γ2 gene and Graves' orbitopathy: the Ala variant confers decreased risk of eye symptoms**

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Introduction
Peroxisome proliferatior-activated receptor-γ2 (PPAR-γ2) plays a crucial role in adipogenesis and has been shown to be involved in the control of immunoregulation and inflammation. Orbital fibroblast differentiation to adipocytes is a PPAR-γ dependent process essential for pathogenic tissue remodeling in Graves' orbitopathy (GO). Genetic variation in PPAR-γ2 gene may modulate expression and/or function of molecule encoded by this gene.
Purpose
The occurrence and associations of the PPAR-γ2 Pro12Ala mutation with clinical manifestation of GO were studied.
Material and methods
The Pro12Ala polymorphism was determined in total 742 Lower Silesia Caucasians including 276 Graves' disease (GD) patients (212 GO patients and 64 subjects without eye changes) and 466 healthy controls using PCR-RFLP technique (with the use of restriction enzyme HpaII).
Results
The distribution of The Pro12Ala polymorphism did not differ between GD patients and healthy subjects. Within the GD group the Ala allele and a presence of Ala variant (Pro12Ala or Ala12Ala genotype) decreased the risk of GO (OR = 0.33, $P$ = 1.2e–5, 95%CI:0.19–0.55 and OR = 0.32, $P$ = 0.00013, 95%CI:0.17–0.58, respectively). Moreover, Ala12Ala genotype was observed only in patients without GO (5.2%, $P$ = 0.007, after Yate's correction). However the distribution of genotypes and alleles in studied marker was similar in patients shared-out according to severity as well as activity status of GO. The female carriers of Ala12 allele statistically seldom develop GO ($P$ = 0.0005, OR = 0.31, 95%CI:0.16–0.61). Additionally, the presence of Ala12 allele increased the risk of active GO in men ($P$ = 0.04 after Yate's correction, OR = 6.47, 95% CI: 1.25–33.52).
Conclusions
For the first time we pointed that the presence of Ala variant (Ala12Ala and/or Pro12Ala genotype) in unique exon B in PPAR-γ2 gene strongly reduces the risk of GO and the Al12Ala genotype was seen only in patients without GO so may be considered as a protective factor.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1591

**Thyroid hormone T3 induces ovarian activity in rat follicles and granulosa cells**

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Background
The menstrual pattern is influenced by thyroid hormones directly through impact on the ovaries and indirectly through impact on SHBG, PRL, and GnRH secretion and coagulation factors. Treating thyroid dysfunction can reverse menstrual abnormalities and thus improve fertility.
Aim
Elucidating the molecular mechanisms underlying the link between thyroid and ovarian function is important for determining the relationship between thyroid status, fertility and menstrual disorders. Our previous work has demonstrated how granulosa cells population can be considered a thyroid hormone target, being its survival induced by T3 under specific circumstances via cell cycle and metabolism regulation.
Materials and methods
To characterize thyroid hormone action in the ovary, the direct effect of triiodothyronine T3 was analyzed *in vitro* using a culture system of rat follicles and granulosa cells obtained directly from female rats, and their growth and function in response to T3 treatment (10–7M) have been evaluated.
Results
Our results showed that both cell growth and follicle dimensions were significantly incremented (up to 40%) by 2–7 days of hormone treatment, as shown by cell growth analyses and follicle dimensions measures. The same cells and follicles were moreover capable of a 50% incremented production of 17 B estradiol, in response to testosterone supply, when treated with 48 h T3 10–7 M. Even the basal production of estradiol was augmented by the hormone presence, as measured by chemiluminescence.

To investigate the mechanisms underlying the observed effects, the major steroidogenic genes were analyzed by RT-PCR, namely FSHR, cyp 19a1, cyp 17, p450 scc, 3BHSD and StAR. All the genes analyzed, and in particular the aromatase and 17B-HSD, which are directly responsible for estradiol production, were significantly upregulated by the 48 h T3 treatment.
Conclusion
In conclusion our data show unambiguously the T3 ability to promote ovarian function in rats, providing interesting results about the T3 ability of directly target steroidogenesis.

Declaration of interest
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## P1592

**Recurrence of Amiodarone induced thyrotoxicosis after reinstitution of this drug**

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Introduction
Amiodarone-induced thyrotoxicosis (AIT) contributes to increased morbidity and mortality in patients invariably having underlying heart disease. In clinical

practice, amiodarone therapy often needs to be restarted. When amiodarone is reintroduced, what happens then?
Objective
The aim of study is to evaluate the progression of thyroid function after reinstitution of amiodarone in patients with a previous history of AIT. Secondarily, to determine the efficacity of thionamides to prevent the recurrence of AIT.
Methods
Retrospectively, between 2000 and 2011, patients with a previous history of AIT, in whom amiodarone needed to be reintroduced, were included. Type and severity of 1st AIT, and thyroid function evolution after reinstitution of amiodarone where investigated.
Results
patients were included in our study. Mean age when AIT diagnosed was $62.2 \pm 16$ years old with male sex predominance. 65.8% of patients had type I AIT. Duration of treatment with amiodarone before developing AIT was shorter in these patients in comparison to patients with type II AIT ($20.5 \pm 20.1$ vs $40.2 \pm 20.0$ months: $P=0.006$). Before reinstitution of amiodarone, 6 patients had radical treatment. In 22 patients, amiodarone was reintroduced without a prophylactic therapy, while a thionamide was introduced in parallel with amiodarone reinstitution in 24 patients. After a mean follow up of $34.9 \pm 39.1$ months, AIT recurred in 14 (29.8%) patients, 12 (25.5%) patients developed hypothyroidism and 20 (48.6%) patients had normal thyroid function. Hyperthyroidism recurred in 62% of patients with amiodarone reinstitution without a preventive treatment, in parallel, only 16% of patients with a prophylactic thionamide suffered from recurrent AIT. Prophylactic thionamide may prevent recurrence of hyperthyroidism ($P=0.06$).
Conclusion
During median follow up of 3 years after reintroduction of amiodarone, the risk of recurrence of hyperthyroidism tends to be high (29.8%) but not compulsory. A prophylactic treatment with a thionamide could prevent or decrease recurrence of hyperthyroidism.
Declaration of interest
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## P1593

**Subclinical and overt hyperthyroidism in a long-term iodine sufficient area of Sweden (Gothenburg) 2003–2005**

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Introduction
The incidence of hyperthyroidism is influenced by iodine nutrition. Sweden is a long-term iodine sufficient (IS) area with an iodination fortification program since 1936.
Methods
In 2003–2005, all referred cases of subclinical (SH) and overt hyperthyroidism (OH) were registered from a targeted population of 631239 individuals in Gothenburg. Information on age, gender, etiology, smoking, thyroid associated ophtalmopathy (TAO), thyroid hormone levels, TSH receptor antibodies (TRab), thyreoperoxidase antibodies (TPOab) and planned treatment was collected at diagnosis. Incidences were calculated. Comparisons between SH and OH for Graves' disease (GD), toxic multinodular goiter (TMNG) and solitary toxic adenoma (STA) were made and for GD also smokers vs non-smokers, $TRab^+$ vs $TRab^-$, $TAO^+$ vs $TAO^-$, and correlations between thyroid hormone levels, age and autoantibodies.
Results
The total incidence of hyperthyroidism was 27.6/100000/year (OH 23.8/100000/year, SH 3.8/100000/year; GD 21.4/100000/year, TMNG 4.3/100000/year and STA 1.8/100000/year). SH was more common among TMNG and STA (40.2% and 45.7%)than in GD (15.1%). SH-GD patients were older, more often smokers and had lower TRab levels than patients with OH-GD. FT4 and T3 levels in GD were higher than in TMNG and STA. FT4, T3 and TRab decreased with age in GD patients (Spearman $-0.23$, $-0.35$, $-0.26$), $P<0.0001$. $TRab^-$ patients had lower T3 than $TRab^+$ patients ($3.7 \pm 1.7$ vs $5.7 \pm 2.8$), $P<0.001$ but similar FT4 levels. TRab was positively correlated to FT4 (Spearman 0.39), $P<0.0001$. TAO occured in 20% of GD patients. $TAO^+$ patients was younger than $TAO^-$ patients. Concentration of FT4 and T3 were similar but TRab was higher in $TAO^+$ patients. Smokers did not have more TAO.
Conclusion
The total incidence of hyperthyroidism was relatively low. The dominating cause was GD with an age-related decrease of thyroid hormones and TRab. The spectra of hyperthyroidism in this long-term IS area probably reflects the future for many countries with shorter history of IS.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1594

**Enhanced expression of the B cell activation factor (BAFF) and baff-receptor (BAFF-R) in the thyroid and in the orbital adipose tissue from patients with graves' disease (GD) and associated orbitopathy (GO)**

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BAFF, a member of the TNF family, promotes autoantibody production by increasing B cell survival and proliferation. We have previously shown that serum BAFF concentrations are increased in GD patients. In the present work, we studied the expression of BAFF and BAFF-R, by immunohistochemistry (IHC) on paraffin embedded sections of: 1. orbital adipose tissue from 22 patients (5 men, 17 women) with GO (3 of them with active GO) associated with GD or Hashimoto thyroiditis in 19 and 2 patients, respectively. One subject was affected with euthyroid GO (EGO); 2. extra-ocular-muscles (EOM) biopsies of 10 patients (2 men, 8 women) with GO (2 of them with active GO) associated with GD; 3. thyroid of 27 patients (20 women, 7 men) with GD and GO. Samples were stained using a panel of antibodies directed against the following antigens: BAFF, BAFF-R, CD3, CD4, CD8, CD20, CD34, CD79, CD1a, CD68, CD163. Thyroid histology showed nodular hyperplasia (NH) in 8 of 27 patients, diffuse hyperplasia (DH) in 19 of 27. Reactivity for BAFF and BAFF-R was found on thyrocytes and on the lymphocytes infiltrating the thyroid. BAFF expression on thyrocytes was higher in DH than NH; conversely, lymphocytes surrounding the areas of NH exhibited a stronger staining for BAFF-R, compared to DH. In 15 of 22 samples, B and T-cells infiltrating orbital adipose tissues expressed BAFF. Interestingly, BAFF staining was stronger on B compared to T lymphocytes. BAFF-R staining was detected in 5 of 22 samples from orbital adipose tissue, but only on B lymphocytes. Orbital fibrocytes expressed BAFF and BAFF-R only rarely (3 of 22 samples). Tissue samples from EOM biopsies were negative either for BAFF or BAFF-R reactivity. In this study we report, for the first time, the increased expression of BAFF and BAFF-R in orbital and thyroid tissue samples from patients with GO and GD. This finding, together with the recent observation of increased serum BAFF levels in GD, suggests an involvement of BAFF and its receptors in the pathophysiology of GD and GO.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1595

**Utility of thiazide diuretics in the management of post- thyroidectomy hypocalcemia: a pilot study**

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Introduction
The incidence of post thyroidectomy hypocalcemia varies from 10–50%. Treatment is either prophylactic or therapeutic calcium and vitamin D supplementation. We evaluated the utility of thiazide diuretics in the management of hypocalcemia prospectively.
Material and methods
This study was conducted in Endocrine Surgery department of a teritiary care teaching hospital. We included 104 patients of total thyroidectomy in the study. 44 cases had normocalcemia (Group A), 29 cases developed biochemical hypocalcemia (Group B) and 31 patients suffered from clinical hypocalcemia (Group C). All the three groups were subdivided in to non- intervention and

intervention groups. 25 mg of thiazide was given orally in intervention subgroup. Results were analysed with SPSS 12.0. ($P$ value $< 0.05$ was taken as significant)
Results
In group A, 4 patients in intervention and 2 in non-intervention subgroups developed biochemical hypocalcemia and 2 in intervention and 3 in non-intervention subgroups developed clinical hypocalcemia respectively. $P$ value was non-significant at 0.116. In group B, 1/15 patients in intervention subgroup and 6/14 patients in non-intervention subgroup progressed to clinical hypocalcemia. This result was 0.03 (statistically significant). In group C, mean duration of calcium infusion was 19 hours (12–36) in intervention subgroup and 27.5 hours (24–72) in non-intervention group. Result was not statistically significant ($P$ value $=0.09$)
Conclusion
These preliminary results could justify the additive role of thiazide diuretic in the secondary prophylaxis of post-thyroidectomy hypocalcemia
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1596

**Maternal Graves' disease induces more perinatal complications and higher risk of thyroid dysfunction at early childhood**

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Objectives
The most common cause of maternal hyperthyroidism during pregnancy is Graves' disease (GD). The aim of this study was to investigate the perinatal complications and the risk of thyroid dysfunction at early childhood of mothers with GD during pregnancy.
Method
Pregnant women with GD ($n=93$) and aged-matched randomly selected healthy pregnant women ($n=140$) were recruited retrospectively. The prenatal and newborn data were collected and analyzed. Toddlers with GD mothers ($n=30$) and non-GD healthy mothers ($n=36$) were also recruited for thyroid function and growth assessments.
Results
Newborns of mothers with GD had significantly higher complications than of non-GD mothers (34.41% vs. 7.86%, $P < 0.05$). Maternal hyperthyrodism was an independent risk factor of stillbirth, infants with a low birth weight and congenital malformation with odds ratios of 9.33, 9.33 and 7.29 respectively. Antithyroid drugs (ATDs) reduced fetal distress and stillbirth of GD mothers from 9.52% and 23.81% to 6.94% ($P < 0.05$) and 4.17% ($P < 0.001$), respectively. Toddlers' serum levels of FT3, FT4, anti-thyroglobulin antibody, and anti-thyroid peroxidase antibody were significantly higher than those of non-GD mothers (all $P < 0.05$).
Conclusion
Pregnancy with GD increased the risk of stillbirth, infants with a low birth weight and congenital malformation. Euthyroidism by ATD treatment reduced GD-induced perinatal complications effectively. Maternal GD may also induce a higher risk of autoimmue thyroid dysfunction at early childhood.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1597

**No Correlations between TSH receptor antibody levels assayed with Elecsys TRAb and the activity or severity of Graves' ophthalmopathy**

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Objective
This study was designed to determine whether TRAb levels are correlated with the activity and severity of Graves' ophthalmopathy (GO).
Subjects and Methods
The subjects were 179 untreated Graves' disease patients. TRAb was assayed with Elecsys TRAb at the initial visit, and an ophthalmic assessment was performed within 3 months. The activity of the ophthalmopathy was assessed by clinical activity scores (CASs) and MRI T2 relaxation times. Exophthalmos was measured with Hertel's exophthalmometer, and the measurements in both eyes were summed (exophthalmos score). Eyelid swelling was evaluated by inspection, scored from 0 to 3 in each eye, and scores were summed (eyelid swelling score). Swelling of each extraocular muscle was evaluated, scored from 0 to 4 by MRI, and the scores were summed (extraocular muscle score).
Results
According to the activity of the ophthalmopathy the numbers of active, inactive, and recovery phase patients were 18, 152, and 1, respectively. CASs ranged from 0 to 3 points, and the numbers of patients with each CAS were 57, 76, 37, and 9, respectively. The median (range) TRAb level of the active phase patients and inactive phase patients was 6.55 IU/L (0.6–40.0) and 13.6 IU/L (0.3–40.0), respectively ($P=0.0298$). The TRAb levels of the patients whose CASs were 0 to 3 were 8.9 IU/L (0.3–40), 13.4 (0.3–40), 18.7 (1–40), and 16 (1.8–40), respectively, and there was no significant difference among them (Wilcoxon test, $P=0.087$).
TRAb levels did not correlate with exophthalmos scores ($r=0.1133$, $P<0.131$), eyelid swelling scores ($r=0.1454$, $P=0.0521$), or extraocular muscles scores ($r=-0.0653$ $P<0.3879$).
Conclusion
The TRAb levels were not correlated with either GO activity or severity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1598

**The influence of recombinant human thyroid stimulating hormone (rhTSH) on the efficacy of radioiodine therapy in patients with toxic nodular goitre with low RAIU**

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The aim of our study was to evaluate the influence of rhTSH on the efficacy of radioiodine therapy (RIT) in patients with toxic nodular goitre(TNG) with low RAIU. Materials and methods: The study was performed on 30 patients with TNG (22 female and 8 male aged 45–78years) referred for 131I therapy. All patients had low RAIU (16–18%) 24 hours after a diagnostic dose of I-131 (4 MBq). All the patients received a single intramuscular dose of 0.05 mg rhTSH (thyrogen). 24 h later diagnostic dose of 131I was administered and thyroid scan with RAIU after 24 and 48 h was estimated. Therapeutic dose of 131I was given on the third day of rhTSH administration. Serum levels of TSH, fT4 and fT3 were determined, 24 h, 72 h after rhTSH administration and on the 3rd day after RIT. The therapeutic activity of 131I calculated by Marinelli's formula and ranged between 280 and 600 MBq. The absorbed dose ranged between 160 and 300 Gy. Follow up control was done every 6 weeks. Thyroid ultrasound, and thyroid scan were done again after12 months of RIT
Results
A significant increase (2–8 fold) in 24hr RAIU was observed after rhTSH administration. The distribution of radioiodine was more homogeneous 48 hours after rhTSH injection. After 12 months 93% of patient were in euthyroidism and 7% (2 patients) develop hypothyroidism. After six months the mean reduction in goitre volume was 20% and 45–50% after twelve months. The medium therapeutic activity of 131I was 280 Mob. Conclusions: Pre-treatment with rhTSH reduce the therapeutic dose of 131I by 50–58% without compromising the result of thyroid volume reduction. rhTSH makes RIT of TNG more effective in the patients with low RAIU.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1599

**Peripheral blood lymphocyte apoptosis and its relationship with thyroid function tests in adolescents with hyperthyroidism due to Graves' disease**

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The aim of the present research was to assess changes in the degree of peripheral blood (PB) lymphocyte apoptosis during methimazole treatment in the group of teenage children.
Material and methods
The percentage of PB apoptotic lymphocytes, assessed by the decrease in mitochondrial transmembrane potential (CMXRos staining), was measured in 30 adolescents at the time of diagnosis, as well as after receiving the normalization of the thyroid hormones levels.
Results
The percentage of apoptotic lymphocytes in previously untreated patients with GD ($5.16 \pm 2.81\%$) was significantly lower ($P = 0.000001$) than the percentage of the apoptotic cells in the same group of patients after receiving methimazole-induced euthyreosis ($10.72 \pm 4.66\%$). There was a correlation between the increase of the mean percentages of apoptotic lymphocytes and the reduction of the FT4 levels ($R = 0.63$, $P < 0.0001$), as well as the reduction of TT3 levels ($R = 0.95$, $P < 0.0001$). The more signs and symptoms accompanied the diagnosis of the GD, the higher increment of the degree of lymphocyte apoptosis was observed during the MMI-treatment ($R = 0.74$, $P < 0.0000001$). The methimazole dosage correlated ($R = 0.85$, $P < 0.0001$) with the percentages of apoptotic cells.
Conclusions
Apoptosis induction of human PB lymphocytes seems to be one of the indicators of proper hyperthyroidism treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P1600

**Individual plasma ghrelin changes evaluated in the same patients in hyperthyroidism, hypothyroidism and euthyroidism**

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Ghrelin is a peptide discovered in 1999 as the first natural growth hormone secretagogue. It also proved to be an essential regulator of metabolism increasing appetite, body weight and stimulating peristalsis. Thyroid plays an important role in the maintenance of energy balance. Thus, correlation between ghrelin and thyroid function is worth consideration. Previous studies suggested, that ghrelin production is increased in hypothyroidism and decreased in hyperthyroism. However, since ghrelin production depends on many factors, including individual features, studies conducted on different groups of patients may not be accurate. We evaluated plasma ghrelin concentrations in the same patients in hyperthyroid, hypothyroid and euthyroid phase, assuming, that such assessment would be the most relevant analysis of plasma ghrelin changes in thyroid disorders.
The study group was selected from the patients with hyperthyroidism treated with radioiodine and followed-up after therapy. Ghrelin level was firstly assessed in hyperthyroid phase. In 12 patients, who developed hypothyroidism after radioiodine, the second measurement was performed. The third analysis was made in euthyroid phase after L-thyroxine replacement therapy. Blood samples were collected from antecubital vein at fast. Plasma ghrelin was measured with a commercial radioimmunoassay.
The study revealed, that in 11 patients ghrelin concentration was the lowest in hyperthyroidism, the highest in hypothyroid phase and reached medium value in euthyroidism. Mean ghrelin levels in all 12 patients were also decreased in hyperthyroidism (TSH $0.006 \pm 0.002$ uIU/ml; ghrelin $144.47 \pm 84.15$ pg/ml) and increased in hypothyroidism (TSH $62 \pm 29.63$ uIU/ml; ghrelin $320.95 \pm 190.16$ pg/ml) in comparison to euthyroid phase (TSH $1.53 \pm 0.81$ uIU/ml; ghrelin $216.16 \pm 111.61$ pg/ml).
Plasma ghrelin changes may indicate its compensatory role in thyroid dysfunction. Low ghrelin level decreases metabolic rate in hyperthyroid patients and high ghrelin concentration leads to appropriate use of energy in hypothyroidism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P1601

**The effect of radioiodine therapy in 950 patients with subclinical hyperthyroidism**

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The aim of our study was to assess the influence of radioiodine (131I) therapy on the achievement of euthyroidism, prevention of adverse effects on the cardiovascular and prevent evolvement to overt hyperthyroidism. Material and methods: We treated 950 patients, aged 30–70 years; 87% of them were female and 13% male; 320 patients with multinodular goitre (MNG), and 630 patients with autonomous nodule (ATN). Some of the patients were treated with antithyroid drugs for 1 to 3 months before I131 therapy (90 patients). Malignant changes were excluded in all nodules by fine needle aspiration biopsy. All the patients had serum TSH levels $< 0.1$ mU/l and effective T-half was more than 3 days at the time of treatment. The activity dose was calculated by the use of Marinelli's formula and ranged between 200 and 600 MBq. The absorbed dose (Gy) ranged between 180 and 300, and was proportional to thyroid volume. Follow up control was done every 6 weeks. Results: Euthyroidism achieved in 99% of patient with ATN and 94% of MNG; 1% of patients with ATN and 6% of patients with MNG develop hypothyroidism. In all of the patients, the symptoms and signs of subclinical hyperthyroidism disappeared (palpitation, tachycardia, atrial fibrillation, exercise tolerance improved, the blood pressure normalised and the quality of life improved). One percent of the patients received 2nd dose of radioiodine. Conclusions: Our result is good and is in the range of the existing literature. The achievement of euthyroidism and the remission of the symptoms and signs of subclinical hyperthyroidism, were due to good diagnosis, well preparation of the patients; accurate measurement of administered activity, effective half-life, and well-organised follow up. We recommend early treatment of subclinical hyperthyroidism, and long period of follow up visits in our department to evaluate the long term effect of radioiodine therapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1602

**Genetic susceptibility to Graves' ophthalmopathy: the role of polymorphisms in anti-inflammatory cytokine genes**

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Background
Various polymorphisms occur in cytokine genes involved in inflammatory processes in Graves' ophthalmopathy (GO). Anti-inflammatory cytokines such as transforming growth factor-β (TGF-β), interleukin-10 (IL-10) and interleukin-4 (IL-4) are among those believed to be involved in the disease process. In this study, we investigated the association between 8 polymorphisms within the mentioned cytokines and GO.
Methods
The following polymorphisms were studied in 50 patients with GO, 57 Graves' patients without GO and 140 healthy individuals using polymerase chain reaction with sequence-specific primers: TGF-β (+869C/T, +915G/C), IL-10 (−1082A/G, −819C/T, −592C/A) and IL-4 (−1098T/G, −590T/C, −33C/T). A corrected $p$ value less than 0.05 was considered statistically significant.
Results
The TGF-β+915C allele (Odds Ratio (OR) = 2.20) and CC genotype (OR = 7.50) as well as +869C allele (OR = 2.21) showed significant correlations with GO. Regarding IL-4 polymorphisms, the −1098G allele (OR = 2.09) and GG genotype (OR = 7.49), and the −33T allele (OR = 2.05) and TT genotype (OR = 4.00) were significantly associated with GO. The IL-10 −819TT genotype (OR = 5.00) was significantly correlated with GO.
Conclusion
This is the first study to show that polymorphisms in anti-inflammatory cytokine genes are associated with susceptibility to GO.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1603

### The T and B cell subsets in thyroids of children with Graves' disease and Hashimoto's thyroiditis

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Background
Autoimmune thyroid disorders (AITD) similar in pathomorphology picture results in hyper- or hypothyreosis. The differences between Graves' disease (GD) and Hashimoto thyroiditis (HT) suggested that changes in the subsets of T-cells may have an influence on the course of this reaction.
Methods
The study involved 90 children: 30 with GD, 30 with HT and the control group – 30 children. After thyroidectomy, standard histological examinations and immunohistochemical reactions in paraffin specimens with monoclonal antibodies against T-cell markers CD3, CD4, CD8 as well as against B-cells - CD79 alpha were performed. Ultrathin sections were examined in the Transmission Electron Microscope.
Results
The autoimmune reaction in GD consists in increased number of CD4+ cells (3.17±4.27%) and plasma cells (22.89±8.61%) producing TRAb and stimulating thyrocytes to activity. The number of CD4+ cells was increased in comparison to healthy children (0.19±0.05%) and to HT (0.93±9.90%). The autoimmune reaction in HT is consist in antibody dependent cytotoxicity in circumstances of a low number of CD4+ cells and an increased number of CD8+cells (20.54±0.68%) in the thyroid tissue in comparison to GD (6.86 ±3.46%) and control group (0.65±0.30%). Plasma cells (31.65±9.11%) in this situation produced the antibodies involved in cytotoxic reactions against thyrocytes.
Conclusion
The Graves'disease was characterised due to increased number of CD4+T cells and increased number CD8+T cells. Hashimoto's thyroiditis characterised by low number CD4+T cells and increased CD8+Tcells. CD8+T cells have the cytotoxic properties only in Hashimoto's thyroiditis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

The percentage of T cells subsets and B cells in Graves' disease and in Hashimoto's thyroiditis in comparison to control group.

| | CD3+T cells | CD4+T cells | CD8+T cells | CD79 a+B cells |
|---|---|---|---|---|
| Graves'disease | 17.79 | 3.17 | 6.96 | 22.89 |
| Hashimoto's thyroiditis | 30.38 | 0.93 | 20.54 | 31.65 |
| Control group | 1.04 | 0.19 | 0.65 | 4.11 |

## P1604

### The thyroid gland is another victim of the insulin resistance syndrome university

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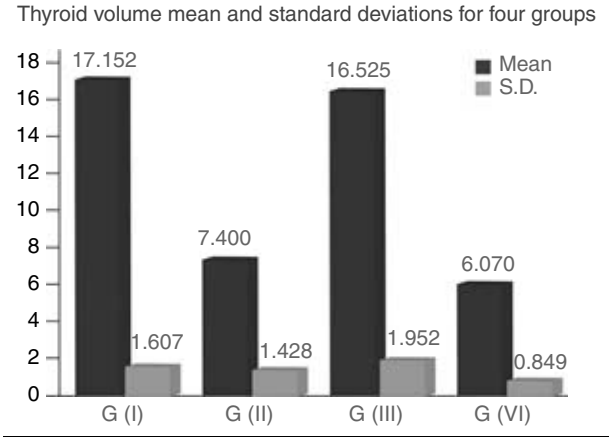
Insulin is a thyroid growth factor that stimulates proliferation of thyroid cells in culture. It has been observed that insulin receptors are over expressed in most thyroid tumors as an early step in thyroid carcinogenesis.
Eighty women (mean age 36.85±4.71 years) were evaluated by a thyroid ultrasound (US), fasting plasma insulin and HOMA IR. Subjects were divided into four groups as follows: G1 (*n*: 24),subjects with IR and obesity; G2 (*n*: 20), subjects with obesity without IR; G3 (*n*: 16), subjects with IR and normal weight; and G4 (*n*: 20) control group (without IR and obesity).
The thyroid volume (TV), measured by US, was in G1, 17.15±1.6 ml; G2, 7.4 ±1.4 ml; G3, 16.53±1.9 ml; and G4, 6.07±0.95 ml. There was no significant difference in TV between G1 and G3, but differences between G1 and G2, and between G3 and G4 were significant at $P<0.05$. The percentage of nodular thyroid glands observed by US in each group was as follows: G1, 58%; G2, 15%; G3, 50%; G4, 5%. Again, the differences between G1 and G2 and between G3 and G4 were statistically significant ($P<0.005$ and $P<0.001$, respectively, for each comparison). Our study also showed that the greater waist circumference;(as one parameter of insulin resistance syndrome by IDF), the greater thyroid volume and the higher incidence of thyroid nodules.
Conclusions
High circulating levels of insulin cause increased thyroid proliferation resulting in a large thyroid volume and the formation of nodules. Thus, the thyroid gland appears to be another victim of the insulin resistance syndrome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding

Clinical data of all groups.

| | Group (1) Mean±SD | Group (2) Mean±SD | Group (3) Mean±SD | Group (4) Mean±SD |
|---|---|---|---|---|
| Thyroid volume $cm^3$ | 17.151±1.6 | 7.427±1.4 | 16.53±1.9 | 6.07±0.84 |
| Thyroid nodules | 14 | 3 | 8 | 1 |
| Percentage of nodules | 58% | 15% | 30% | 5% |




## P1605

### Four families with nonautoimmune hyperthyroidism harboring the same thyrotropin receptor germline mutation (Asn406Ser) in Kumamoto Prefecture, Japan

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Nonautoimmune hyperthyroidism (NAH) due to activating mutation of the TSH receptor (TSHR) gene is extremely rare. We report 4 unrelated families with NAH experienced for the last two years in Kumamoto Prefecture, Kyushu, Japan. TSHR gene mutation site of these four families are all the same: p.Asn406Ser (heterozygous). Thyroid functions observed in these families were subclinical hyperthyroidism or overt hyperthyroidism except for one case with euthyroidism. Thyroid autoantibodies (TRAb, TPOAb, TgAb) were all negative.
[Family 1] We have already reported about this family (Clin Endocrinol (Oxf).2011).
[Family 2] 29-year-old male showing hyperthyroidism with large goiter(estimated weight:84.2g).
[Family 3] 59-year-old male showing subclinical hyperthyroidism with with nodules in the thyroid gland which was diagnosed as papillary carcinoma.
[Family 4] 40-year-old female showing subclinical hyperthyroidism with nodule in the thyroid gland which was diagnosed as papillary carcinoma. Her son had overt hyperthyroidism.
[Conclusion] This mutation(p.Asn406Ser) has not been found outside the southern Kumamoto Prefecture. Many pedigrees of NAH may have accumulated in this area.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1606

**Antigen mimicry between aeroallergens and thyroid antigens can modify the levels of thyroid hormones and antibodies in thyroid autoimmunity**

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The allergic sensitization represents an environmental factor for thyroid autoimmunity and highlights a common pathway between autoimmunity and allergy. Elevated IgE levels are connected to hyperthyroid Graves' disease. The allergic sensitization in thyroid autoimmunity seems mainly to be an associated immune reaction.
Allergic sensitization to 20 aeroallergens and the levels of thyroid hormones and antibodies were studied in 121 patients with Graves' disease, 104 patients with Hashimoto's thyroiditis and 65 healthy subjects. AllergyScreen test with quantitative evaluation was applied for the detection of allergic-specific IgE levels. The levels of TSH, $FT_4$, $FT_3$ and anti-thyroid peroxidase (TPO), anti-thyroglobulin (Htg) and TSH receptor antibodies were measured.
The house-dust mite II (D2) allergic sensitization was more frequent in thyroid autoimmunity than in controls (odds ratio (OR): 2.97, $P<0.002$ in Graves' disease; OR: 3.41, $P<0.01$ in Hashimoto's thyroiditis). The allergic sensitizations to alder (T2, OR: 2.85, $P<0.03$), birch ($T_3$, OR: 3.27, $P<0.03$), ragweed (W1, OR: 2.7, $P<0.01$) and grass-mixture (Gx, OR: 2.58, $P<0.04$) were higher in Graves' patients than in those with Hashimoto's thyroiditis. The mugwort (W6) allergic sensitization was associated with increased levels of TSH receptor antibodies ($11.7\pm13.5$ U/l vs $7.9\pm17.3$ U/l, OR: 6.47, $P<0.03$). The birch ($T_3$) and the ragweed (W1) allergic specific IgE levels were associated with increased anti-TPO (OR: 3.88, $P<0.04$) and increased $FT_4$ (OR: 2.73, $P<0.04$) in Graves' disease or lower TSH levels (OR: 8.27, $P<0.004$) in Hashimoto's thyroiditis, respectively. The modifying effect of allergic sensitization did not depend on the presence of allergic symptoms.
The results in matching of amino acid sequences demonstrated a high percentage of identities (42–50%) between W1, W6, $T_3$ aeroallergens and thyroid antigens (TSH receptor, TSH, TPO, Htg).
In conclusion, the role of antigen mimicry between ragweed, mugwort, birch allergens and thyroid antigens may be responsible for the alteration in the levels of thyroid hormones and antibodies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1607

**Diagnostic accuracy of elastography in thyroid nodule ultrasound evaluation. A prospective multicenter trial**

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Background
Real-time elastography (RTE) was reported to improve the diagnostic accuracy of B-mode ultrasound (US) examination of thyroid nodules but the results on selected series of patients are still controversial.
Aim of the study
To blindly evaluate on a consecutive series of solid thyroid nodules, devoid of confounding factors, the diagnostic accuracy of RTE and to compare it with the traditional B-mode features.
Materials and methods
From September 2010 until June 2011, 323 consecutive solid thyroid nodules underwent fine-needle aspiration biopsy (FNA) at three thyroid referral centers in Rome. B-mode US and RTE examinations (Mylab 70, Esaote, Genoa, Italy) were performed before FNA and images were stored and blindly reviewed by six experienced endocrinologists. A RTE score (from I, soft, to IV, hard) was assigned on the basis of color pattern, according to a previous classification. Twenty-six nodules (8.0%) with non-diagnostic FNA were excluded and 297 cases were enrolled in the study. Patients with indeterminate, suspicious and malignant cytology were operated upon. Nodules with benign cytology had a clinical and US control after six months.
Results
Ninety-five nodules (32%) were malignant and 202 (68%) benign. The PPV for malignancy provided by the presence of at least one conventional US risk factor (marked hypoechogenicity, microcalcifications, spiculated margins and intranodular vascularization) was 98%, and the NPV due to the absence of any US risk factor was 34%, with a 71% diagnostic accuracy. The PPV for malignancy of class III and IV RTE scores, as a whole, was 60% and the NPV was 85%, with a 75% diagnostic accuracy. When evaluated separately, marked hypoechogenicity, microcalcifications, spiculated margins and intranodular vascularization showed a 90%, 75%, 61%, and 65% diagnostic accuracy, respectively.
Conclusions
In unselected solid thyroid nodules, and in absence of confounding sonographic factors, RTE showed a diagnostic accuracy similar to the single traditional US risk factors. The PPV for malignancy provided by the presence of at least one US suspicious finding, however, was superior to that of combined class III and IV RTE scores.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1608

**FOXA1 and FOXA2 oppositely regulate human type 1 Iodothyronine deiodinase gene in liver**

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Type 1 iodothyronine deiodinase (D1), a selenoenzyme that catalyzes the bioactivation of thyroid hormone, is expressed mainly in the liver. Its expression and activity are modulated by several factors, but the precise mechanism of its transcriptional regulation remains unclear. In the present study, we have analyzed the promoter of human D1 (hDIO1) gene to identify factors that prevalently increase D1 activity in the human liver. Deletion and mutation analyses demonstrated that a forkhead box (FOX)A binding site and an E-box site within the region between $-187$ and $-132$ bp are important for hDIO1 gene promoter activity in the liver. EMSA demonstrated that FOXA1 and FOXA2 specifically bind to the FOXA binding site and that upstream stimulatory factor (USF) specifically binds to the E-box element. Overexpression of FOXA2 decreased hDIO1 gene promoter activity, and short interfering RNA-mediated knockdown of FOXA2 increased the expression of hDIO1 gene mRNA. In contrast, overexpression of USF1/2 increased hDIO1 gene promoter activity. Short interfering RNA-mediated knockdown of FOXA1 decreased the expression of hDIO1 gene mRNA, but knockdown of both FOXA1 and FOXA2 restored it. The response of the hDIO1 gene promoter to USF was greatly attenuated in the absence of FOXA1. Taken together, these results indicate that a balance of FOXA1 and FOXA2 expression modulates hDIO1 gene expression in the liver.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This work was supported, however funding details unavailable.

## P1609

**Dyrk1A (dual-specificity thyrosine (Y)-phosphorylation regulated kinase 1A) overexpression is linked to congenital hypothyroidism in Down syndrome**

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Introduction
Trisomy 21 or Down Syndrome (DS) patients have a predisposition for Congenital Hypothyroidism which can aggravate their mental status.
Hypothesis
The presence of three copy of Dyrk1a gene, localized in chromosome 21 in Humans, is responsible for a thyroidal dysgenesis.
Our aim is to understand the molecular mechanisms underlying this condition.

Methods
The transgenic Dyrk1a (TgDyrk1a) mouse, our DS murine model, contains three copies of the Dyrk1a gene and was obtained through electroporation of a Bacterial Artificial Chromosome containing the entire gene with its own regulatory sequences. We studied their thyroidal phenotype in young adults (8–13 weeks old) by histology, immunohistochemistry and blood $T_4$ hormonal dosages, reflecting the thyroidal function. We compared the thyroidal molecular phenotype of the TgDyrk1a and wild type mice: RNA levels of molecules involved in the thyroidogenesis were studied by qRT-PCR at different embryonic stages.
Results
The average surface of thyroidal follicles in young adult TgDyrk1a mice is smaller (TgDyrk1a: 2164 $\mu m^2$ versus wild type: 1420 $\mu m^2$; $P=0.005$; $n=6$). They presented also a lower plasmatic $T_4$ (TgDyrk1a: 2.4 ng/ml versus wild type: 3.7 ng/ml; $P=0.019$; $n=14$). The overexpression of Dyrk1a in the thyroids leads to an elevation of RNA level expression of Nkx2-1, Foxe1, Thyroperoxidase and Thyroglobulin, involved in thyroidogenesis, at E13.5 and E17.5.
Conclusion
Our first results show an abnormal thyroid function and histology in young adult TgDyrk1a mice and an overexpression of thyroidal developmental molecules. To further understand the molecular mechanism linking Dyrk1a overexpression to altered thyroid folliculogenesis and function we are studying some candidates as direct targets of Dyrk1a using thyroidal cell lines.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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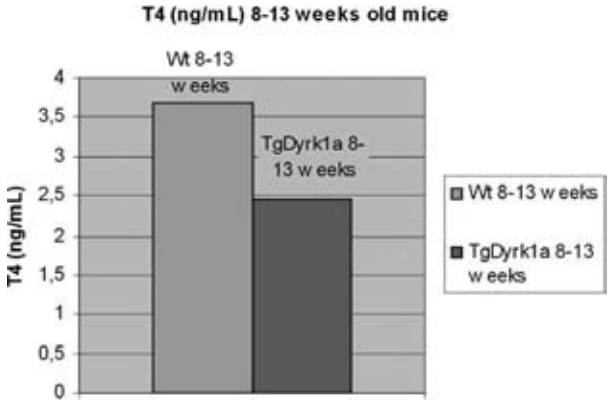


## P1610

**Impaired absorption of thyroxine in occult celiac disease**

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Some case report suggested that increased need for thyroxine may occur in overt celiac disease (CD). However, overt CD (with typical gastrointestinal symptoms) only represents 10 to 20% of cases of CD, being the majority represented by the atypical CD and other occult forms, in which extra intestinal symptoms prevail. Few data on the need for oral $T_4$ in patients with occult CD are available. In this study we analyzed the replacement $T_4$ dose in 68 hypothyroid patients with isolated Hashimoto's thyroiditis (HT) and in 35 patients bearing both HT and atypical occult CD. We have evaluated the ability of the same dose of $T_4$ to reach target TSH in 21/35 patients before and after gluten free diet (GFD). In the remaining 14/35 CD patients, noncompliant with GFD, we have analyzed replacement $T_4$ dose and compared with the one observed in the 68 patients with hypothyroid HT without CD. In patients with isolated HT, the desired serum TSH (median = 1.02 mU/l) was reached in all patients after $5 \pm 2$ months of treatment at a median $T_4$ dose of 1.31 μg/kg per day. After similar period and dose of $T_4$, higher levels of TSH (median = 4.20 mU/l) have been observed in patients with HT and CD. In 21 CD patients, target TSH (median TSH = 1.25 mU/l) has been attained after $11 \pm 3$ months of GFD without increasing $T_4$ dose (1.32 μg/kg per day). In the remaining 14 patients, noncompliant with GFD, target TSH has been achieved but at higher $T_4$ dose (median = 1.96 μg/kg per day; +49%; $P=0.0002$) than in hypothyroid patients without CD. Our data shows that absorption of thyroxine is impaired even in occult celiac disease and this effect is reversed by GFD or overcome by increasing $T_4$ dose. Malabsorption of $T_4$ may be a tool to unveil the presence of occult forms of celiac disease in $T_4$ treated patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This work was supported, however funding details unavailable.

## P1611

**Frequency of PDE8B gene polymorphisms in patients affected by sporadic and familiar nonautoimmune subclinical hypothyroidism**

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Background
Nonautoimmune subclinical hypothyroidism is characterized by elevated serum levels of TSH in the presence of normal thyroid hormone levels and absence of anti-thyroid antibodies. As a result of a genomic-wide study, a strong association between three polymorphic variants in exon 1 of human phosphodiesterase 8B (PDE8B) gene (rs4704397, rs6885099 e rs2046045) and serum levels of TSH has been recently reported. The aim of the present study was to evaluate the frequency of PDE8B gene polymorphisms in a group of patients affected by sporadic or familial nonautoimmune subclinical hypothyroidism.
Methods
The study group comprised 113 patients affected by sporadic (101) or familial (12) nonautoimmune subclinical hypothyroidism with elevated serum TSH levels (medium serum TSH $8.997 \pm 12.48$ μU/ml) and normal free circulating $T_3$ ($FT_3$) and $T_4$ ($FT_4$) levels. Genomic DNA was obtained from whole blood of patients using standard procedures to genotype patients for specific single nucleotide polymorphism (SNP) of PDE8B gene by TaqMan SNP genotyping assay and to sequence the entire coding region of the TSH receptor (TSHr) gene.
Results
The ancestral allele associated with increased TSH level was present in 82/113 patients (73%) for rs4704397, in 79/113 patients (70%) for rs6885099 and in 84/113 patients (74%) for rs2046045. However, similar values of serum TSH were detected in patients with minor or major allele for each polymorphism. Genetic analysis revealed the presence of TSHr gene mutations at the heterozygous state (D36H, P52T polymorphic variants; P68S, R109Q and P162A mild inactivating mutations) in 17/113 patients.
Conclusion
A prevalence of the minor allele of PDE8B gene SNPs associated with elevated serum levels of TSH was demonstrated in patients affected by sporadic or familial nonautoimmune subclinical hypothyroidism, however significant differences in circulating TSH in patients with minor or major alleles for each polymorphism were not identified demonstrating the lack of association between the polymorphisms and circulating TSH levels in these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1612

**MicroRNA expression profile helps to distinguish benign and malignant thyroid nodules starting from cells of fine needle aspiration**

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Objective
MicroRNAs (miRNAs) are small endogenous non-coding RNAs that pair with target messengers regulating gene expression. They are involved in biological processes including development, organogenesis, tissue differentiation, cell cycle and metabolism. Changes in miRNA levels occur in human cancers, including thyroid cancer. Fine needle aspiration (FNA) with cytologic evaluation is the most reliable tool for malignancy prediction in thyroid nodules, but cytologic

diagnosis remains indeterminate for 20% of nodules. In this study we evaluated the expression of miR-146b, miR-155, miR-187, miR-197, miR-221, miR-222 and miR-224 to distinguish benign and malignant thyroid nodules.
Methods
The study included 88 samples obtained by FNA of thyroid nodules from 86 patients (45 benign, 43 malignant). miRNA expression was evaluated by quantitative RT-PCR and statistical analysis of data was performed.
Results
All miRNAs increased in malignant nodules with respect to benign ones, but only the expression of miR-146b, miR-155, miR-187, miR-221, miR-222 and miR-224 significantly raised. Using data mining techniques we obtained a criterion able to classify a nodule as benign or malignant on the basis of miRNAs expression values. The decision model based on the expression of miR-146b, miR-155 and miR-221 was valid for 86/88 nodules (97.73%). To evaluate how much general is the criterion in correctly classifying a nodule not present in our study group, we adopted cross-validation techniques, obtaining a reliability of 78.41% (Sensitivity = 79.07% and Specificity = 77.77%).
Conclusions
The expression profiles of three miRNAs allowed to distinguish benign from malignant thyroid lesions starting from FNA, and may improve the accuracy of cytological analysis.
Declaration of interest
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## P1613

**Effect of combination therapy with potassium iodide and 15 mg of methimazole for Graves' hyperthyroidism**

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Introduction
Iodide administration is generally recommended in combination with anti-thyroid drugs when a rapid return to normal thyroid hormone levels is required, such as in the treatment of patients with 'thyroid storm'. However, there are few reports concerning the combination therapy for many patients with Graves' hyperthyroidism. In this paper, we studied the effects of potassium iodide (KI) combined with 15 mg of methimazole (MMI) to the initial treatment for hyperthyroidism, and to the changes in TRAb titers and goiter sizes during 2 years treatment.
Patients and methods
One hundred and thirty-two Japanese patients, who had moderate or severe thyrotoxicosis, were divided into two groups. The patients in group A (50 cases) were treated with 50 mg of KI and 15mg of MMI once daily, and those in group B (82 cases) were treated with 15 mg of MMI alone. There were no differences in the initial data between the two groups. The mean duration of KI administration was $11.4 \pm 6.9$ weeks in group A. In both groups, the MMI doses were gradually reduced to a maintenance level after the patients became euthyroid.
Results
1 After 4 and 8 weeks of treatment, normal $FT_4$ was observed in 42% and 80% of patients in group A, and in 30% and 65% of patients in group B, respectively. The mean time required to become euthyroid was $5.9 \pm 3.5$ weeks in group A, and $7.3 \pm 3.7$ weeks in group B. The difference was statistically significant ($P = 0.008$) . 2. The mean TRAb level before therapy was $55.0 \pm 21.7$% in group A and 52.6 $\pm 22.8$% in group B. Those in both groups significantly decreased after 6 months of treatment. The percentage of TRAb-positive patients decreased from 100% before therapy to 56% after 1 year and to 43% after 2 years of treatment in group A, from 96% to 60% and to 45% in group B, respectively. The mean goiter size before therapy, shown by cm in diameter, was $5.5 \pm 1.1$ cm in group A, and 5.3 $\pm 1.1$ cm in group B. After 2 years of treatment, the mean goiter size was $5.1 \pm 0.8$ cm in group A, and $5.2 \pm 1.2$ cm in group B, respectively. There were no differences in TRAb levels or goiter sizes between the two groups during therapy.
Conclusions
These results demonstrate the combination therapy is adequate for the treatment of patients with Graves' hyperthyroidism, especially who have moderate or severe thyrotoxicosis, without increasing doses of MMI and risks of adverse reactions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1614

**Estrogens block the apoptotic action of tgfb in human thyrocytes**

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Introduction
Multinodular Goiter (MNG) is a thyroid neoplastic disease thirteen times more common in women than in men. While a local effect of estrogens at cellular levels could affect there can be other causes to explain the gender difference in incidence such as diet, environmental factors, genetic causes or alterations in metabolic pathways.
Cellular effects of estrogens in human thyrocytes although previously studied are not completely clarified. Some reports suggest that they increase cell proliferation while others do not.
TGFb is a cytokine that has an important role in the normal function of the thyrocyte. It has two independent actions: an antiproliferative and a pro-apoptotic action. Previous results in our group, show that in the absence of other growth factors TGFb reveals its pro-apoptotic action and such action is related to a decrease in the levels of p27Kip1.
Aims
To study the effect of estrogens in the apoptotic action of TGFb in human normal thyrocytes in comparison with MNG thyrocytes.
Materials and methods
We used primary cultures of normal human thyrocytes (NT) and MNG from our bank of human thyrocytes in culture (BANTTIC). Our follicular cells in culture maintain the thyroid phenotype (TaqMan) and express alpha and beta estrogen receptors (immunohistochemistry). They were treated with TGFb or vehicle in the presence of a range of physiological concentrations of b-estradiol or vehicle following a time-course.
Apoptosis was measured by Hoescht. p27Kip1 levels were quantified by Western blot.
Results
In NT and MNG, the presence of physiological doses of estradiol (0.01 to 1 nM) blocked the pro-apoptotic effect of TGFb. In parallel, estradiol blocked the effect of TGFb on the repression of p27Kip1 levels.
Conclusions
Our results suggest an antiapoptotic action of estrogen against TGFb. This action may be mediated through p27Kip1.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1615

**TRAb level and ophthalmopathy index as predictive factors of poor response and of severity of Graves ophthalmopathy in patients treated with anti-thyroid drugs and radioiodine**

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Introduction
In a prospective study we determined factors affecting GO activity and poor response to glucocorticoid therapy in patients treated with radioiodine (131-I group) or with anti-thyroid drugs (ATD group).
Material and methods
The ATD and 131-I groups consisted of 168 patients (mean age $52.2 \pm 11.2$ years) and of 46 patients (mean age $52.1 \pm 13.3$ years), respectively. SoluMedrol pulses (8.0 g) were applied, followed by orbital irradiation (20 Gy). Levels of TSH, $FT_4$, and TRAb, CAS and IO were evaluated in all patients prior to treatment, and 1, 6, and 12 months after.
Results
Age, sex, GO duration, IO, CAS and $FT_4$ levels were similar in patients of both groups. In the 131-I group TRAb levels were significantly higher over the course of observation ($P < 0.05$). As evaluated using logistic regression, pre-treatment TRAb concentration was related to severe GO ($IO \geq 5$) only in the 131-I group ($RR = 1.047$, 95% $CL = 1.003–1.092$, $P \leq 0.05$). Poor responders were 69/167 of ATD and 13/46 of 131-I patients who required further oral glucocorticoids following SoluMedrol pulses. Only pretreatment TSH and IO were predictive of poor response ($P < 0.05$), with $IO \geq 6$ threshold, as based on discriminant analysis (sensitivity 0.47, specificity 0.71). In the ATD group, IO was found to be correlated with TRAb concentration at 0, 1, and 6 months ($P < 0.05$; e.g. for TRAb-0 vs IO-0, $r = 0.326$). In the 131-I group, correlation was stated between TRAb concentration and IO over the time of observation ($P < 0.05$, e.g. TRAb-12 vs IO-12, $r = 0.436$).

Conclusions
Pretreatment TRAb is predictive of risk of severe GO only in 131-I patients. Pretreatment TSH and IO are risk factors of being a poor responder to glucocorticoid therapy in ADT and 131-I patients. Correlation between TRAb and IO was found in both groups.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1616

**A possible common link between Graves' disease and allergic immune response**

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Background
The elevation of serum immunoglobulin E (IgE) has been recently reported to be associated with Graves' disease (GD). Interleukin 13 (IL-13) is a major cytokine involved in IgE synthesis and therefore may be a potential candidate gene influencing the clinical course of the GD. The aim of this study are to evaluate whether the atopic parameter such as total IgE is related to TSH receptor antibody (TRAb, thyrotropin-binding inhibitory immunoglobulin: TBII) and whether IL-13 gene polymorphisms are associated with the course of GD.
Methods
In 405 patients with GD, we investigated the history of common allergic diseases by questionnaire and measured total IgE and TRAb. We also examined IL-13 gene single-nucleotide polymorphisms in the 5' promoter region at position −1112 (C to T change, C-1112T) and in exon 4 at position −2044 (G to A change, G2044A).
Results
Log-transformed TRAb was higher in patients who had clinical symptoms of asthma ($2.30 \pm 1.44$ vs $1.79 \pm 1.36$, $P = 0.011$) and treatment history of it within a year ($3.37 \pm 2.15$ vs $1.84 \pm 1.36$, $P = 0.014$). Log-transformed TRAb was related positively with log-tranformed total IgE ($r = 0.136$, $P < 0.01$). Subdividing GD patients according to the clinical symptoms of asthma, −1112T/T + C/T genotypes had significantly increased in patients with asthma symptoms (42 vs 26%; $\chi^2 = 5.788$; $P = 0.016$). Subdividing them according to the treatment history of asthma within a year, −2044A/A + G/A genotypes had significantly increased in patients with treatment history (100 vs 51%; $\chi^2 = 4.687$; $P = 0.030$).
Discussion
TRAb was significantly related with total IgE, known as an atopic marker. TRAb and IL-13 gene polymorphisms were both significantly increased in patients with clinical symptoms or treatment history of asthma. Our results suggest that there might be a possible common link between autoimmune thyroid disease and allergic immune response.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1617

**Subclinical hyperthyroidism (SH) in the elderly: symptoms, depression and quality of life (QoL) and relationship with TSH concentration in a prospective cohort of 110 patients**

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SH is defined by a decrease of TSH with normal thyroid hormone (TH) concentrations. The clinical picture is not clear, whether these patients may have symptoms or alterations of QoL. It is well known that in overt hyperthyroidism, symptoms may be absent in old patients. In SH, one study in young patients has reported an increased symptom score and a slight decrease of QoL but these results have not been confirmed in middle-aged subjects. We report the initial data of the Pirathes study, an ongoing randomized study comparing radioiodine treatment vs monitoring in SH. We particularly analyzed the results regarding the 'grade' of SCH as some authors have proposed to distinguish patients with TSH below 0.1 and patients with $0.1 \leq TSH < 0.4$ mU/l.
Inclusion criteria were: age $> 50$, durably $TSH < 0.4$ mU/l, normal TH, signs of thyroid autonomy on a thyroid scan and sinusal heart rhythm. After randomization, patients are seen every 4 months. At each visit, they fill 3 questionaries: one on thyroid related symptoms, one on anxiety and depression (HADS) and one on health-related QoL (SF36). We report here the data at inclusion of the first 110 patients included.
Patients (M27, F83) had a mean age of $64.6 \pm 8.8$, and a TSH $0.09 \pm 0.09$ mU/l. 72.6% had a $TSH < 0.1$, 26.4% between 0.1 and 0.4 mU/l. 28.8% could considered as symptomatic, mainly with symptoms of hyperthyroidism but 11% of the whole population had rather symptoms of hypothyroidism. HADS anxiety score was $8.6 \pm 3.6$ and HADS depression score $4.5 \pm 3.7$. SF36 was comparable to that of the general population (PCS $47.0 \pm 8.1$, MCS $45.5 \pm 11.9$). There were no differences between 'grade 1' and 'grade 2' patients for BMI, heart rate or blood pressure. Patients with $TSH < 0.1$ had a tendancy to be more anxious and more depressive ($-0.7$ and $-1.0$ respectively) and to have a decrease of QoL but the differences were significant only for PF and RP components of the SF36. Symptoms were also more pronounced in these patients. HADS, QoL and symptoms scores were highly correlated.
Our study shows that elderly patients with SH have few symptoms and no clear alteration of QoL. However, patients with $TSH < 0.1$ have a tendancy to be more anxious and depressed and to have slight alterations in Qol and this could bring a clinical basis to the recent concept of 'grading' SH.
Declaration of interest
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## P1618

**Thyroid function of Japanese elderly**

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Serum TSH is the most sensitive index of thyroid function in the absence of hypothalamic–pituitary disease.
However, TSH reference limits are controversial, particularly the upper limit. District and age subpopulations have unique TSH distribution. We investigated characterization of thyroid hormones in Japanese elderly. 859 participants (mean age 74.3 years) over 60 years without known thyroid disease were surveyed.
Percentages of people whose TSH were over 5.0 mU/l were respectively 9.2, 18.7, and 30.1% in male and 11.2, 15.4, and 16.4% in women among 60s, 70s, and over 80 years. A progressive increase in prevalence of TSH above 5.0 mIU/l with age existed.
Of 744 participants who were assayed concentrations of free $T_4$, 703 subjects were within limits of free $T_4$. Additionally, TSH, free $T_4$, and free $T_3$ of 610 subjects were assayed at the same time. A progressive decrease was observed in the concentration of free $T_3$ with age in both sex, and TSH mildly increased with age.
In elder people, especially over 80 year male, these tendencies were clearer. Conversion from $T_4$ to $T_3$ might be decreased in Japanese elderly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1619

**Assessment of cognitive function in hypothyroidism using P300 evoked potentials**

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The P300 wave is an event related potential which is used to assess cognitive function, it is recorded as positive deflection in voltage at latency of roughly 300 ms. P300 latency suggests that shorter latency times are related to cognitive performance. This study was undertaken to evaluate objectively effect of hypothyroidism on latencies of ERPs and response to thyroxine replacement.
Biochemically proven new onset cases with hypothyroidism were enrolled into study. After detailed history and physical examination and routine biochemical investigations P300 cognitive potentials were recorded using a Nicolet Viking Select neuro-diagnostic system version 10.0. Study was done in electrophysiology lab in Osmania Medical College.
Results
Patient characteristics (i.e age, sex, BMI) of both cases and controls were comparable. Cases consisted of 2 groups overt hypothyroid cases −24, mean TSH values in them was 94.1.subclinical −21 cases in whom mean TSH value was 12.3.
Mean P300 latencies of all cases at Cz was 342.42±29.5, and at Pz was 345.4 ±30. Mean P300 latencies of controls at Cz was 296.4±34 and at Pz was 297.9±33.Difference between cases and controls was highly significant p value $<0.001$. Mean P300 values in overt cases was 362.6±32.9 at Cz, and at Pz it was 362.5±33.9. Mean P300 values in subclinical cases was 319.3 +/30.9at Cz, and at Pz it was 316.4±27.9. P300 values in overt cases was highly significant compared to controls. Whereas p300 values in subclinical cases vs controls was just significant.
There was statistically significant difference in p300 values (at Cz and Pz) before and after treatment in overt ($P$ value $<0.001$)as well as subclinical cases ($P$ value $<0.009$).
P300 latency prolongation in both clinical and subclinical hypothyroid cases shows that cognitive function is affected adversely even in subclinical hypothyroid cases. Most psychometric tests assessing cognitive function can identify gross defects in cognition,hence these will not be able to detect subtle changes in subclinical hypothyroidism. Such subtle defects may not be of importance in elderly individuals but willaffect performance of younger individuals in day to day life.
This test of auditory evoked potentials p300 may be useful to identify patients with subclinical hypothyroidism who will benefit with treatment as against those who can be followed up.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1620

**Successful treatment of hyperthyroidism is associated with significant risks of overweight/obesity independent of the treatment modality employed**

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Introduction
Obesity is a growing health concern in developed countries. Patients undergoing treatment for hyperthyroidism frequently express concerns regarding excessive weight gain, especially when offered treatment with I-131.
Methods
We investigated 1047 patients with overt hyperthyroidism attending a specialist thyroid clinic to determine the extent of weight changes and to identify risk factors for weight gain following treatment. Weight differences were calculated by comparing weight at presentation and at discharge from clinic.
Results
(69.4%) patients gained ≥5% of their presenting body mass (mean gain 9.9 ±0.2 kg; mean BMI increase 3.6±0.08 kg/m$^2$) during 22.03±0.42 months. 44.2% of patients with normal BMI at presentation became overweight or obese and 44.6% of overweight patients developed obesity at the end of treatment. Weight gain was most intense during the initial 6 months of treatment but continued relentlessly during 36 months when body weight had increased >10% ($P<0.001$). At discharge, the proportions of obese men and women were significantly higher when compared with age and gender specific population of the West Midlands ($P<0.001$). Similar proportions of subjects gaining weight were observed in those treated with thionamides and subjects receiving one or multiple doses of I-131 (weight gain in 66.8%,70.5%,72.3% respectively, $P$=NS) . The reporting of weight loss prior to presentation (n=702, AOR: 3.0, $P<0.001$) , higher presenting serum $fT_4$ concentrations (AOR: 1.01 per 1 pmol/l, $P<0.001$), longer treatment duration (AOR: 1.02 per month, $P=0.001$), and male gender (n=226, AOR: 1.44, $P=0.05$) were independently associated with increased probabilities of weight gain. Patients' age, smoking status, ethnic origin, weight status at presentation, the treatment modality and the development of hypothyroidism were not associated with increased likelihoods of weight gain.
Conclusion
Cure of hyperthyroidism is associated with marked weight gain and significantly increased risks of becoming overweight or obese, independent of the treatment modality employed. Subjects with more severe hyperthyroidism, those reporting prior weight loss, patients requiring longer treatment and men are particularly at risk of gaining weight.
Declaration of interest
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## P1621

**Oral anticoagulant therapy: is it the same in hyperthyroid patients?**

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Aim of this study was to evaluate the effectiveness and safety of oral anticoagulant therapy in patients with atrial fibrillation and hyperthyroidism compared to those presenting the same cardiac disorder, but with normal thyroid function.
Material and methods
The study included 61 patients admitted to the Endocrinology Department in Elias Hospital between January 2011–October 2011 with mean age of 64 years: 25 patients had primary hyperthyroidism and atrial fibrillation, 36 patients had atrial fibrillation with normal thyroid function, used as a control group. The patients were followed during the hospitalization (mean period 10 days) regarding the thyroid function tests (TSH, Free $T_4$) the international normalized ratio (INR) and the dose of oral anticoagulant taken (acenocumarol).
Results
All patients (20 women and 5 men) in the hyperthyroid group had a supressed TSH (below 0.01 UI/l) and a near upper limit values of free $T_4$. They were recently diagnosed with either Graves's disease (18 pts) or toxic multinodular goiter (7 pts) and received antithyroid therapy at admitance in the hospital. The acenocumarol dose at the admitance was an average of 0.36±0.19 mg daily with an average INR of 1.8±1.1.
In the control group (23 women and 13 men refeered for thyroid disfunction but proved to have normal TSH and free $T_4$ and no history of thyroid disfunction), the average dose of accumarol was 0.71±0.2 daily at the admitance with an average INR of 2.16±0.6.
We found a significantly lower dose of acecumarol needed in the hyperthyroid patients (0.3 comparing to 0.7 mg) comparing to controls with no significant differences in the INR values between groups. Taking into account the dynamic of the INR values during the follow up, we found a significantly higher variations of the INR values in the hyperthyroid group (from 1.8 to 2.7 average). In this group we have noticed 5 cases of minor adverse effects comparing to none in the control group during the follow up period.
Conclusion
Achieving and maintaining target levels of efficiency and safety of oral anticoagulant treatment throughout the hyperthyroidism might be more difficult due to important fluctuations of INR values during the antithyroid treatment.
Therefore hyperthyroid patients require more frequent monitoring of INR because imbalances especially in the sense of overdose are unpredictable and can have serious consequences.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1622

**Thyroid hormone abnormalities in haemodialyzed patients: low triiodothyronine as well as high reverse triiodothyronine are associated with increased mortality**

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Objective
Numerous abnormalities of thyroid hormones in end-stage renal disease (ESRD) have been described. Our aim was to analyze the impact of these abnormalities on survival.
Design
In 167 haemodialyzed ESRD patients, TSH and thyroid hormone levels (including reverse $T_3$, i.e. $T_4$, $fT_4$, $T_3$, $fT_3$, $rT_3$) were determined. The patients were then prospectively followed up for up to 5 years and the possible impact of any observed abnormalities on their mortality was studied.
Results
Only 16.8% patients had all six tests within the reference range. The pattern of nonthyroidal illness syndrome was found in 56.3%. Low $T_3$ was particularly common (44.3%), and clearly associated with increased 6- and 12-month mortality and decreased overall survival (log-rank test, $P=0.007$). Independent of $T_3$ levels (Spearman correlation, NS), increased $rT_3$ was more frequently observed (9.9%) than expected from the literature, and was also related to increased mortality and decreased survival (log-rank test, $P=0.021$).
Conclusions
Increased $rT_3$ may be more common in ESRD patients than previously expected, and together with decreased $T_3$ it may serve as an indicator of poor prognosis in subsequent months.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1623

**Perioperative clinical outcomes after robot-assited thyroid surgery by transaxillary approach**

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Background
Many endoscopic minimaly invasive procedures have been developed and are used now in thyroid and parathyroid surgery. However, the endoscopic approach is more technically demanding and due its limitations, endoscopic thyroidectomy remains limited in application and practiced in a relatively small number of centers. Recently robotic technology has been also applied to thyroid surgery. The aim of this study is to present our experience and to demonstrate the technical feasibility, intraoperative safety and efficacy of robotic thyroidectomy.
Methods
The technique that we are practicing use the unilateral transaxillary approach without gas insufflation. We used the da Vinci SI Surgical Robotic System for all interventions and we performed 35 total unilateral lobectomy and 7 total thyroidectomy. Patients were diagnosed with unilateral thyroid nodules and 7 of them, whit bilateral thyroid nodules. For each patient we analyzed the clinical characteristics, tumor size, pathologic type, operative time, amount of drainage, duration of hospital stay, postoperative complications, postoperative neck and anterior chest pain, and cosmetic satisfaction.
Results
All operations were performed successfully without any need for conventional open conversion. The mean overall operation time was 155 min respectively 80 min console time. The mean tumor size was 3.5 cm. There was one temporal brachial plexus neuropraxia and 4 wound seroma. There were 2 malignant thyroid lesions at the final histopathological results. In this situation we perform total thyroidectomy by open cervicotomy during the same hospitalisation.
Conclusions
Robotic technology overcame some technical limitations associated with conventional endoscopy. Robotic thyroidectomy by gasless transaxillary method is feasible, safe, and provided good outcomes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1624

**Hyperthyroidism in patients with acute atrial fibrillation attended at an emergency room**

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Introduction
Atrial fibrillation (AF) is the most common cardiac complication of hyperthyroidism, with significant mortality and morbidity resulting from embolic events. In patients who attend at an emergency room (ER) with acute AF, the thyroid hormones aren't usually evaluated given the low prevalence of hyperthyroidism in the general population.
Aims
To assess the prevalence of thyroid dysfunction in adult patients who presented at an ER with acute AF.
Methods
Prospective study of consecutive patients with acute AF that attended our hospital's ER; excluded those with hypothalamic or pituitary disease, pregnancy, corticosteroid or dopamine therapy, hemodynamic instability. Selected as controls patients in sinus rhythm who presented at the ER on the same period of time. All patients answered a brief questionnaire (age, medications in use, medical history, saline restriction), underwent clinical examination, electrocardiogram and blood samples for thyroid function tests. Those with an altered serum TSH level were subsequently reevaluated for confirmation of thyroid dysfunction.
Results
Total of 105 individuals, 61 patients (acute AF) and 44 controls (sinus rhythm). Cases had a higher prevalence of thyroid dysfunction (37.7% vs 9.1%, $P=0.001$), total hyperthyroidism (24.6% vs 2.3%, $P=0.002$), overt (14.8% vs 0%, $P=0.008$) and subclinical hyperthyroidism (9.8% vs 2.3%, $P=$n.s.). There was a negative correlation between serum TSH levels and heart frequency of the arrhythmia ($\rho=-0.36$, $P=0.004$) and also between serum TSH levels and the number of anti-arrhythmic drugs necessary for treatment ($\rho=-0.313$, $P=0.014$). The odds-ratio of an acute AF patient having hyperthyroidism was 14.02.
Conclusions
The high prevalence of hyperthyroidism in patients with acute AF attended at our ER suggests that routine thyroid testing is required. Serum TSH level negatively correlates with heart frequency, emphasizing the importance of thyroid function in conversion to sinus rhythm.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1625

**Alterations in adiponectin concentration in hyper- and hypothyroid patients; possible underlying mechanisms and role of metformin treatment in experimental animals**

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Recent reports on the interplay between adiponectin, thyroid hormones and metformin have given conflicting results. Our study aimed to investigate adiponectin concentration in thyroid disorders and to assess the possibility of involvement of adiponectin in the regulation of thyroid hormone production, also to clarify the role of pituitary-thyroid axis in the modulation of adiponectin production and to assess the interplay between metformin treatment, thyroid function and adiponectin. The study was designed on 2 modules; In the human 30 study females were included in 3 groups; control, hyperthyroid and hypothyroid. Body mass index (BMI), serum adiponectin, thyroid profile, total cholesterol

(TC), high density lipoprotein(HDL) and HOMA-IR were measured. In the animal study, female albino rats divided into control, experimentally-induced hyperthyroid, experimentally-induced hypothyroid, hyperthyroid + metformin, hypothyroid + metformin groups. gC1q gene expression (in thyroid tissue), adiponectin, TSHr, PPARγ in adipose tissue were measured. Results: increased serum adiponectin, decreased BMI, unchanged HOMA-IR or lipid profile in hyperthyroid patients. Decreased serum adiponectin and HDL, increased TC and HOMA-IR in hypothyroid patients. increased adiponectin expression in hyperthyroidism rats and its decrease in hypothyroidism without observed weight changes with unchanged TSHr expression in different thyroid states. In hypothyroidism decreased PPARγ expression which was improved by metformin, which also improved thyroid function and adiponcectin expression. Decreased gC1q expression in thyroid disorders and its increase by metformin. Conclusion: increased adiponectin concentration and expression in hyperthyroidism and their decrease in hypothyroidism. These observed changes in adiponectin could contribute to changes in HOMA-IR and lipid profile detected hyper and hypothyroidism. Adipose tissue TSH receptors are not involved in the modulation of adiponectin production. In case of hypothyroidism, there is a link between decreased adiponectin secretion and expression, decreased PPARγ receptors and dyslipidemia. Metformin improved thyroid function in hypothyroidism and has an antiapoptotic effect in both thyroid disorders.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1626

### A liquid formulation of L-thyroxine (L-T4) solves problems of incomplete normalization/suppression of serum TSH caused by proton pump inhibitors (PPI) on conventional tablet formulations of L-T4

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Background

PPI are known to diminish the intestinal absorption of L-T4, which is commonly ingested as tablets of L-T4 sodium salt. However, a liquid formulation is available (Tirosint, oral solution, IBSA Farmaceutici Italia s.r.l., Italy), in which the hormone is solubilized in 28.8% ethyl alcohol. One ml of this formulation (=28 drops) contains 100 mcg L-T4 sodium salt in 243 mg ethanol. Because this formulation contains L-T4 (which is a lipophilic hormone) already solubilized in an organic solvent, as opposed to L-T4 contained in a powder within a tablet, there could be the advantage of virtually skipping the dissolution in the stomach, and subsequent quick, direct arrival of L-T4 in the upper intestine segments (duodenum, jejunum) where most of it is absorbed.

Patients & methods

Upon consent, 1 woman under replacement therapy [100–125 mcg/day] and 9 patients (7 women and 2 men) under TSH suppressive therapy [82–135 mcg/day], all 10 with PPI-induced malabsorption of LT4, were switched to Tirosint oral solution while maintaining the daily dose of L-T4 and continuing ingesting the PPI as before. The PPI were: lansoprazole, omeprazole or pantoprazole. Post-switch serum TSH was re-assayed after a minimum of 2 months.

Results

In the hypothyroid woman, TSH fell to 2.0–2.7 mU/L down from 5.0–7.3. In the other 9 patients, TSH fell to $<0.01$ to 1.97 mU/L (median: 0.10; mean $\pm$SD: $0.68 \pm 0.91$), down from 0.28–2.95 (median: 0.81; mean $\pm$SD $1.02 \pm 0.74$; $P=0.0004$ by ANOVA after log10-transformation), and with 65% of the post-switch TSH levels at 0.10 mU/L or below compared to 0% (zero%) of the pre-switch levels ($P<0.0001$ by Fisher's exact test).

Conclusion

The liquid formulation of L-T4 is an extremely effective means to circumvent the problem of incomplete absorption of the L-T4 caused by the PPI-induced increase of the gastric pH.

Declaration of interest

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## P1627

### Serum chemokine CXCL10 is increased in chronic autoimmune thyroiditis associated with autoimmune gastritis

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Autoimmune thyroiditis (AT) may occur as a single disease or associated with further endocrine and non endocrine autoimmune diseases (NEAD). Chronic autoimmune gastritis (CAG) is the more frequently associated NEAD. A prevalent Th1-representative pattern of cytokines and chemokines has been described in isolated autoimmune thyroiditis. However, the behaviour of chemokines when AT is associated with CAG is not known. Aim of the study has been to measure serum levels of CXCL10 in patients with chronic autoimmune thyroiditis in association with CAG. Serum CXCL10 was assayed in 95 consecutive AT patients, in 95 gender and age-matched healthy volunteers (Controls, C) and in 45 patients with autoimmune thyroiditis plus CAG. All patients studied were free from: a) active infectious diseases in the last three months; b) treatment with drugs known to interfere with immune system, namely cytokines, interferon, corticosteroids, NSAIDs, amiodarone, lithium; c) pregnancy and lactation over the previous 6 months; d) the presence of acute or chronic systemic diseases. High CXCL10 levels (defined as a value higher than mean+2SD of controls; i.e. $>103$ pg/ml) were observed in 3% of controls, in12% of patients with isolated AT and in 45% of patients with AT+CAG, ($P<0.0001$). Hence, the mean serum levels of CXCL10 were significantly higher in patients with AT, isolated or associated with CAG, than in control subjects ($C=49 \pm 27$ pg/ml, $AT=74 \pm 29$ pg/ml, $AT+CAG=126 \pm 121$ pg/ml; $P=0.003$). Moreover, AT+CAG patients also showed significantly higher CXCL10 levels than those with isolated AT (Bonferroni-Dunn: $P=0.0004$). In conclusion, in patients with AT and autoimmune gastritis, circulating CXCL10 concentrations are significantly higher than in patients with isolated autoimmune thyroiditis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1628

### Association between preoperative value of TSH and pathological findings in nodular thyroid disease

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Higher serum TSH has been recently postulated as a predictor of thyroid malignancy in nodular thyroid disease. In addition, some reports have demonstrated its association with more advanced stages of differentiated thyroid cancer (DTC).

Material and methods

We collected retrospectively preoperative serum TSH from patients who underwent total thyroidectomy in the period 2005–2011 in a single tertiary center. We recorded demographic data, nodule number, tumour size and final anatomic pathology. Patients with Graves disease or primary hypothyroidism under treatment previous to surgery were excluded, and so were cases with medullar or anaplastic thyroid cancer.

Results

524 patients including 187 with malignancy; 453 females, 154 with malignancy (34%), as 33 of the 71 males (46.5%). Patients with cancer were younger ($51.6 \pm 14.7$ yr) than those with benign nodules ($55.7 \pm 14.3$ yr, $P<0.001$). It was uninodular disease in 17.2% of benign disease and 34.2% of diagnosed as malignancy ($P<0.0001$). Preoperative mean TSH levels were higher in patients with malignancy vs. benign pathology ($2.53 \pm 2.5$ vs. $1.50 \pm 1,8$; $P<0,0001$). Out of the 187 cases of DCT, 48 were incidental microcarcinomas (INCM), with mean TSH of $2.10 \pm 1.82$, still different from bening cases ($P=0.004$). Excluding these, the mean TSH was $2.68 \pm 2.69$ in the remaining DTC ($P<0.001$ vs INCM). There was difference in mean TSH between follicular adenomas (FA) (n:65; $2.51 \pm 3$) and hyperplastic goitres (n:264; $1.21 \pm 1.2$); but no between FA and CPT ($P=0.25$). Median TSH in DTC shows a tendency to be higher with bigger tumour size (1.78 vs 2.14; $\leq 2$ vs $>2$ cm; $P=0.099$).

Conclusions
Our series, as others published, depicts benign lesions having lower TSH level, with difference between pathologic subtypes, while DTC, and similarly to FA, being associated with higher level. The correlation among histotype, tumour size and TSH level suggests a role of TSH in the carcinogenesis of DTC and pathogenesis of FA.
Declaration of interest
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## P1629

**Clinical presentation (symptoms and complications) of hyperthyroidism in a cohort of 1,144 patients: results of the Thyrdel study**

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Symptoms and physical signs are variable and non specific in hyperthyroidism (HT). We report the results of a large study conducted among 263 French endocrinologists. Each investigator was asked to include all consecutive patients seen for HT during the study period. For each patient, symptoms, physical signs, biological data and treatment were recorded. The endocrinologists included 1,144 patients with HT: 802 Grave's disease (GD), 121 multinodular goitres (MNG), 112 iatrogenic HT (mainly amiodarone), 69 toxic adenoma and 40 thyroiditis. 18.3% had subclinical HT (GD 11.0%, MNG 49.5%, iatrogenic HT 7.9%, toxic adenoma 61.4%, thyroiditis: 35.3%).
Mean age was 48.7 years (ranging from 42.7 for thyroiditis to 63.6 for MNG), 76.3% were females (78 to 83.5% for every etiology except for iatrogenic: 27.2%) Most patients (81.9%) were diagnosed because they had clinical signs of HT except those with iatrogenic HT (mainly diagnosed by specific screening): 72.1% had palpitations, 74.5% had fatigue, 28.4% had digestive symptoms, 51.8% had thermophobia/sweating/polydipsia, 65.5% tachycardia, 16.7% had arrythmia (more frequently among iatrogenic HT: 55.4% and MNG: 24.2%), 66.5% weight loss (on average $-6.3$ kg), 32.2% had a clinical goiter. Symptoms were more frequent among GD, toxic adenoma and thyroiditis. Palpitations, thermophobia/sweating/polydipsia and weight loss (but not the other signs) were more frequent among patients whose TSH was below 0.1 mU/L compared to patients with $0.1 < \text{TSH} < 0.4$ mU/L ($P < 0.05$). Patients older than 65 years had less thermophobia/sweating/polydipsia symptoms, slower heart rate but more cardiac arythmia compared with younger subjects. More than 2/3 of patients with subclinical HT had at least one symptom of HT, they had a significantly higher heart rate but no symptom appeared discriminant with overt HT.
This large cohort study will improve our knowledge on epidemiological and clinical presentation of HT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1630

**Beta (CCL2) and alpha (CXCL10) chemokines modulation by cytokines and by peroxisome proliferator-activated receptor-alpha agonists in Graves' ophthalmopathy**

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Introduction
No study has evaluated the effect of cytokines on the prototype beta chemokine (C-C motif) ligand (CCL)2 in Graves' ophthalmopathy (GO), nor of peroxisome proliferator-activated receptor (PPAR)alpha activation on this chemokine secretion in fibroblasts or preadipocytes in GO.
Design and methods
We have tested the interferon (IFN)gamma and tumor necrosis factor (TNF)alpha effect on CCL2, and for comparison on the prototype alpha chemokine (C-X-C motif) ligand (CXCL)10, and the possible modulatory role of PPARalpha activation on these chemokines secretion in normal and GO fibroblasts or preadipocytes in primary cell cultures.
Results
The present study shows that IFNgamma alone, or in combination with TNFalpha was able to stimulate the secretion of CCL2 in primary orbital fibroblasts or preadipocytes from patients with GO, at levels similar to those observed in controls. IFNgamma and TNFalpha stimulated also CXCL10 chemokine secretion as expected. The presence of PPARalpha and -gamma in primary fibroblasts or preadipocytes from patients with GO has been confirmed. PPARalpha activators were able to inhibit the secretion of CXCL10 and CCL2, while PPARgamma activators were confirmed to be able to inhibit CXCL10, but had no effect on CCL2. PPARalpha activators were stronger inhibitors of chemokines secretion than PPARgamma agonists.
In conclusion, CCL2, and CXCL10, are modulated by IFNgamma and TNFalpha in GO. PPARalpha activators are able to inhibit the secretion of the main prototype alpha (CXCL10) and beta (CCL2) chemokines in GO fibroblasts or preadipocytes, suggesting that PPARalpha may be involved in the modulation of the immune response in GO.
Declaration of interest
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## P1631

**Thyroid function in large elderly population – results of multicenter study performed in Poland**

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Multidimensional studies of medical aspects of ageing were performed in 6 provinces of Poland, the protocol included cross sectional analysis of thyroid function parameters.
The sample was randomly chosen and included 4,051 respondents (49.2% females).
Group consisted of 3,502 elderly subjects aged 65–90+y, divided into approximately equal six age subgroups; and 549 younger cohort, aged 55–59 y.
Thyroid function was assessed by TSH measurement (IRMA, reference range 0.2–4.5 mIU/ml.). Clinical data were not included.
Mean TSH concentration in the population was $2.55 \pm 6.7$ mIU/ml; median 1.6. Gender related differences were found. TSH was higher in women than in men ($2.9 \pm 8.6$ vs $2.2 \pm 4.6$; $P < 000018$); median respectively:1.7 and 1.55. In 25% of the cohort TSH exceeded 2.5 mIU/ml. TSH did not differ between age subgroups.
About 11% of the population had abnormal TSH suggesting thyroid dysfunction.
Among elderly subjects 7.9% were assessed as hypothyroid (10% women and 5.6% men) and 2.9% as hyperthyroid (3.5% women and 2.4% men).
In the youngest group 7.65% were hypothyroid and 1.8% hyperthyroid.
Upon the simultaneous fT4 measurements, thyroid dysfunctions were classified as subclinical in 98% hypothyroid and in about 70%. of hyperthyroid subjects. Most of cases of advanced thyroid dysfunction were found among the oldest subjects.
Conclusions
This large population study revealed thyroid dysfunction in over 10% elderly subjects. There is some possibility of misinterpretation of TSH resulting from the influence of extrathyroidal factors; yet wide reference range of TSH used in this study decreased this risk.
This study reminds the need of careful attendance to the dysfunctions in elderly patients, especially that symptomatology of thyroid disease in this age groups can be misleading.
Declaration of interest
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## P1632

**Late manifestation of subclinical hyperthyroidism after goitrogenesis in an index patient with a N 670 S TSH receptor germline mutation causing familial non-autoimmune autosomal dominant hyperthyroidism (FNAH)**

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In the 27 families with FNAH the onset of hyperthyroidism varies from 18 months to 60 years. Also the manifestation of goiters is variable in these families.
A 74 year old women first presented at the age of 69 years in 9/2006 with tachyarrythmia and hypertension. After the initial treatment of her hypertension and oral anticoagulation for her intermittant atrialfibrilation 3/2007 a thyroid workup revealed a suppressed TSH of 0.029 (normal < 0.4 mU/l) and normal fT3 and fT4. TPO, TSH-receptor and thyreoglobulin antibodies were negative. Thyroid ultrasound reveiled a thyroid volume of 102 ml with several nodules with diameters of up to 2.6 cm right and up to 1.8 cm left. Szintigraphy showed a homogeneous Tc uptake of 1.95%. She was treated with 1 GBq I 131 in 6/2007 which normalised her thyroid function untill 12/2007 (TSH 3.05 mU/L). Her brother and sister do not suffer from thyroid diseases. Her 45 year old daughter and her 16, 12 and 11 year old grandsons do not have symptoms of hyperthyroidism and display normal TSH, fT3 und fT4.
Because of the diffuse Tc uptake and the negative TPO, TSH-receptor and thyreoglobulin antibodies her TSH receptor gene was analysed in DNA extracted from EDTA blood.in spite of her negative family history for hyperthyroidism. Sequencing revealed a N 670 S TSH receptor germline mutation. This TSH receptor germline mutation's constitutive activity could previously only be demonstrated in HEK but not in COS cells (Mueller et al. 2009).
This case illustrates the necessity to analyse patients with hyperthyroidism accompanied by diffuse Tc uptake and negative TPO, TSH-receptor and thyreoglobulin antibodies (so called antibody negative Graves' disease) for TSH receptor germline mutations. Moreover, it illustrates that TSH receptor germline mutations may first lead to long standing nodular goitrogenesis before the late manifestation of subclinical hyperthyroidism.
Declaration of interest
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## P1633

**The alterations of the endothelial function, haemostatic and inflammatory parameters in subclinical and overt hyperthyroidism**

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Subclinical hyperthyroidism (SH) is characterised by supressed serum TSH concentration accompanied by fT3 and fT4 levels maintained within their reference ranges. The vascular endothelium as a specific target of thyroid hormones is strongly affected in hyperthyroidism. We aimed to compare the alterations of the endothelial function, haemostatic and inflammatory parameters in patients with SH in comparison with overt hyperthyroidism (OH) due to Graves' disease and toxic nodular goiter. In the present study, we determined serum levels of soluble forms of ICAM-1, VCAM-1, vWF, IL-6, IL-12, IL-18 and CRP to elucidate a possible role of those parameters as markers of endothelium dysfunction (ED). 117 patients were included into the study: 56 patient with OH, 61 with SH and 39 euthyrotic controls (CG). In our study fibrinogen, vWF, PAI-1 and VCAM-1 levels increased significantly in study groups compared with the CG. We also found an increase in serum CRP, IL-6, IL-12 and IL-18 levels in OH and SH groups. In SH group we observed the highest levels of serum CRP and IL-6. Similar values for sICAM-1, E-selectin were demonstrated in study and CG. These results may reflect a relative hypercoagulability state both in subjects with SH and OH. In summary, our results suggest that hyperthyroidism is accompanied by the endothelial damage, which depends both on the cause of thyrotoxicosis and on degree of hyperthyroidism. Elevated concentrations of the markers of the endothelial damage may serve as a confirmation that persons with hyperthyroidism are extremely predisposed to the occurrence of the cardiovascular diseases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1634

**Prediction of remission rate in the patients with Graves' disease after treatment with a minimum maintenance dose of Methimazole**

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Introduction
According to the guideline for medical treatment of Graves' disease by the Japan Thyroid Association in 2011, discontinuation of antithyroid drug is recommended when normal thyroid functions have been maintained for a certain period ($\geq$6 months) after treatment with a minimum maintenance dose (mentioned below). We prospectively studied the remission rates in the patients with Graves' disease.
Methods
The subjects were 108 patients with Graves' disease, who were newly diagnosed from 2003 to 2008 (mean age, 46.2$\pm$13.2 years old, 22 men and 86 women). They were treated with methimazole (MMI) at the initial dose of 15 mg/day. MMI doses were gradually decreased to minimum maintenance dose (5 mg every other day).In this study, the duration of minimum maintenance dose was defined as 12 months. We finally discontinued MMI when their serum FT4 and TSH had been kept within normal range for 12 months. After discontinuation of MMI, they were followed every 4–6 months to confirm continuous remission.
Results
Eighty-six patients could reach minimum maintenance dose. The percentage of patients who reached minimum maintenance dose was 46% at 1 year, 68% at 2 years and 78% at 3 years. In 63 patients, in whom MMI was stopped after therapy for 23$\pm$8 months, remission rates were 86% at 1 year, 74% at 2 years and 67% at 3 years after MMI discontinuation. The levels of TRAb (TRAb-CT) and the ratio of TRAb positive patients at the time of drug cessation were 13% and 46% in remission group after 2 years. In relapse group within 2 years, those were 15% and 46%, respectively. There were no differences between the two groups.
Conclusion
The present study indicates that a minimum maintenance dose of MMI to keep euthyroid state for 12 months is a useful and practical procedure for treatment of Graves' disease.In this protocol, TRAb might give little additional informations for prediction of remission.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1635

**Predictors of relapse of Graves' disease after thionamide drugs treatment in clinical practice**

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Background
One of the main drawbacks of primary treatment of Graves' disease (GD) with thionamide drugs (TD) is the high relapse rate estimated to be 50–60%. Fear about side effects, specially agranulocitosis and hepatic complications, is other drawback of this treatment option.
Objective
To evaluate the clinical and/or biochemical characteristics that might predict relapse in patients with GD treated with TD.
Design and patients
Clinical records of 264 hyperthyroid patients due to GD referred to our hospital outpatient clinic between 1998 and 2008 were reviewed. They were treated with a course of TD at least 6 months. TD were stopped when they had undetectable thyroid-stimulating antibodies (TSAbs) and/or were euthyroid. At the end of the treatment, patients were followed-up for at least 1 year.
Results
214 patients were females. Mean age at diagnosis was 40.38$\pm$13.68 years (14-80). Mean treatment duration was 19.9$\pm$6.2 months (6–51). Only seven patients were treated with propylthiouracil. Mean time of follow-up after stopping TD was 30.5 months. Relapsing Graves' hyperthyroidism was observed in 154 (58.3%) patients. In univariate analysis, relapse was associated with younger age ($P<0.05$), higher level at diagnosis of free thyroxine (T4) ($P<0.05$) and free triiodothyronine (T3) ($P<0.001$) and higher TSAbs levels at the end of the TD course ($P<0.0001$). By multivariate analysis only initial free T4 and free T3 levels and TSAbs level at withdrawal were indepently associated with relapse. The percentage of relapsing patients at 12, 24 and 60 months after discontinuation of TD was 60.1%, 82.5% and

94.4%. Only 36 patients (13.6%) have maintained euthyroidism after 60 months of TD withdrawal. No major adverse reaction was observed. Minor adverse effects occurred in 11 patients (4.2%).
Conclusions
Hyperthyroidism relapse in patients with GD treated with TD in our study group was associated with analytical features of severe hyperthyroidism at diagnosis and higher TSAbs levels before stopping TD. Moreover, a low percentage of patients were in remission after 60 months of TD discontinuation. This may help early consideration of alternative therapy for those patients who have aggressive disease at diagnosis and/or who are TSAb-positive before TD withdrawal. In our study, TD showed to be safe, with a low rate of minor adverse effects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1636

**The significance of TSH receptor blocking antibody levels in gestational stage**

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Background & objectives
There are multiple forms of functional TSHR autoantibodies which stimulate or inhibit the thyroid. The TSH-Receptor blocking antibody (TSBAb) exists in the serum of Graves' disease, which is functionally different from TSH receptor stimulating antibody (TSAb). TSBAb that blocks the action of TSH is also associated with idiopathic myxedema and some Hashimoto's disease. We measured TSBAb levels and evaluated the pathogenetic significance of TSBAb in gestational women.
Subjects & methods
Thirty two Japanese pregnant women were enrolled to measure TSBAb, TSAb, thyroglobulin antibody (TgAb) and anti-thyroid peroxidase antibody (TPOAb), TSH, free T4 and Free T3. Thyroid function was evaluated with TSH levels (standard values; 0.55 to 4.18 μU/l). The biological activity of TSBAb were assayed with porcine thyroid cell suspensions using cAMP measurement by RIA.
Results
Demographic data were $30.2 \pm 6.2$ years olds for age, $12.6 \pm 5.3$ weeks for the gestational stage, thirteen women on hyperthyroidism, four women on hypothyroidism and fifteen women on euthyroidism. TSBAb levels in pregnant women were not correlated with the gestational periods. In thirteen hyperthyroid and fifteen euthyroid state TSBAb were within normal range, whereas three of four hypothyroid women demonstrated high levels of TRBAb (more than 45.6%). Two hypothyroism subjects with high titer of TRBAb, TgAb and TPOAb.
Speculation & conclusions
In the gestational stages thyroid function varies from hypo- to hyper-function of thyroid, our results suggested that TSBAb might play an important role in hypothyroidism of the pregnant women with or without Hashimoto's disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1637

**Evidence of humoral response to extracellular matrix proteins in Grave's diseace and thyroid associated ophtalmopathy**

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Thyroid Associated Ophtalmopathy (TAO) is considered one of the most difficult pathologies to treat and a big threat for Graves Disease (GD) patients. This has enhanced the interest in finding a marker that can predict the onset and the prognosis of TAO. It has been demonstrated that GD is a chronic inflamed tissue that there is a remodelling of the ECM structure which elaborate inflammatory signals itself and also promotes the recruitment of cells from the bloodstream; these events can lead to ECM self-epitopes exposition and induce autoimmune phenomena.
Aim of this study, is to determine whether the occurrence of anti-ECM autoantibodies is associated with GD and TAO.
We studied 50 outpatients affected from GD (24 affected by TAO) and 40 healthy donors: sera were assayed for the presence of autoantibodies to CI, CIII, CIV, CV, LM and FN. When compared with HD control sera, GD sera resulted significantly positive to all individual anti-ECM antibodies of the IgG and IgM isotype with exception of the anti-LM IgM antibodies. Remarkably, anti-CIII autoantibodies were significantly associated with GD TAO+ patients ($P = 0.045$).
Our results demonstrated that among the anti-ECM autoantibodies in GD patients those reacting with CIII were able to discriminate $TAO^+$ from $TAO^-$ GD patient cohort.
In conclusion our results suggest an involvement of anti-ECM antibodies also in GD and a possible specific role of anti-CIII IgG in TAO GD patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1638

**Thyroid dysfunction in rheumatoid arthritis**

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Introduction
Rheumatoid arthritis, the most common inflammatory arthritis, is commonly seen with thyroid hormone autoantibodies and thyroid dysfunction.In this survey we evaluate the thyroid function and the prevalence of thyroid autoantibody, autoimmune thyroiditis and other forms of thyroid dysfunction to determine whether these patients should be screened for thyroid disease.
Material and methods
Our study included 224 rheumatoid arthritis patients (195 female, 29 male), all fulfilling the revised ACR criteria for the diagnosis of RA who consecutively admitted to Rheumatology clinic of university based, internal medicine centre of Razi hospital in Rasht, the capital city of Guilan province in north of Iran. Mean age was $49.05 \pm 13.53$. For each patient, demographic data were recorded. Each patient was evaluated for the personal and family history of thyroid disease. All patients underwent tests for Free T4, Free T3, TSH, Anti Thyroid Peroxidase (Anti TPO), and Anti Thyroglobulin (Anti Tg).
Result
(19.6%) patients had positive family history for thyroid disease. Laboratory evidence for thyroid dysfunction in the form of hypothyroidism was obtained in 42 (18.8%) patients who all were women. Hypothyroidism was more common in Patient with a positive family history of thyroid disease which was statistically significant. We could not detect such a relation regarding to sex and age. In this study no case of hyperthyroidism was detected. Autoimmunity was detected in 39 (17.4%) patients. All cases were women which was statistically significant ($P < 0.008$).Physical examination and thyroid sonography revealed simple goitre, multinodular goitre, simple nodule in 3(1.3%), 3(1.3%) and 2(0.8%) cases respectively.
In 19 (43.2%) patients with thyroid disease, the family history for thyroid disease was positive. When contrasting patients with positive family history of thyroid diseases with patients without such a family history, the results were statistically significant only in the case of hypothyroidism ($P < 0.02$). A statistically significant relationship between sex and thyroid abnormalities was found only in the case of autoimmunity ($P < 0.008$).
Conclusion
Thyroid dysfunctions are common in rheumatoid arthritis. It seems wise to evaluate these patients for the investigated abnormality especially the autoimmunity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1639

**The usable markers of atherosclerosis measured by Doppler Ultrasound of lower limb artery in treatment- naïve hypothyroid females**

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Background
It has been shown that hypothyroidism is associated with atherosclerosis. The presence of atherosclerosis process could be registered by use of some Doppler Ultrasound parameters (DUP) – intima-media complex thickness (IMCT) and systolic velocity (SV). In this study we present initial DUP on the lower limb artery in treatment-naïve hypothyroid females and the influence of thyroid hormone levels and some metabolic components.
Methods and design
In this study 45 female patients were involved and divided into three groups regarding the thyroid hormone levels at the time of presentation: subclinical hypothyroid group, clinical hypothyroid group, and control euthyroid group. At the initial visit, some of metabolic components were measured: BMI, systolic and diastolic blood pressure, blood glucose and lipid levels. All patients were referred for DUP measurement on the proximal segment of right superficial femoral artery (RSFA). Data were statistically analyzed by using SPSS for Windows.
Results
An average IMCT and SV at presentation were $0.86 \pm 0.21$ mm and $0.53 \pm 0.13$ m/sec, respectively. The values obtained for SV were not differ between groups, while IMCT values were find to be different ($F = 8.212$, $P < 0.01$), with no contribution of difference between subclinical and clinical hypothyroid group. The patients average age ($48 \pm 11$ years) influenced significantly on IMCT ($r = 0.494$, $P < 0.01$). The overall initial average levels for TSH and fT4 were 7.21 mIU/L and $12.7 \pm 3.2$ pmol/L and both hormones significantly influenced on IMCT ($\rho$TSH = 0.584; $\rho$fT4 = −0.41; $P < 0.05$), but not on SV. The levels of triglycerides, glucose, cholesterol and its fractions ware not found to influence on IMCT.
Conclusion
This study clearly indicates that abnormal IMCT is a better marker of atherosclerosis in treatment of naïve hypothyroid patients than SV. Since TSH and fT4 levels may influence IMCT, both hormone levels should be used as the valid markers of serious atherosclerosis presence, in contrary to measured metabolic components.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector

## P1640

**Maternal-neonatal relationship of iodine metabolism in perinatal period: Changes in urinary iodine excretion in Japanese mothers and newborn infants**

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In the perinatal period iodine deficiency results in growth and mental impairment in fetus and newborns. However, there are limited data on iodine metabolism in mother and infants during this critical period.
Objective
To assess the changes in urinary iodine concentration (UIC) in mothers and neonates.
Methods
From July 2010 to October 2011, 155 pregnant women($31.1 \pm 2.1$ years) without thyroid disease and their term infants ($38.7 \pm 4.7$ weeks) were consecutively studied. The spot urine and blood samples were collected in the third trimester of pregnancy and one day before birth, the 2nd and 30th postpartum day. Neonatal urine was collected using a small self-adhesive sterile bag in the first, 5th and 30th postnatal day. The subjects with thyroid antibodies or serum TSH and FT4 values out of the reference interval were excluded.
Results

i) In neonates ($n = 95$), median UIC was 121.0 μg/L in the first voided urine, then increased to 223.0 μg/L in the 5th day and reached to 271.0 μg/L in the 30th day.
ii) Maternal median UIC was 166.5 μg/L in the third trimester and not significantly different from that in one day before birth (158.5 μg/L). At the 2nd postpartum day urinary iodine excretion abruptly increased to 256.0 μg/L, then decreased to 115.0 μg/L at the 30th postpartum day($n = 150$).
iii) The maternal UIC in one day before birth was well correlated with that of their neonates at birth (Spearman $r = 0.214$, $n = 126$).
iv) At one month the median UIC of the formula-fed infants was 108.0 μg/L and significantly lower than that of breast-fed infants (246.0 μg/L).

Conclusion
In Japan, an iodine-sufficient area, newborn infants have sufficient iodine store at birth and excrete as much iodine as adults' level after 5th postnatal day. Major iodine source is breast milk and the iodine store might be not depleted at least one month after birth.
pp: post partum
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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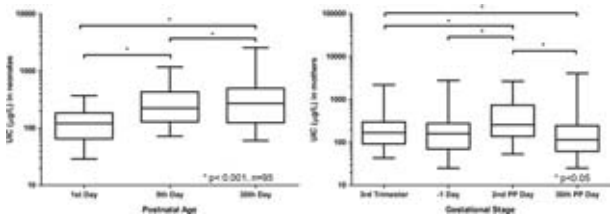

## P1641

**Is Japan an iodine excess country? Current iodine status assessed by urinary iodine and food frequency questionnaire**

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Japan has been regarded as a nongoitorous country due to the regular intake of iodine-rich food. However, nationwide information on current status of iodine intake is not available because there is no survey system for iodine nutrition in Japan.
Objective
The objectives of this study is to characterize current iodine status in Japan.
Methods
Since 2002 we have conducted studies on iodine nutrition in the Metropolitan area including Tokyo, Chiba and Kanagawa prefectures. Spot urine samples were collected in healthy subjects without known thyroid diseases and urinary iodine concentration (UIC) was measured by the ammonium persulfate digestion on microplate method based on the Sandell Kolthoff reaction. Serum TSH, free thyroxin and thyroid autoantibodies were measured if blood samples are available. Dietary iodine intake (DII) was assessed by using an iodine-specific food frequency questionnaire developed and verified by us.Iodine content was calculated on the basis of data from the standard tables of food composition in Japan 2010.
Results
Median UICs according to age groups are summarized in the table. Median daily DII in healthy adults was 471.0 μg /day (IQR:256.7, 859.8) and there was no significant difference in DII value between male (453.1 μg /day) and female (503.5 μg /day). The questionnaire data correlated well with urinary iodine excretion (Spearman $r = 0.32$).
Conclusion
According to WHO/ICCIDD criteria for iodine deficiency disease median UICs of 100–199 μg/L at population level indicate adequate iodine intake and optimal

nutrition and the median UIC over 300 μg/L in local schoolchildren is regarded as excessive. Our results indicate that current iodine status in Japanese is sufficient, not excessive, although the data is local.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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Iodine concentration in spot urine

| Age Groups | n | Age (years) | UIC (μg/L) | |
|---|---|---|---|---|
| | | | Median | IQR |
| Term Newborn infants | 146 | 0 day | 109.0 | 61.8, 177.8 |
| | 139 | 4th day | 225.0 | 127.0, 432.0 |
| Infants | 103 | 30 days | 256.0 | 121.0, 495.0 |
| Schoolchildren | 654 | 9.6 | 281.6 | 173.5, 555.4 |
| Adults | 325 | 47.7 | 213.0 | 126.0, 425.0 |
| Pregnant Women* | 683 | 30.9 | 219.0 | 124.0, 436.0 |
| Postpartum Women** | 532 | 30.9 | 135.0 | 78.0, 262.0 |

IQR: Interquartile range, * 21.2 weeks of gestation, **34.0 days after birth

## P1642

### Determinants of children's iodine nutrition and epidemiological features of children's goiter and nodule in iodine-excessive areas

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Introduction

Excessive iodine intake can cause iodine overnutrition, goiter and other thyroid disease. This study is to probe the determinants of children's iodine nutrition and their goitrous and nodular prevalence in areas with excessive iodine in drinking water in Hebei province of China. Methods Three townships were selected by simple random sampling among the 8 townships with median water iodine being 200–300 μg/l in Hengshui prefecture of Hebei province. No less than 200 children aged 8–10 years old were randomly selected in each of the 3 townships to measure their thyroid volume by ultrasound, and to detect the urinary iodine of half of them. Drinking water samples were also collected by systematic sampling in the villages where these children lived to measure the iodine content. Results The median urinary iodine of 326 children aged 8–10 years in these 3 townships ranged from 478.4 to 571.3 μg/l, and the portions of urinary samples with iodine content over 300 μg/l varied from 77.9% to 86.6%. Children's median urinary iodine had a linear correlation with median water iodine in 12 villages of these 3 townships($R=0.83$, $F=22.0$, $P=0.001$), and the linear regression equation was as followed: urinary iodine $=318.1+0.829$*water iodine. A total of 452 children aged 8–10 years was measured by ultrasound in these 3 townships, identifying 37 cases of goiter with goiter rate being 8.2%. There was no significant difference in goiter rates across gender and age groups. In 2 out of the 3 townships, 15 cases of nodules were detected with average detection rate being 5.6%. The detection rates across gender and age groups were not significantly different. Conclusions Children's urinary iodine in these areas was excessive which mainly derived from iodine excess in drinking water. Goiter prevailed in these areas, and a high detection rate of thyroid nodules was also identified.

Declaration of interest

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## P1643

### Control of 217 hypothyroid women during pregnancy

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Introduction

The new guidelines for the management of hypothyroidism (HP) during pregnancy recommend a tighter treatment with levothyroxine (Lt4) to reach TSH values similar to those of pregnant women without thyroid dysfunction (TSH $<2.5$ mU/l in the 1st trimester-T- and TSH $<3$mu/l in the 2nd and 3rd T).

Objectives

To assess the degree of control of HP in women followed during pregnancy according to the current criteria and the possible association between control of TSH in each T and: miscarriage%, preterm deliveries% and few gestational complications frecuencies (pregnancy induced hypertension-HTA-, gestational diabetes-GD-) and birth weight.

Patients and methods

Study of 217 pregnant women with primary HP followed in our hospital for Lt4 adjustment.We describe miscarriage %, preterm deliveries%;few gestacional complications% and birth weight.

Results

23.2% of women did not take a minimum 150 μg daily iodine supplements in their first assessment. % of women with TSH $<4.3$ mU/l (normal superior reference range -NSR-in non-pregnant population) changed from 78.7% before pregnancy to 64.8% in the 1st T, 84.8% in the 2nd T and 95.1% in 3rdT ($P<0.05$) . % of pregnant women with TSH $<$ NSR for pregnant population was significantly lower: 31.3%, 63.9% and 86.8% in the 1st, 2nd and 3rd T respectively. This % was higher in those patients submitted earlier (1st T:76%) than late (in 3rd T: 91%, $P<0.05$). There were no significant differences in preterm deliveries %, HTA, GD comparing women with TSH values within the control goals to those with TSH above goals in any trimesters. We found a non significant trend toward greater % of preterm deliveries with high TSH values in 2 nd T. Mothers% whose newborns weighed $<2300$ g was higher in those with 3erT TSH$>4.3$ mU/l vs TSH $\leq 4.3$ (20 vs 2.7%, $P=0.04$) and with TSH 3erT$>3$ vs $<3$ mUi /l (11.5 vs. 2.3%, $P=0.05$).

Conclusion

Early assesment of pregnant women is necessary to achieve goals of control (possibly in the preconceptional period). The high level of controlled patients% in 3erT could explain the lack of significant differences in the% of complications.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1644

### Vitamin D deficiency is not associated with thyroid autoimmunity

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Context

Vitamin D deficiency has been identified as a risk factor for a number of autoimmune diseases including type 1 diabetes and multiple sclerosis.

Objective

We hypothesized that low levels of vitamin D are related to the early stages of autoimmune thyroid disease.

Design

Two case-control studies were performed. Study A: cases were subjects from the Amsterdam AITD cohort (euthyroid women who had 1st or 2nd degree relatives with overt AITD) who at baseline had normal TSH and no thyroid antibodies; controls were healthy women examined at the same time period. Study B: cases and controls were subjects from the Amsterdam AITD cohort who at baseline had normal TSH and no thyroid antibodies and during follow up developed TPOAb (cases) or remained without thyroid antibodies (controls). Controls in both studies were matched for age, BMI, smoking status, estrogen use, month of blood sampling and in study B for the duration of follow up.

Results

Study A: The 78 cases had a higher serum 25(OH)D concentration than the 78 controls ($21.0\pm7.9$ ng/ml vs. $18.0\pm6.4$ ng/ml, $P=0.01$). The prevalence of 25(OH)D deficiency ($<20$ ng/ml) was lower in cases than in controls (48.7% vs. 64.1% respectively, $P=0.05$); the same was true for the prevalent rates of 25(OH) D deficiency and insufficiency ($<30$ ng/ml) (83.3% vs. 94.9%, $P=0.02$).

Study B: The 25(OH)D levels in ng/ml were $22.6\pm10.3$ vs. $23.4\pm9.1$ at baseline and $21.6\pm9.2$ vs. $21.2\pm9.3$ at follow-up (67 cases vs. 67 controls, NS). The frequency of 25(OH)D deficiency ($<20$ ng/ml) at baseline was comparable in cases and in controls (49.2% vs. 34.3% respectively, $p=0.05$); the same was true for the prevalent rates of 25(OH)D deficiency and insufficiency ($<30$ ng/ml) (79.1% vs. 86.5%, $P=0.25$). Similar figures were obtained at the time of seroconversion.

Conclusions
Early stages of thyroid autoimmunity (in study A genetic susceptibility and in study B development of TPOAb) were not related with low vitamin D levels.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1645

### Iodine prophylaxis in pregnant women: effects on offspring and the mother's thyroid hormones

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Background and aims
Use of potassium iodide (PI) supplements during gestation in mild to moderate iodine deficiency areas remains a controversial issue.
Objective
Comparing the pharmacological effect of iodized salt versus iodide potassium supplements on the maternal thyroid function and neurodevelopment (Bayley Scales) of the progeny.
Methods
One hundred and thirty one pregnant women were included from the first trimester (below 10 weeks of amenorrhea) with random allocation in three groups: A) Iodized salt B) 200 mcg of iodide potassium (PI) per day and C) 300 mcg of PI per day.
Main outcome measures
Thyroid function parameters (TSH, FT4, FT3 and urinary iodine excretion) and Bayley-III Scales of Infant Development in children.
Results
The thyroid function tests in three trimesters of gestation and postpartum have not shown significant differences between the three treatment groups, except in urinary iodine excretion (higher in group treated with 300 mcg PI/day). In direct scores of psychometrical scales, we found higher scores within the sons whose mothers were supplied with lodizet salt at 300 mcg PI/day. We found a positive relation between motherbreed urinary iodine, mental scales and Bayley tests in every age group; However, there is not any relation between urinary iodine and psychomotor scale.
Conclusions
Actually It is necessary to maintain an accurate iodic nutrition on these women in fertile age, that is why the usage of pharmacological supplements of 200–300 mcg of PI is well recommended in iodine deficient areas.The use of pharmacological supplements of PI is safe and doesn't alter the maternal thyroid function, even improves the urinary iodine excretion. But if you use iodized salt regularly, you may not have need for iodine supplementation in the future.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1646

### Low prevalence of postpartum hyperthyroidism in women after radioiodine treatment for Graves' disease before the pregnancy

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Context
Postpartum hyperthyroidism (PH) is one of the postpartum thyroid disorders that occurs during the first year postpartum and PH is caused by destructive thyroiditis and recurrent Graves' disease. PH is an exacerbation of an underlying autoimmune thyroiditis that is aggravated by the immunological rebound that follows the partial immunosuppression that occurs during pregnancy. The prevalence of PH among women with Graves' disease (GD) who had been treated with radioiodine (RI) before their pregnancy has never been investigated.
Objective
Our objective was to determine the prevalence of PH among women who had been diagnosed with GD and undergone RI therapy before their pregnancy.
Subjects and methods
We reviewed the cases of women with GD who became pregnant between January 1, 1999 and December 31, 2010, and selected the 188 women who had undergone RI therapy before the pregnancy as the subjects of this study. Of the 188 women, 110 were on replacement therapy with levothyroxine (25–175 μg/day, mean 87.5 μg/day) (Group 1) and 78 were euthyroid without medication (Group 2). The control subjects were 107 women who became pregnant during 2009 who had been treated with antithyroid drugs and were in remission throughout the pregnancy. All subjects were followed up for at least one year after delivery. We monitored the TSH receptor antibody (TBII) level of all of the women during pregnancy and the postpartum period.
Result
Transient hyperthyroidism was observed in one of the 110 women in Group 1 (0.9%) and in three of the 78 women in Group 2 (3.8%). No cases of permanent hyperthyroidism were observed in either Group 1 or Group 2. PH was observed in 59 of the 107 women (55%) in the control group. In 41 of the 57 women it was transient hyperthyroidism, and in the other 18 it was recurrent Graves' disease and required medication. After delivery the TBII level increased in 15 cases in Group 1, in 19 cases in Group 2, and in 34 cases in the control group.
Conclusion
The results of this study provided evidence that RI treatment before pregnancy is effective in preventing PH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1647

### The outcome of radioiodine therapy after five years in patient with non-toxic nodular goitre

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The aim of our study was to evaluate the short term efficacy of radioiodine therapy (RIT) to reduce thyroid volume with minimal risk of hypothyroidism in patients with non-toxic nodular goitre. Material and Methods: We treated 120 patients, aged 20–76 years; (88%) of the studied groups were female and (12%) male. Initial 24 h RAIU was ranged between 22–44% and effective half-life was more than 3 days. Thyroid volume ranged between 42–170 ml. Malignant changes were excluded in all nodules by fine needle aspiration biopsy. The activity dose was calculated by the use of Marinelli's formula and ranged between 200–800 MBq. The absorbed dose ranged between 150 and 260 Gy. Thyroid ultrasonography, and thyroid scan was done before, after 12 month and yearly for four year of RIT. Follow up control was done every 6 weeks in the first year, then every 6 months. Results: After 12 months of radioiodine therapy a mean thyroid volume reduction of 46% was achieved. Euthyroidism persist in 91% of patients, and hypothyroidism develop in 9% of patients. After 3 years of RIT 10% of patient develop hypothyroidism. After 5 years a mean thyroid volume reduction of 49% was achieved and 11% of patients develop hypothyroidism. All patients were highly satisfied; the compressive symptoms relieved. Conclusions: Radioiodine is non-invasive, safe and cost effective method of therapy for reduction of non-toxic goitre and should not be restricted to elderly patients, or to patients with high surgical risk, but should be used as first choice in every patient with non toxic nodular goitre with thyroid volume $> 40$ ml. Surgery should be reserved as first choice if malignancy is suspected. The reduction of thyroid volume with low percent of hypothyroidism, were due to well accurate measurement of administered activity, relatively high effective half-life, and well-organised follow up.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1648

**Langerhans cell histiocytosis of the thyroid and pituitary gland**

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Langerhans cell histiocytosis is a disease caused by clonal proliferation of a group of antigen-presenting cells known as Langerhans cells. Its manifestations range from isolated lesions to multisystem disease usually involving bone, skin and the pituitary stalk. Thyroid involvement is rare.

A 44-year old woman presented to the joint thyroid clinic with a short history of neck swelling, confirmed to be a smooth goitre on clinical examination. Initial blood tests results were: TSH 0.24 mu/l (0.30–4.20), T4 8.2 pmol/l (9–19), TPO antibody negative.

A neck ultrasound demonstrated a diffuse thyroid abnormality with generalised hypoechoic echotexture, widespread microcalcification and level 6 lymphadenopathy. No focal lesions were identified. FNA of the thyroid was suggestive but not diagnostic of medullary carcinoma. Carcinoembryonic antigen was 6.5 ug/l (1–5), but calcitonin was undetectable.

Total thyroidectomy was recommended by the MDT. However, dissection of the left lobe was technically challenging and a left hemi-thyroidectomy only was performed as the Recurrent Laryngeal Nerve failed to stimulate post lobectomy. As the pathology was unknown at the time it was decided not to embark upon surgery on the right side.

Histologically, histiocytes were seen on a background of numerous eosinophils and a lymphoid infiltrate. Immunohistochemistry revealed positivity for CD1a and S-100. These features were in keeping with a definitive diagnosis of LCH.

Following surgery she developed multiple skin lesions suggestive of multifocal involvement and more recently she has been found to have pituitary dysfunction (Table 1).

LCH initially presenting in the thyroid as seen in our case is extremely rare. The management of this multisystem disease remains a challenge.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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Pituitary profile

| Investigation | Result | Reference range |
|---|---|---|
| Cortisol | 34 nmol/l | 160 – 550 |
| TSH | <0.05 mu/l | 0.30–4.20 |
| T4 (on thyroxine replacement) | 12.1 pmol/l | 9–19 |
| T3 | 5.2 pmol/l | 2.5 – 5.7 |
| LH | <0.5 IU/l | |
| FSH | 1.8 IU/l | |
| Oestradiol | <70 pmol/l | |
| Prolactin | 1784 IU/l | 125 – 625 |
| IGF-1 | 3.7 nmol/l | 13.0 – 64.0 |
| Sodium | 152 mmol/l | 135 – 145 |

## P1649

**Celiac disease in patients with autoimunne thyroiditis – pilot study**

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Introduction

Benefit of general screening for concomitant autoimunne diseases in patients with chronic autoimunne thyroiditis (AIT) is serious problem from medical, even from economic point of view. Some authors advice for screening, some advert to mass occurence of AIT and warn about huge expenses. Situation vary from one country (region) to another.

Aim

To assess incidence of celiac disease in patients with chronic autoimunne thyroiditis and find any risk factors.

Methods

200 consecutive patients with autoimunne thyroiditis was selected for the study. Including criteria was positive sonographic findings of AIT and/or positive antibody/ies (anti-thyreoperoxidase, anti-thyreoglobulin). In all patients was measured level of anti-transglutaminase antibodies and IgA, as well as current level of anti-thyreoperoxidase antibodies. Enterobiopsy was performed in patients with positive anti-transglutaminase and sample was assessed according to Marsh's clasification.

Results

Valid data was gained from 186 pacients, 134 women and 52 men. 9 patient has positive anti-transglutaminase antibody and 8 patients (4.3%) has significant histologic changes for celiac disease. There was no difference in gender, weight (BMI), presence of other autoimunne disease (primarily diabetes mellitus type 1), dose of levothyroxine, little, but not significant difference in age −(33.0 [25.0–38.5] years in celiac group vs. 24.5 [27.3–41.8] in healthy group; $P=0{,}09$) and significant difference in anti-thyreoperoxidase levels (396 [210–967] IU/ml in celiac group vs. 152 [35–443] IU/ml in healthy group; $P=0.05$). No difference was observed in anti-thyreoglobulin antibodies.

Conclusion

Small number of patients made comprehensive statistical arrangement difficult, but this preliminary results indicate usefullnes of celiac disease screening in asymptomatic patients with AIT, especially with elevated anti-thyreoperoxidase antibodies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1650

**Is thyroid volume (TV) in children related to the urinary iodine concentration (UIC) in urine spot sample?**

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Goiter prevalence (GP) and median UIC in population studies are both considered markers of iodine nutrition. Controversy exists which of them is more reliable.

Aim

To assess correlation between UIC and thyroid volume in Polish schoolchildren.

Material and methods

9402 Polish schoolchildren (51.3% girls, 48.7% boys) aged 6–12 years were examined in 1999–2011 ('Thyromobil' action). 40.9% of children were born at least one year after implementation of obligatory iodine prophylaxis (IOIP). TV was assessed ultrasonographically, GP was evaluated according to both 1997 and 2004 WHO reference values. To avoid age influence TV(ml)/BSA(m2) ratio (TVBR) was calculated. UIC in urine spot samples was measured by Sandel-Kolthoff's method.

Results

Median UIC was 96.18 mcg/l. Median TVBR was 3.97 ml/m2. GP for age (GPA) was 5.5% and 55.7%, for BSA 4.9% and 56.5% according to 1997 and 2004 reference, respectively. GPA was 5.7% and 5.2% according to 1997 reference, and 56,8% and 54.5% according to 2004 reference, for UIC $<$ and $\geq$100 mcg/l, respectively. GPA was 6.5% and 4% according to 1997 reference, and 56.6% and 58.7% according to 2004 reference, for children born before and after IOIP, respectively. Median TVBR was 4.1 and 3.8 ml/m2 ($P<0.0001$) for children born before and after IOIP, respectively. Median UIC was 91 and 104.3 mcg/l ($P<0.001$), for children born before and after IOIP, respectively. Median UIC was 96.5 and 91.9 mcg/ml ($P=0.43$), and 97.8 and 95.0 mcg/l ($P=0.025$) for children without and with goiter according to 1997 and 2004 age reference, respectively. TVBR was 4.05 and 3.97 ml/m2 ($P<0.0001$) for UIC $<$ and $\geq$100 mcg/l, respectively. Negative, statistically significant correlation between TVBR and UIC was observed ($r=-0.033$, $P<0.005$).

Conclusions

Statistically significant correlation between UIC and TV was confirmed. However, neither 1997 nor 2004 WHO reference values seem to reliably estimate influence of iodine prophylaxis on TV.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1651

**Evaluation of health related quality of life and general symptoms in Hashimoto's thyroiditis: are factors other than thyroid function effective? Analysis of preliminary results**

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Introduction
Factors affecting health related quality of life (HRQOL) in Hashimoto's thyroiditis (HT) are controversial and research on the topic is limited. This study aimed to evaluate the factors that may affect HRQOL and admitting symptoms in a group of patients with HT.
Methods
For the evaluation of HRQOL, SF-36, Beck anxiety scale and Beck depression scale were performed on subsequent 57 cases with HT admitting to outpatient clinic of Endocrinology department of Baskent University Hospital. Also, according to the admitting symptoms of the patients, they were asked to score between 0 and 10 expressing severity for each of the nine most frequent symptoms. Thyroid function tests, thyroid autoantibodies were studied, and thyroid sonography was performed to each patient.
Results
The most frequent symptoms mentioned were dysphagia, dyspnea, feeling of swelling in the neck, difficulty in collared clothing, snoring, night sweating, headache, lightheadedness, and pain in the neck. According to SF-36 form assessment, there was a correlation between thyroid volume and mental health ($P<0.05$; $r=-0.31$) and physical role limitation ($P<0.05$; $r=+0.30$) subscales. As for general symptom evaluation, headache and light-headedness were more severe in patients who were not on L-$T_4$ than those on L-$T_4$ ($P<0.05$ and $P<0.01$ respectively). Corelation analysis revealed a positive relation between the symptom of difficult breathing and thyroid volume ($P<0.01$; $r=+0.39$).
Conclusion
Determining almost no relation between HRQOL scales and thyroid hormone levels, but detecting some relations between some scales and thyroid volume may be meaningful in HT cases, i.e. possible contributions of morphological factors. The final results of our ongoing study may reveal us more on the topic.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1652

**Proptosis reduction in patients with Graves' orbitopaty (go) at 12 weeks after intravenous steroid therapy is associated to disease inactivation**

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We have retrospectively studied the clinical outcome of 58 patients (14 men and 44 women) with autoimmune thyroid disease and active GO, treated with high dose intravenous methylprednisolone (MP; cumulative dose 7.5 g). Ophthalmological assessment was performed at baseline and at 6, 12 and 24–30 weeks after the first MP infusion. In 43/58 patients we have also studied NR3C1gene polymorphism, which has been associated to different sensitivity to steroids. The therapeutic outcome has been assessed as: i) reduction of clinical activity score (CAS) $\geq 2$ point or ii) of proptosis $\geq 2$ mm or iii) improvement of diplopia according to the Gorman score, in relation to age, gender, duration of thyroid or orbital disease, smoking habits, serum parameters (TRAb, TSH, $FT_3$, $F_4$ concentrations) and genetic data (NR3C1gene polymorphisms). A significant clinical improvement was observed in 67% of patients at 6 weeks and in 80% at 12 weeks; this rate decreased to 70% at 24 weeks as a consequence of relapse of GO in 8 patients (13%). At 12, but not at 6 and 24 weeks, the reduction of the CAS, observed in 80% of patients, was more evident in non-smoking patients (62.5%) than in smokers (37.5%; $\chi^2$; $P<0.045$). A decrease of proptosis $\geq 2$ mm was also significantly associated with the reduction of CAS $\geq 2$ in 34.3% of the patients, who at 12 weeks had a significant response to treatment ($\chi^2$; $P<0.003$). No significant changes in the Gorman score were observed after treatment. No association was found between the therapeutic response to MP and clinical, serological and genetic parameters. Our data show that: i) the therapeutic response of MP is observed in as many as 70% of patients; ii) treatment may be more responsive in non-smoking patients and iii) proptosis reduction is the ophthalmological parameter better associated to inactivation of GO after MP.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1653

**Evaluation of the effect of radioactive iodine treatment on urea breath test**

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Introduction
*Helicobacter pylori* (HP) infection is the most significant cause of gastritis and related morbidities. Radioactive iodine treatment (RAIT) is an important option in the treatment of hyperthyroidism. In addition to thyroid gland, RAI accumulates in stomach in significant amounts. In our study, we planned to investigate whether RAIT has an effect on carbon-14-urea breath test (UBT) in patients with hyperthyroidism.
Materials-method
The study included 71 patients that were planned to give RAIT due to hyperthyroidism. Patients that used antibiotics in the last month and proton pump inhibitor in the last week, pregnant women, individuals with stomach operation history were excluded from the study. The entire patients were implemented UBT an hour before RAIT for the detection of HP infection. Patients whose test came out positive were repeated UBT one month following the treatment.
Results
Of the 71 patients, 62 had positive UBT before RAIT. Of the 62 patients, 52 were female and 10 were male, and the mean age was $53.9 \pm 12$. Following an average 15 mci (10–20) RAI dose, UBT became negative in 10 out of 62 patients (17.3%). The rate of negativity was found to be 17.3% in females and 10% in males; and significant difference did not occur ($P=1$). When the patients were divided into groups as 20 mci (group 1), 15 mci (group 2), and 10 mci (group 3) based on RAI doses, UBT negativity rates were 7 (25%), 2 (10.5%), and 1 (6.7%), respectively, and the difference was not statistically significant ($P=0.206$).
Conclusion
RAIT in low doses does not have an effect on UBT. However, UBT negativity rate increases parallel to the treatment dose. Further studies that investigate the relation between HP radiosensitivity and RAI dose are needed.
Keywords: *Helicobacter pylori*, radioactive iodine treatment, carbon-14-urea breathe test.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1654

**Review of a thyrotoxicosis shared-care scheme: treatment choice and outcomes**

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Since 1994, after initial outpatient review (OPD) for thyrotoxicosis, we have minimised OPD attendance and provided shared-care advice to primary care physicians on thyroid function tests (TFT) and anti-thyroid drug (ATD) and levothyroxine ($T_4$) dose titration using our electronic patient record (EPR). Using EPR data we analysed treatment (Rx) choice, effectiveness and outcomes, classifying patients as autoimmune (+ve TPO or other Graves' features), nodular, mixed or other (no recorded features of either).
Results
2696 patients (13–94 years; 77%♀), 55 079 TFTs (median 18, max 97/patient), 13 840 patient-years observation (median 4.1 y, max 17.4 y), median 2.9 y from last OPD. Rx choice was patient-driven after appropriate information was given: overall 90% had ATD (84% carbimazole; 11% PTU; median first course

1.50 y; mean # courses 1.6, median 1), 21% radioiodine (RAI) and 7% thyroidectomy. Rx and outcomes are summarised in table. RAI was used more often in nodular disease, and fewer patients became hypothyroid, but many cases of every aetiology chose to continue ATD as an alternative. 'Long-term' ATD (stated strategy or actual use >1000d) was used in 20% and ongoing in 12%; longest ongoing Rx was 24.8 y duration.

$T_4$ ($tT_4$ or $fT_4$)/TSH/Both were normal in 92/59/55% of 55,079 TFTs reported. Control was best in patients on no Rx (94/66/63%) compared to patients on $T_4$ alone (95/58/55%) or ATD (90/54/50%). Control on long-term ATD (92/65/60%) was better than shorter-term courses (89/51/47%) but best control was observed in the minority of TFTs on a block and replace regime (95/63/61%). Control was not better in TFTs taken after RAI: 91/51/48% overall; 86/49/43% on no Rx; 95/56/53% when on $T_4$ Rx.

Conclusions

Thyrotoxicosis can be effectively monitored by a specialist clinic using EPR whilst minimising the need for patient attendance in hospital. Good control of $T_4$ was observed but achieving a normal TSH is more challenging. Long term use of ATD is a valid patient treatment choice which achieves TFT control better than RAI in our hands.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1**

| | | Anti-thyroid drugs | | | Radioactive iodine | | | | Latest F/U | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cases | % Rx | Courses | Long-term | % Rx | % Hypo | Days[a] | Thyroidectomy | On ATD | On $T_4$ |
| Autoimmune | 1137 | 93% | 1.8 | 21% | 18% | 79% | 302 | 8% | 32% | 22% |
| Nodular | 318 | 79% | 1.3 | 27% | 40% | 34% | 712 | 12% | 36% | 17% |
| Mixed | 107 | 91% | 1.7 | 28% | 31% | 67% | 444 | 17% | 24% | 35% |
| Other | 1134 | 89% | 1.5 | 15% | 18% | 74% | 197 | 4% | 30% | 20% |

[a]Mean days from RAI to hypothyroidism.

## P1655

### Left-shifted set point of calcium/PTH-relation in patients with Grave's thyrotoxicosis

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Patients with Grave's disease (GD) are more prone to develop low serum calcium values than patients operated on because of goitre after total thyroidectomy. Moreover, some patients with GD achieve hypercalcemia probably due to active bone disease. We have used citrate-calcium clamping (CiCa) in order to investigate the calcium homeostasis in detail in patients with Grave's disease.

Material and method

Our series of patients undergoing total thyroidectomy for GD ($n=$xx) and gioter ($n=$yy) were scrutinized for postoperative hypocalcemia and need for calcium and/or vitamin D substitution. CiCa-clamp (Schwarz P, Sörensen HA 1992, 1993) were used in 11 patients and 24 controls to quantify the secretion of PTH in relation to ionized plasma calcium level. The wet point equal to the plasma ionized calcium concentration at which 50% of the maximal secretion of PTH is inhibited, as well as other CiCa-related parameters were calculated.

Results

The rate for having hypocalcemia was 48% in our patients overall, but patients with GD have lower calcium levels, 16% had < 2.00 mmol/l compared to 4.5% in the goiter group; need more calciumsubstitution during the hospital stay (6.5 vs 5.1%) and at discharge (33.2 vs 12.9%).

The GD group have a significant more left sided set point than the normal group, 1.14 vs 1.20 mmol/l $P<0.001$ (Mann–Withney $U$ test) as well as an increased response in release of PTH to hypocalcemic stimulus.

Conclusion

Patients with GD have a left-shifted calcium/PTH set point compared to normal controls. We also noted a faster and higher level of PTH response to hypocalcemic stimuli in GD, presumably related to known derangements in these patients, such as high bone turnover. The CiCa response mimicks that of obese patients in which vitamin D insufficiency has been proposed as an underlying cause.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1656

### Correlation between thyroid hormone values and carotid intima media thickness in patients with subclinical hypothyroidism

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Purpose

The aim of the study was to investigate the influence of TSH and free thyroxin ($fT_4$) hormones on carotid intima media thickness (cIMT).

Material and methods

Sixty nine consecutive patients who attended to the Department of University Clinic of Endocrinology, Diabetes and Metabolic disorders with newly diagnosed subclinical hypothyroidism (ScH) were evaluated for mean and maximal cIMT. ScH were defined as elevated TSH with normal $fT_4$ serum values.

Results

Mean TSH, $fT_4$, cIMT, and max cIMT were: 7.9±3.6 mU/l, 14.5±2.8 pmol/l, 0.61±0.1 mm, and 0.65±0.1 mm, respectively. Statistically significant positive correlation were evaluated between TSH value and mean and max cIMT ($r=0.28$, and $r=0.29$, respectively, $P<0.05$). While $fT_4$ statistically significant negative correlated with mean and max cIMT ($r=-0.35$, and $r=-0.33$, respectively, $P<0.01$).

Conclusion

Thyroid hormone values are correlated with carotid intima media thickness in patients with subclinical hypothyroidism.

Declaration of interest

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Key words: carotid intima media thickness, thyroid-stimulating hormone, subclinical hypothyroidism.

## P1657

### miRNA expression of the thyroid after goiter formation and involution using different iodine regimens

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Background

Thyroid damage is known to occur during experimental iodine-induced goiter. MicroRNAs (miRNAs) are a class of small non-coding RNAs regulating gene expression. Dysregulation of specific miRNAs can interfere with tissue homeostasis. The purpose of this study was to investigate the dynamic expression profiles of miRNAs during adequate or mild-excess iodine supplementation in the iodine-deficient thyroid.

Methods

Hyperplastic goiter was induced in rats by feeding a low-iodine (LI) diet for 12 weeks. Subsequently, involution of hyperplasia was obtained by administering an adequate/1- (Group 1) or 2-fold (Group 2) physiological dose of iodine for 4 weeks with a control group receiving adequate iodine intake. MiRNAs expression was analyzed in all the groups using miRNA microarray technique.

Results

In the LI group, during hyperplastic goiter formation, there were 20 miRNAs decreased and 8 increased compared to the control group. Among them, miR-708 was downregulated up to 93% and miR-144* was upregulated 8.7-fold.

After involution of the glands of Group 1 and 2, 7 common miRNAs still remained dysregulated similar to the hyperplastic phase, but 8 common miRNAs recovered to levels as found in the control group. Notably, Group 1 had more recovered miRNAs than Group 2.

Compared to the control group, some new dysregulated miRNAs emerged in Group 1 and 2 after involution. Of these, there were 6 identical miRNAs in group 1 and 2, but the amount of new misexpressing miRNAs in Group 2 was obviously larger than in Group 1. In Group 2, miR-878 was even 6-fold higher than in Group 1.

Conclusions
Our results indicate that misexpression of miRNAs is involved in iodine-induced goiter formation by a low-iodine regimen and that supplementation with adequate iodine could be more helpful to restore homeostasis than mild-excess iodine. However, further functional studies of some specific miRNAs are needed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1658

**Autoimmune thyroid disease and cardiovascular risk factors**

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Aims
To examine whether Graves' disease (GD) and Hashimoto thyroiditis (HT) are associated with insulin resistance (IR) and other cardiovascular risk factors. Patients and methods: We analyzed 479 patients with autoimmune thyroid disease, 354 (74%) with HT and 126 (26%) with GD, 94% woman, with a mean age of $46 \pm 16$ years. The patients in both groups were properly treated in order to normalize TSH, $FT_3$ and $FT_4$ levels. We recorded thyroid function tests, BMI, IR markers comprising the homeostasis model assessment for insulin resistance, the quantitative insulin sensitivity check index, the hepatic insulin sensitivity index, the whole-body insulin sensitivity index, and the insulinogenic index. A 75-g OGTT was performed and measurements of plasma glucose, insulin, and C-peptide were obtained at 0 min ($'$), 30$'$, 60$'$, 90$'$ and 120$'$. We also recorded the levels of total cholesterol (TC), HDL, LDL-cholesterol, triglycerides (TG), apolipoprotein B (ApoB), ApoA1, lipoprotein (a), homocysteine, C-reactive protein, folic acid and vitamin B12 levels. Statistical analysis was performed with the Mann–Whitney $U$ test and Spearman's correlations tests. Results are expressed as mean $\pm$ S.D. A two-tailed $P \leq 0.05$ was considered significant.
Results
We found that patients with HT had significantly higher levels of BMI ($26.5 \pm 5.3$ vs $24.8 \pm 4.7$ kg/m$^2$, $P=0.03$); C-peptide at 30 min ($7.3 \pm 2.8$ vs $6.6 \pm 2.5$ ng/ml; $P=0.03$); C-peptide at 60 min ($10.5 \pm 4.2$ vs $9.3 \pm 3.3$ ng/ml; $P<0.01$). In patients with HT we also found significantly higher levels of LDL-cholesterol ($124 \pm 31$ vs $116 \pm 33$ mg/dl; $P<0.01$), TG ($116 \pm 70$ vs $104 \pm 75$ mg/dl; $P=0.034$), ApoB ($97 \pm 23$ vs $86 \pm 24$ mg/dl; $P<0.001$), B12 vitamin ($467 \pm 308$ vs $372 \pm 174$ pg/ml; $P=0.02$), anti-thyroglobulin antibody ($141.7 \pm 179.0$ vs $121.2 \pm 160.3$ UI/ml, $P=0.01$). We found a negative correlation between TSH and TRAb levels ($r=-0.180$; $P<0.001$). In the whole group TSH positively correlated with TC ($r=0.097$; $P=0.04$) and ApoB ($r=0.117$; $P=0.02$). Conclusions: After treatment to normalize thyroid function, HT patients have a higher cardiovascular risk than GD patients, associated with overweight, subclinical inflammation and atherogenic lipid profile.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1659

**The syndromes of thyroid autoimmunity among patients with antiphospholipid syndrome**

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Background
Literature data regarding the prevalence of the syndromes of thyroid autoimmunity (AIT) in antiphospholipid syndrome (APS) are rather limited. However, quite frequently it is hypothesized on common pathophysiologic mechanisms underlining these disorders.
Objectives
The aim of this investigation was to evaluate the frequency of disorders of thyroid function and occurrence of anti-thyroid antibodies (ATAs) among patients with antiphospholipid syndrome (APS) included in the Serbian National APS Registry.
Methods
Started in January 2006, Serbian National APS Registry currently records information about 267 patients (206 females) in its database. These patients had a mean age of $44.89 + 12.95$ years and mean disease duration of $4.41 + 4.07$ years. Authors are planning to analyze serum levels of TSH, free thyroxine ($fT_4$), anti-thyroglobulin antibody (TgAb), anti-thyroid peroxidase antibody (TPOAb) and thyroid receptor antibody (TRAb) in all of these patients. Now, we are presenting initial results obtained from 68 patients (comprising 25.46% of all patients included in Serbian National APS Registry) who undergone endocrinology screening during last year.
Results
Thyroid involvement was observed in 30.9% (21 patients, 20 females) of studied patients with APS. All of these patients were positive for at least one of the anti-thyroid antibodies, TgAb or TPOAb. On the contrary, no one of them was found positive for TRAb. We found no case of active hyperthyroidism in this sample, but according to previous medical data six of them had a history of treatment for hyperthyroidism. Hypothyroidism requiring substitution treatment was found in 14.8% (ten patients) of patients with APS. All of these patients were positive for at least one of the anti-thyroid antibodies.
Conclusion
Coexistence of the syndromes of thyroid autoimmunity and antiphospholipid syndrome in significant percent of patients is obvious. However, mutual genetic and/or pathophysiologic mechanisms connecting antiphospholipid syndrome and autoimmune thyroid diseases have yet to be proven.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1660

**Anti neutrophil cytoplasmic antibody levels in graves patients using propylthiourasil**

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Aim
Antineutrophil cytoplasmic antibody (ANCA) positivity is usually determined in medium and large vessel vasculitis. This antibody has two different forms. Cytoplasmic ANCA (PR3-ANCA) occurs against proteinase-3, while perinuclear ANCA (MPO-ANCA) occurs against myeloperoxidase and bactericidal-permeability increasing proteins. The presence of ANCA is mostly seen in systemic vasculitis and also in drug induced vasculitis. There is no sufficient data about prevalence of positivity ANCA and development of antithyroid antibodies after treatment. In this study our aim was to investigate MPO-ANCA and PR3-ANCA levels in Graves's patients using propylthiourasil (PTU).
Material and method
Graves patients (9 men, 41 women) using PTU and 37 healthy control group were included in the study. ANCA levels of patients and control groups were evaluated.
Results
Mean level of PR3-ANCA in Graves group was significantly higher than control group ($P=0.025$). No significant difference was found between levels of MPO-ANCA ($P>0.05$). Positive correlation was observed between Anti-TPO, Anti-Tg with PR3-ANCA in patient group (respectively, $P=0.001$, $r=0.47$; $P=0.03$, $r=0.310$). Longer duration of treatment in the patient group showed increased levels of PR3-ANCA ($P=0.024$, $r=0.314$). PR3-ANCA levels in Anti-Tg positive patients were higher than those with negative ($P=0.018$). MPO-ANCA and PR3-ANCA were positive in two Graves patients while only MPO-ANCA positive in two patients.
Conclusion
PTU may cause ANCA positivity but vasculitis not develop in all cases. In our study; higher ANCA levels were found in Graves's patients receiving long term PTU treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1661

**A rare case of interferon-α-induced hyperthyroidism in a patient with chronic hepatitis C with granulocytopaenia and transaminasemia treated successfully with radioiodine**

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Background
Conventional management of Interferon-α-Induced Hyperthyroidism (IIH) with radioactive iodine (RAI) may be used when treatment with β blockers or antithyroid drugs (ATD), proves ineffective or is contraindicated.
Case presentation
We present a 38-year-old woman who has been treated with combined pegylated interferon alpha (INF-α) and Ribavirin for chronic hepatitis C. Destructive thyrotoxicosis appeared after four months of continuous IFN-α therapy and a β blocker was prescribed. Initially, the patient presented normal TSH 2.4 μIU/ml, however during therapy with INF-α, TSH diminished to 0.05 and thyroid hormones were elevated: $fT_4$ 23.1 pmol/l, $fT_3$ 7.2 pmol/L. Ultrasound examination showed completely irregular and greatly decreased echogenicity of the thyroid gland. The radioiodine uptake (RAIU) was deeply decreased to 2 and 3% at 5 h and 24 h, respectively. The thyroid scintiscan showed lack of isotope accumulation. Hypothyroidism developed and L-thyroxine was prescribed. The following year, hyperthyroidism reoccurred with TSH 0.08 μIU/ml, $fT_4$ 26.4 pmol/l, $fT_3$ 8.2 pmol/l, positive TSHR-Abs 6.2 (normal $<2$ IU/l) and mild Graves' Ophthalmopathy (GO). RAIU values were 23% at 5 h and 46% at 24 h. Thyroid scintiscan showed diffuse goiter. At this point β blocker was introduced and ATD was started. After three months of therapy an increased level of aminotransferases and granulocytopaenia were observed. Hence, the patient received RAI and glucocorticosteroid, while INF-α therapy was continued. After approximately 4 months, hypothyroidism reappeared with insignificantly raised TSH level. One year later the patient was euthyroid and required no further treatment.
Conclusions
Our report suggests that: i) radioiodine therapy might be an effective and safe method of treatment in cases of IIH with mild GO. ii) IFN-α therapy need not be discontinued in patients with IIH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1662

**Elastography in the differential diagnosis of thyroid nodules**

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Objective
Despite the publication of a recent meta-analysis of elastography in thyroid nodules, further work is necessary on this issue in different populations. In this study we aimed to evaluate the clinical value of elastography in nodular goiter in mild iodine defficient region without excluding according to nodule charecteristics.
Materials and methods
This prospective study was conducted between April 2010 and December 2011 in Yildirim Beyazit Diskapi Research Hospital Endocrinology outpatient clinic. Five hundred and twenty eight nodular goiter patients underwent included whom thyroid fine-needle aspiration biopsy (FNAB) were included in our study.
Results
There were 471 females and 57 males and their age ranged from 45 to 78 years. Total 586 nodules were evaluated in these patients. Elastography score and index were measured with real time ultrasound elastography (Hitachi EUB 7000 HV machine with using 13 MHz linear transducer). The area under the curve (AUC) for the elasto score (AUC) was 0.90 ($P<0.0001$) and AUC for the strain index was 0.945 ($P<0.0001$). We suggest that strain index reflects malignancy better than the elasto score. We conclude that 3.5 (68% sensitivity and 99% specifity) is cut-off point for elasto score. For strain index we conclude that 3.9 (73% sensitivity and 99% specifity) is cut-off point.
Conclusion
Strain ratio and strain index measurements of thyroid nodules in our population is efficient and increase the diagnostic performance of the sonography.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1663

**Assessment of left atrial mechanical function in thyroid dysfunction**

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Introduction
The objective of this study was to investigate left atrial mechanical function (LAMF) and to associate these measurements with diastolic function in subclinical (SHT) and overt hypothyroidism (OHT).
Methods
Twenty-six newly diagnosed patients with SHT (20F, 6M, mean age 42.2 $\pm$12.5 years), 21 patients with OHT (17F, mean age 40.2$\pm$8.5 years) and 28 healthy volunteers (control group, 21F, 42.4$\pm$11.2 years) were enrolled in this study. Standart M-mode measurements, mitral Doppler flow analysis, tissue Doppler parameters at lateral, septal and right ventricular annulus. Diastolic functions with both conventional and tissue Doppler method were measured. Left atrial maximal volume, minimal volume and volume at atrial systole measured using the disc method. Parameters reflecting left atrial mechanical function were measured.
Results
Active emtptying volume (AEV) and active emptying fraction (AEF) were found to be significantly increased in the OHT and SHT group compared to controls. Passive emptying volume and passive emptying fraction were lower in the OHT and SHT group compared to controls but did not reach statistically significance. Conduit volume was significantly lower in the OHT and SHT group compared to controls. The E/A ratio were significantly lower in the SHT and OHT groups compared to the controls. The lateral and septal E/Em were significantly higher, septal Em/Am was significantly lower in the OHT and SHT group compared to the controls. A correlation analysis revealed that the E/A ratio was negatively correlated with LAAEV and LAAEF ($r=-0.28$; $P=0.02$, $r=-0.29$; $P=0.012$ respectively). The E/Em ratio measured at the septal annulus was positively correlated with AEV and AEF ($r=0.24$; $P=0.04$, $r=34$; $P=0.003$ respectively). There was a negative correlation between the septal Em/Am ratio and AEF ($r=-0.26$; $P=0.03$).
Conclusion
LAMF are impaired in patients with thyroid dysfunction. We consider that impairment of LAMF is primarily caused by seconder to impairment of left ventricular diastolic functions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1664

**Prevalence, risk and clinical features of subclinical hyperthyroidism in patients with type 2 diabetes**

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Introduction
Both subclinical hyperthyroidism and type 2 diabetes (T2D) have been associated with an increase in cardiovascular disease risk and mortality. We aimed to assess the prevalence of newly diagnosed subclinical hyperthyroidism in a cohort of patients with T2D, and also to analyse the relationships between diabetes-related characteristics and the presence of subclinical hyperthyroidism.

Methods
Nine hundred and thirty-three diabetic patients without previous history of thyroid disease (45.4% females; mean age, 66.3 year; median duration of diabetes, 10 years; mean HbA1c, 7.8 ± 1.6%) were evaluated. A sample of 911 non-diabetic subjects without known thyroid dysfunction was studied as control group. Serum concentrations of thyrotropin were measured in all subjects. When thyrotropin values were lower than 0.40 mU/l serum concentrations of free thyroxine and triiodothyronine were also quantified.
Results
Subclinical hyperthyroidism was found in 22 female (4.3%) and 15 male (3.5%) diabetic patients. No case of overt hyperthyroidism was found. In comparison with control subjects, the relative risk (OR and 95% confidence interval) for subclinical hyperthyroidism was 1.67 (0.98–2.85). This OR was significant in women (3.69 (1.56–8.71), $P=0.001$), but not in men (0.78 (0.38–1.61)). In comparison with patients without hyperthyroidism, patients with subclinical hyperthyroidism were older, had longer duration of diabetes, had a higher percentage of goiter, a higher percentage of subjects treated with diet, and a lower percentage of subjects treated with oral antidiabetics. Furthermore, fasting glucose levels were lower in patients with subclinical hyperthyroidism. Logistic regression analysis showed that age and the presence of goiter were significantly related to subclinical hyperthyroidism in patients with T2D.
Conclusion
The risk for subclinical hyperthyroidism is increased in women with T2D. Advanced age and the presence of goiter are significantly and independently related with the presence of subclinical hyperthyroidism in diabetic population.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1665

**Softgel capsules of levothyroxine in the treatment of patients with gastric disorders: preliminary results**

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Increased need for thyroxine has been described in several gastrointestinal disorders (*H. pylori* infection, gastric atrophy, celiac disease), even in their occult or symptomless forms. A transitory or stable impairment of gastric acidity, by increasing pH value, seems to interfere with thyroxine availability, highlighting a novel role for the stomach in the following intestinal $T_4$ absorption. Distinctive dissolution profiles of different $T_4$ preparations (tablets and/or softgel capsules) have been described and softgel $T_4$ capsules performed better than tablets in alkaline pH. In patients with gastric disorders, where larger doses of thyroxine tablets are required, softgel capsules of $T_4$ may help to reach the therapeutic target and this represented the aim of our study. To this end, we have enrolled patients in therapeutic thyroid homeostasis, presenting with impaired gastric acid secretion and longlasting $T_4$ treatment (>5 years) with the same brand of tablets. All these patients had been advised and agreed to take oral thyroxine under fasting conditions, waiting at least 1 h before eating. Patients bearing additional conditions interfering with thyroxine treatment (e.g. drugs, intestinal disorders, pregnancy, etc.) were excluded from the study. A total of 30 patients (28F/2M; median age = 51 years; median $T_4$ dose = 2.05 μg/kg per day) met these criteria and switched from the usual tablets treatment to the softgel $T_4$ capsules at a significantly lower dose of thyroxine (median $T_4$ dose = 1.77 μg/kg per day; $P=0.0082$). Thyroid function and TSH were measured after 3, 6, 12 and 18 months from the treatment switch. Most of patients (18/30; 60%) showed a TSH increase after 3 months of treatment, with no change in $FT_4$ levels. However, after 6 months, TSH returned to the baseline values in about 2/3 of patients despite the reduced dose of $T_4$. Thyroid hormones were not changed during the study ($P$ = NS). These preliminar findings strongly suggest that treatment with softgel $T_4$ capsules may reach the therapetic goal at a lower dose than a $T_4$ tablet preparation in patients with impaired gastric acid secretion.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1666

**Serum thyrotropin, leptin concentrations, thyroid autoimmunity and smoking status, in an euthyroid, iodine-sufficient, Mediterranean population with different body mass index**

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Introduction
A positive correlation between serum thyrotropin concentrations (TSH) and body mass index (BMI) has been demonstrated. Some studies not shown this, in euthyroid subjects, or observed only if thyroid autoimmunity (TA) is present or in non-smokers (NS). Leptin (Lp) could be the major link between BMI and TSH.
Design
To analyze the relationship between TSH, free thyroxine ($FT_4$), Lp, TA (peroxidase and/or thyroglobulin antibodies) and smoking status in a representative sample of euthyroid, iodine–sufficient, non-hospitalized population of Catalonia, with different BMI. Data collected included if each person smoked or had smoked. Glycemia and insulinemia were also determined and HOMA index, calculated.
Results
Eight hundred ninghty four adults (390 men) of 44.87 ± 15.03 years and BMI 26.19 ± 4.61 kg/m$^2$ (17.01–52.70) with normal TSH (0.33–3.96 mIU/l) and $FT_4$ (0.87–1.90 ng/dl), and median urine iodine concentration 150.0 μg/l were studied. Lp correlated directly with BMI ($P=0.00$). There was no correlation between TSH and BMI. In men, TSH correlated directly with Lp ($P=0.00$) and in women, directly with Lp ($P=0.00$) and HOMA ($P=0.03$) and inversely, with $FT_4$ ($P=0.02$). Only in smoker men, TSH correlated directly with Lp ($P=0.01$) and HOMA ($P=0.02$). In smoker women, TSH correlated directly with Lp ($P=0.00$) and in NS women, inversely with $FT_4$ ($P=0.04$). The multivariate analysis showed that in men, the significant predictors of TSH variations are Lp, directly ($P=0.02$) and age, inversely ($P=0.01$). In women, the predictors are Lp and the presence of TA, directly ($P=0.04$; 0.00) and $FT_4$, inversely ($P=0.01$).
Conclusions
Leptin is a predictor factor of TSH concentration variations, in euthyroid subjects. Other predictor factors are different in men and women. The smoking status can influence the relationship between TSH and some predictor factors. These data should be taken into account before drawing conclusions about the parameters that influence TSH concentrations in euthyroid people with different BMI.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1667

**Resistance to thyroid hormone: clinical, biochemical and genetic features of Mediterranean population**

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Introduction
Resistance to thyroid hormones (RTH) is a genetic disorder caused in 85% of cases by mutations in thyroid hormone receptor beta gene (TRβ). The current information about it in Spanish population is scarce, limited to case reports or short series of patients. Thus, we aimed to describe the clinical, biochemical and genetic features of Mediterranean patients with RTH referred to our institution (one of the referral centres in Spain) for genetic testing during the last 15 years.
Material and methods
One hundred and sixty-six blood samples of Mediterranean patients (164 Spanish and 2 Greek patients) were received for RTH genetic testing between January

1997 and December 2011. Genetic testing was performed by PCR amplification followed by sequencing of exons 7, 8, 9 and 10. Clinical and biochemical features were obtained from available information sent by referring hospitals.
Results
In all, mutations were identified in 49 patients (28 probands and 21 relatives). 64.6% were women, and mean age at diagnosis among probands was $33.2 \pm 20.5$ years. The following clinical features were recorded: goitre in 50%, hyperkinetic behaviour in 32%, and tachycardia in 29%. Up to 19% of the probands had suffered some type of ablative therapy (radioiodine or thyroidectomy) before diagnosis. As for biochemical features, mean TSH was $10.2 \pm 21.5$ mUI/l (NV 0.4–4 mUI/l), and mean $fT_4$ was $2.75 \pm 0.9$ ng/dl (NV 0.8–2 ng/dl). We found seven new mutations not previously described: p.Ile276del, p.Arg320Ser, p.Phe451Leu, p.Pro452Arg, p.Leu456fsx9, p.Glu457Gly and p.Phe459Leu. The most frequent mutation in our population was p.Arg243Gln, present in three families. Surprisingly, none of our patients harboured the mutation p.Arg338Trp, which is the most common in the published series.
Conclusions
Clinical and biochemical features of our sample of Mediterranean population with RTH are similar to those previously described in the medical literature. However, genetic findings differ, with the identification of seven new mutations in TRβ gene.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1668

**Diagnosis and therapeutic practices of hyperthyroidism in france. results of a survey on 263 endocrinologists and 1214 patients (the thyrdel study)**

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There are considerable variations in diagnosis and therapeutical practices in hyperthyroidism (HT) between countries. Few of these differences rely on a scientific basis. we present the results of a study conducted among a representative sample of 263 French endocrinologists. All consecutive patients seen for HT during the study period were included. Symptoms, biological data and treatment were recorded. 1214 patients with hyperthyroidism were included, 1144 had an identified etiology: 802 Graves' disease (GD), 121 multinodular goitres (MNG), 112 iatrogenic hyperthyroidism (mainly amiodarone), 69 toxic adenoma and 40 thyroiditis. Antithyroid antibodies were measured in about half of the population (antiTPO 48.2%, antiR-TSH 56.3%). Ultrasonography was performed in 93.8% whereas scintigraphy was done in only 40.3% of the patients. Therapeutic management depended on the etiology. For the first episode of GD, antithyroid drugs (ATD) alone were the first line treatment in 90.9% of the patients, combined to surgery in 6.1% and with radioiodine in 2.9%. Surgery was preferred to radiodiodine in MNG (51.7 vs 22%) and toxic adenoma (57.3 vs 23.5%).
Euthyroidism was achieved after 3 months in 64.4% of GD, whereas 17.1% were still hyperthyroid and 18.4% were hypothyroid. Block and replace antithyroid regimen was used in 54.6% of the patients. After 3 months, 74.2% patients were euthyroid in the group block and replace vs 64.2% patients in the group with ATD alone ($P = 0.009$). For MNG, toxic adenoma and thyroiditis, around 75% of patients were euthyroid after 3 months, whereas it was the case for only 59.4% of patients with iatrogenic HT.
These results show large discrepancies between practices among a representative sample of French endocrinologists compared with recent American guidelines. Further studies are needed to determine the optimal management of HT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1669

**The impact of TSH on diffusion of thyroglobulin through follicular lumina: possible relation to hormone formation**

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Although the physico-chemical properties of colloid are not usually considered among the factors regulating thyroid function, it is conceivable that decreasing the viscosity of the intrafollicular colloid may facilitate diffusion of thyroglobulin (Tg) molecules within the lumina. The resulting increase in the number of contacts between Tg molecules and peroxidase at the apical membrane may promote the coupling reaction. Rats on a hormonal diet were given 0.5 μg $T_4$/ml of drinking water or 20 μg $T_4$ s.c. for 2 days up to 4 weeks to suppress endogenous TSH. 0.5 IU TSH was then injected together with 125 J. Animals were killed at intervals from 30 min up to 8 h. One thyroid lobe was used for autoradiography, the other for analyses of labeled iodoaminoacids by paperchromatography. Eight experiments with slightly varying design were performed. Within 4 h the total 125J uptake is not increased by TSH ($0.22 \pm 0.03\%$ (S.E.M.) vs $0.31 \pm 0.09\%$ in controls). However, in rats treated with $T_4$ s.c. for 2 days the percentage of 125J–$T_4$ rises from $3.6 \pm 0.2$ to $9.7 \pm 0.07\%$ within 1 h after TSH. While in controls 80% of all follicles with a diameter $> 30$ μm show the classical ring reaction, TSH decreases the relative fraction of ring labeled follicles to 30%. After long term preparation by $T_4$ given in the drinking water, the 125J–$T_4$ rises from $2.5 \pm 0.09$ to $7.0 \pm 1.0\%$ within 4 h after TSH. Whereas in controls, ring labeling was still present in 40% of the follicles, virtually all follicular lumina were homogeneously labeled in TSH treated animals.
Since $T_4$ formation is simultaneously accelerated, the enhanced contact between intraluminal Tg molecules and the membrane bound peroxidase may be the mechanism by which TSH promote early $T_4$ synthesis before iodine uptake is increased.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1670

**Iodine supply and prevalence of thyroid autoimmunity and autoimmune thyroiditis in children and adolescents between 1 and 16 years old in our city**

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Introduction
Thyroid autoimmunity is related to iodine supply. Iodine nutrition has improved in our country, but the prevalence of autoimmune thyroiditis in our children is unknown.
Objectives
To determine the status of iodine nutrition in children and adolescents in our city. To calculate local prevalence of thyroid autoimmunity and autoimmune thyroiditis in pediatric ages.
Design
Cross-sectional epidemiological study.
Subjects and methods
By a multistage probability sampling 1387 children and adolescents aged between 1 and 16 were selected. Physical examination was carried out including neck palpation. Parents were asked about eating habits, social and demographic aspects. Urinary iodine, free thyroxine, TSH and antiperoxidise and antityroglobulin antibodies were measured. Thyroid autoimmunity was diagnosed when any antibody was positive and autoimmune thyroiditis when autoimmunity was associated with impaired thyroid function or goitre. To study the relation between thyroid autoimmunity and urinary iodine with independent variables we used binary and multiple logistic regression and multiple lineal regression.

Results
Median urinary iodine was 199.5 μg/l. The prevalence of thyroid autoimmunity and autoimmune thyroiditis were 3.7% (2.4–5.0) and 1.4% (0.4–2.4). Thyroid autoimmunity is associated with female sex (OR 2.78; $P<0.001$) and age (OR 1.30; $P<0.001$). Iodine status is associated with milk and dairy products ($P<0.001$) and vegetable intake ($P=0.021$), but not with use of iodated salt at home ($P=0.1$).
Conclusions
The iodine supply in children and adolescents in our city is optimal. Milk and dairy products are the most important iodine source. Thyroid autoimmunity and autoimmune thyroiditis are prevalent in pediatric ages in our city mainly in females and older subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1**

| | Thyroid autoimmunity<br>Prevalence (% and confidence interval at 95%) | Autoimmune thyroiditis<br>Prevalence (% and confidence interval at 95%) |
|---|---|---|
| Total group | 3.7 (2.4–5.0) | 1.4 (0.4–2.4) |
| Males | 2.3 (1.1–3.5) | 0.8 (0.0–2.8) |
| Females | 5.0 (3.4–6.6) | 1.9 (0.9–2.9) |
| Prepubertal | 2.4 (1.2–3.6) | 0.5 (0.0–1.8) |
| Pubertal | 6.8 (4.2–9.4) | 3.0 (1.4–4.6) |
| 12–16 years old | 6.2 (4.9–7.5) | 3.2 (0.0–6.5) |
| 6–12 years old | 4.6 (2.6–6.6) | 1.2 (0.0–2.4) |
| 1–6 years old | 0.6 (0.0–2.6) | 0.0 (0.0–0.9) |

## P1671

### Prevalence of thyroid diseases in a population of non-endocrine tumors in north-east Italy

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Few data on prevalence of thyroid diseases in neoplastic groups of patients has been reported. A slight increased prevalence of hypothyroidism and autoimmune thyroiditis in patients with breast cancer and cutaneous melanoma are been described.
Aim
Of the study was to evaluate the prevalence of thyroid diseases in a group of non-endocrine neoplasm consecutively evaluated by the oncologist and endocrinologist in a iodine-sufficient area of north-east Italy. Three hundred twenty two patients (Group A)(128 women and 194 men, aged 38–88 years, median 65 years) were observed at the Department of Medicine (Endocrine Unit and Day-Hospital Unit) between 2007 and 2010 and compared with a group of three hundred consecutively hospitalized patient for non-oncological diseases of similar age and sex (Group B). 64 pts of group A were affected by pulmonary, 56 by breast, 84 by colon, 35 by gastrointestinal-biliary-pancreatic and 32 by urogenital cancer; 13 pts were affected by other neoplasms. Thyroid diseases were present in 9.9% of patients (32 pts) of group A (female 13.2% and male 7.7% of total group; 2.1% hypothyroidism, 2.4% autoimmune thyroid disease, 0.6% nodular toxic goiter and 6.0% multinodular nontoxic goiter) compared with 14.3% (43 pts) of group B (female 13.0%, male 5.4%; $P<0.01$; 4.1% hypothyroidism, 5.0% autoimmune thyroid disease, 1.0% nodular toxic goiter and 4.2% multinodular nontoxic goiter) . The prevalence of thyroid disease was 9.3% in pulmonary, 12.5% in breast, 7.1% in colon, 11.4% in gastrointestinal–biliary–pancreatic and 17.1% in urogenital cancers, 0% in other neoplasms.
In conclusion, these data shows that in this group of patients the prevalence of thyroid diseases is lower to that of a general non-oncologic hospital population and this may be due to the fact that patients were predominantly male. The higher prevalence of thyroid disease is observed in the urogenital and mammary cancer.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1672

### Serum concentrations of thrombospondin-1 and adiponectin in patients with hyperthyroidism before and after normalisation of thyroid function

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Background
Hyperthyroidism is a common endocrine disorder that is linked to increased cardiovascular morbidity and mortality. Thrombospondin, the matricellular protein, is an important player in the process of cardiac remodelling with complex effects on cardiovascular disease (e.g. prothrombotic and antiangiogenic effects vs potential protective effects in heart failure). Adiponectin is an adipose tissue-derived hormone that shows beneficial effects on atherogenesis, endothelial function, vascular remodelling and insulin resistance. Effects of treatment of thyrotoxicosis on serum adiponectin and thrombospondin concentrations have been poorly investigated, so far.
The aim of this study was to evaluate the circulating levels of adiponectin and thrombospondin-1 in patients with hyperthyroidism before and after normalisation of thyroid function.
Methods
We studied 15 patients (four males), (age $51.8\pm15.3$ (mean $\pm$ S.D.) years) with hyperthyroidism due to Graves' disease, toxic adenoma or toxic multinodular goitre, treated with thiamazole. Thyroid function was normalised after 6–10 weeks. Patients were evaluated at time of diagnosis and again after normalisation of thyroid function with appropriate therapy. Serum concentration of free $T_3$, free $T_4$, TSH, adiponectin and thrombospondin-1 were measured in all subjects before and after treatment with thiamazole.
Results
There was a significant decrease in free $T_3$ and free $T_4$ concentrations (from $8.74\pm4.79$ to $3.54\pm2.40$ pg/ml, and from $4.48\pm2.21$ to $1.02\pm1.07$ ng/ml, $P<0.001$), for free $T_3$ and free $T_4$ respectively. There were, however, no significant differences in adiponectin ($30807.3\pm12996.2$ *vs* $32755.3\pm16638.8$ ng/ml, $P=$NS) and thrombospondin-1 levels ($37400.7\pm11097.7$ vs $40850.7\pm9426.4$ ng/ml, $P=$NS) between patients evaluated at time of diagnosis and again after normalisation of thyroid function.
Conclusions
Our results suggest that reductions of cardiovascular risk due to immediate effects of treatment of thyrotoxicosis are unlikely to be directly related to changes in serum adiponectin and thrombospondin-1.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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## P1673

### Changes in serum homocysteine, lipid, vitamin B12, folate levels and glomerular filtration rate before and after treatment in overt hypothyroidism

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Overt hypothyroidism is associated with an increased risk for cardiovascular disease. We aimed in this study, to assess the changes in renal function, serum lipids, vitamin B12, folate and homocysteine levels before and after treatment in hypothyroid patients.
Fifty-four (male/female;7/47) hypothyroid patients enrolled to study. We measured homocysteine(tHcy), total cholesterol(TC), triglyceride(TG), HDL-cholesterol (HDL-C), creatinine, folic acid, and vitamin B12 levels in both hypothyroid and euthyroid state. Table 1 summarizes the clinical and laboratory data of patients before and after L-$T_4$ replacement. Homocystein levels in hypothyroidism ($9.67\pm5.24$ mmol/l) were significantly higher than in euthyroid state ($8.16\pm3.38$ mmol/l, $P=0.038$). Serum creatinine was higher and GFR was lower before treatment. Following L-$T_4$ replacement, renal functions significantly improved. After achieving the euthyroid state, folate levels significantly increased although vitamin B12 remained unchanged. Serum lipids were significantly high before treatment. After L-$T_4$ replacement, lipid levels statistically significant decreased. We demonstrated a negative correlation between GFR and lipids and a positive correlation with tHcy and lipids in hypothyroid state. All of these correlations became not significant after L-$T_4$ replacement.
In conclusion, our study confirmed previous reports on the effects of thyroid status to cardiovascular risk factors namely hyperlipidemia and hyperhomocysteinemia.

Furthermore, we concluded that, there was a strong correlation between serum lipids and both of GFR and tHcy in only hypothyroid status.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1** Clinical and laboratory characteristics of the patients in hypothyroid and euthyroid state.

| | Hypothyroid state($n=54$) | Euthyroid state($n=54$) | *P* value |
|---|---|---|---|
| TSH (μIU/l) | 37.05±34.15 | 2.32±1.55 | <0.001 |
| $fT_4$ (ng/dl) | 0.64±0.33 | 1.31±0.29 | <0.001 |
| Creatinine (mg/dl) | 0.78±0.23 | 0.68±0.13 | <0.001 |
| Homocysteine (μmol/l) | 9.67±5.24 | 8.16±3.38 | 0.038 |
| GFR (ml/min) | 98.42±24.32 | 111.48±20.79 | <0.001 |
| Total cholesterol (mg/dl) | 227.75±56.56 | 196.92±36.34 | <0.001 |
| LDL-C (mg/dl) | 140.97±45.32 | 115.37±33.01 | <0.001 |
| HDL-C (mg/dl) | 52.94±16.35 | 48.83±15.72 | 0.001 |
| Folate (ng/ml) | 7.61±2.76 | 8.53±3.19 | 0.017 |

## P1674

### Decreased echogenicity and increased vascularization of fetal thyroid on 2D-ultrasonography caused by mother's Graves' disease

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Fetal ultrasonography is recommended in pregnant women with elevated TSH receptor antibodies (TRAK) or treated with antithyroid drugs in attempt to recognize fetal thyroid dysfunction. We report two cases of pregnant women with Graves' disease in whom fetal thyroid ultrasonography was especially useful in diagnosing child involvement.

A 33-year-old woman at 30-week pregnancy with 3-months history of hyperthyroidism treated with propylothiouracil (PTU) 150 mg daily. Patient TSH was 0.033 μIU/ml (normal, 0.4–4.0 μIU/ml), $fT_4$ 9.24 pmol/l (normal, 11.5–22.7 pmol/l), $fT_3$ 6.1 pmol/l (normal, 2.8–6.5 pmol/l), TRAK 17.28 IU/ml (positive results >1.8 IU/ml). Fetal ultrasonography identified enlarged hypoechoic thyroid gland with increased peripheral vascularization. Fetal hormones concentrations obtained through cordocentesis were indicative for subclinical hypothyroidism: TSH 18.5 μIU/ml (normal, 2.4–12.8 μIU/ml), $fT_4$ 11.0 pmol/l (normal, 9.7–16.7 pmol/l), $fT_3$ 1.17 pmol/l (normal, 1.1–3.7 pmol/l).

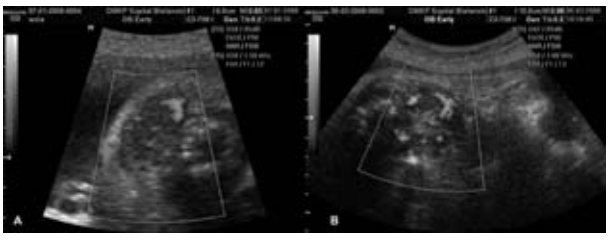
The second patient, 30-year-old woman at 35-week pregnancy with 5 months history of hyperthyroidism and inadequate PTU treatment. Her TSH was <0.001 μIU/ml, $fT_4$ 27.8 pmol/l, $fT_3$ 18.1 pmol/l, TRAK 35.5 IU/ml. Fetal ultrasonography revealed hypoechoic goiter with increased central vascularization. The diagnosis of the mother and fetal hyperthyroidism was established.

Our results show that fetal thyroid gland when affected by transplacental passage of of maternal TRAK can demonstrate the same characteristic ultrasound pattern of Graves' disease as in adults: enlargement, hypoechogenicity and hypervascularization. In accordance to previous observation the mode of blood flow within fetal thyroid enables discrimination between fetal hyperthyroidism and hypothyroidism caused by ADT overdosing. The 2D- ultrasound images of fetal goiter with increased blood flow are demonstrated.

Decreased echogenicity, increased thyroid size and increased peripheral vascularization of the fetal thyroid (A); decreased echogenicity, increased thyroid size and increased central vascularization of the fetal thyroid (B).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.



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## P1675

### Measurement of TSH, $FT_4$ and $FT_3$ with immunoassays based on LOCI technology

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Background

Measurements of TSH, free tetraiodothyronin ($FT_4$) and triiodothyronin ($FT_3$) are essential to achieve assessment of thyroid function. Luminescent Oxygen Channelling Immunoassays (LOCI) have recently been developed for the measurement of biomarkers of thyroid function. Such homogeneous immunoassays allow a faster delivery of the results. The aim of our study were therefore to determine the reference values for LOCI TSH, $FT_4$ and $FT_3$ assays and to compare LOCI assays with our routine assays.

Method

Serum samples from 129 healthy individuals (mean age: 54 years, 45 females and 84 males) were evaluated with the LOCI TSH, $FT_4$ and $FT_3$ assays on the Dimension Vista (Siemens). In addition, patients' samples from our laboratory routine flow ($n=177$) were used for method comparison with the DxI assays (Beckman Coulter), a 'conventional' heterogenous immunoassay.

Results

The reference intervals obtained from the healthy volunteers group were for LOCI assays: .27–2.89 μU/ml for TSH, 0.80–1.30 ng/dl for $FT_4$ and 2.61–4.06 pg/ml for $FT_3$. The LOCI assays were significantly correlated with our routine assays ($r=0.987$, $P<0.0001$ for TSH; $r=0.924$, $P<0.0001$ for $FT_4$ and $r=0.514$, $P<0.0001$ for $FT_3$). For the method comparison, the Passing and Bablok regression analysis showed intercepts of −0.015; 0.098 and 0.297 for TSH, $FT_4$ and $FT_3$ respectively. The slopes were 1.107 for TSH, 1.333 for $FT_4$ and 1.075 for $FT_3$. No significant deviation from linearity for these three parameters was observed. Bland and Altman plots revealed mean differences of −1.8 for TSH, 0.36 for $FT_4$ and 0.47 for $FT_3$.

Conclusion

Our study has provided reference values for the LOCI TSH, $FT_4$ and $FT_3$ assays. Furthermore, we have demonstrated a good agreement between LOCI assays and 'conventional' immunoassays.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1676

### Thyroid function tests in acute kidney injury

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Methods

A prospective study in 35 patients hospitalized for AKI for two consecutive years was carried out. TFT (serum thyrotropin, TSH; free thyroxine, $FT_4$; and total triiodothyronine, $T_3$ concentrations) were measured in each patient on three occasions: at admission, at hospital discharge and at their first outpatient visit.

Results

Total prevalence of alterations in TFT was 82.9% ($n=29$). Of those, euthyroid sick syndrome (ESS) with low $T_3$ only was the most common ($n=13$, 37.1%) derangement. In the whole group of patients TSH (0.93 (0.35–2.27) μU/ml) and $FT_4$ (1.2±0.3 ng/dl) were normal, whereas $T_3$ was low (0.7±0.1 ng/ml). TSH, $FT_4$ and $T_3$ were similar in different types of AKI. In the simple regression analysis we only found a negative correlation between TSH and serum urea concentrations ($\rho=-0.382$; $P=0.024$). At hospital discharge (median hospital stay 6 days (2–10)), TFT showed significant changes only in $T_3$ concentrations (0.8±0.3 ng/ml, $P=0.013$). At this point, the percentage of patients with normal TFT increased from 17.1% at baseline to 40% at discharge and then to 66.7% at their first outpatient visit. We found no association between the presence and type of alterations in TFT and clinical (sex, age, personal history of diabetes and/or hypertension, number and type of drugs used, signs and symptoms, and degree, type and etiology) and

prognostic (hospital stay, recovery of renal function, need of renal replacement therapy, residual chronic renal failure and mortality) factors associated to AKI.
Conclusion
Over 80% of AKI patients exhibit alterations in TFT. The commonest derangement is ESS (~70%), mainly low $T_3$ syndrome, which is present in about one third of the patients with altered TFT. ESS recovers spontaneously as renal function improves. The presence of TFT alterations seems not to be associated with clinical and prognostic implications in AKI patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1677

### Spectrum and prevalence of thyroid diseases detected by ultrasonographic examination in west black sea region of Turkey, the MELEN study

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Objective
Aim of the study was to investigate the spectrum and prevelance of goiter and thyroidal diseases by ultrasonography in moderately iodine deficient area.
The MELEN study is a prospectively designed survey on the prevalence of cardio metabolic risk factors and thyroid diseases in Turkish adults. A total of 2298 subjects with a mean age of 50 (age range 18–92) were interviewed. Thyroid ultrasonography was performed and interpreted by the same experienced physician, using the same equipment with a 5–12-MHz linear-array transducer. Goiter prevalence was defined according to Gutekunst's criteria. After an overnight fast, blood samples were collected from all the study subjects for the determination of serum free thyroxine, TSH were measured.
Results
The rate of goiter showed a significant female predominance (35% in women and 23% in men, $P<0.001$). According to ultrasonographic examination, the most common thyroid disease was multinodular goiter (MNG; 42%), followed by nodular goiter (NG; 14.6%). Taking into account that subjects had been operated possibly due to NG/MNG, the crude prevalence of nodular disease in the region reached up to 65%. The rate of normal thyroid gland was only 27.4%. According to TSH values, thyrotoxicosis (TSH $<0.35$ μIU/ml) rate was 12.9% and subclinical and overt hypothyroidism (TSH $>$ 4.5 μIU/ml) rate was 7.1%.
Conclusion
In an iodine-deficient community, a progressive increase with age in goiter prevalence, thyroid nodularity, and functional autonomy was observed. Thyrotoxicosis and nodular thyroidal diseases are more important and predominant thyroid diseases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1678

### Optimal timing of thyroxine dose adjustment for treating patients with primary hypothyroidism: what is the estimated time required to reach stable TSH levels?

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Aim
Serum TSH is the target value by which adequate thyroid hormone supply can easily be monitored in patients with primary hypothyroidism. It is controversial when TSH should be measured before thyroxine dose adjustments are made, 4–8 weeks are recommended. To define the time when dose adjustments of thyroxine are feasible more clearly, we looked at the time required to reach stable TSH levels.
Methods
We prospectively studied a case series of patients with newly diagnosed hypothyroidism (TSH $>10$ mU/l and $fT_4<12.3$ pmol/l). Treatment was initiated with standardised doses of thyroxine, 0.05 mg/day if there was a history of cardiac disease and 0.1 mg/day in all other cases. Blood pressure, pulse, weight, TSH, $fT_4$, $fT_3$, cystatin C and creatinine were recorded once weekly on the same day for a total of 8 weeks. Thyroxine dose was kept constant until the change in TSH between one week and the next was $<2$ mU/l, and it was increased by 0.025 mg every 8 weeks until TSH normalised.
Results
Twelve (six male) patients, 44.6 + 11.6 (mean + S.D.) years old with a median TSH at baseline of 57.6 mU/l (range 11.2–151.8 mU/l) and a median cystatin C at baseline of 0.77 mg/l (range 0.47–1.3 mg/l) gave informed consent. The patients were observed for a minimum of 8 weeks and a maximum of 24 weeks (eight patients for 8 weeks, one patient for 16 weeks and two patients for 24 weeks), resulting in 19 observation periods. Nine patients were euthyroid by the end of the study, the remaining three patients could not participate for a further 8 weeks due to work responsibilities. After adjusting for the number of observation periods for each patient, the mean time to achieving stable TSH (defined as a change in TSH of $<2$ mU/l per week) was 3.45 weeks (95% CI, 2.44–4.46 weeks).
Conclusion
TSH does not seem to change significantly after a mean of 3.5 weeks after an alteration of thyroxine dose. Dose adaptations can be made after 4 weeks of treatment without having to wait 6–8 weeks.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1679

### The concept of ultrasonographic thyroid pattern: re-evaluation after 10 years

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Aim
To re-analyze the concept of echographic thyroid patterns (ETP) and clinical diagnostic in thyroidology, proposed 10 years ago.
Material/method
i) Between 1996 and 2012, $>25\,000$ thyroid ultrasound, linear probes, 7.5 MHz. ii) Description – eight ETP: pattern 0 – lack of thyroid, 1 – hypoechogenous pseudonodular; 2 – hypoecogen homogenous; 3 – micronodular hypoechogenous; 4 – macro ($>$ 10 mm; ±micro) nodular; 5 – neomogenous hypo/hyperechogenous pseudonodular; 6 – micronodular anechogenous; 7 – hyperecogenous diffuse (normal). iii) Patients: 1092 HT, 5 'sero-negative' thyroiditis (T S-N), 61 thyroiditis with hyper-ATG-emia (T-ATG), 70 idiopathic mixedema (IM); 67 Graves-Basedow disease (GBD) without HT; 995 control. D. Fiability analysis = specificity 2011/specificity 2003.
Results
i) Number echographies/pattern/disease in table.
ii) Sensitivity, specificity, predictive positive value and accuracy for P1: 62,64; 89,73; 88,79; 74,73.
iii) Test X2 (7 degree of freedom): $\gg 24.36$ $<P\ll 0.001$. iv) Reliability 96%.
Conclusions
i) From sensitivity, specificity and predictive positive value analysis, the classification proposed in 2003 (*Rom. J. Endocrinol. Metab.*, 2003, **2**(3) 40. OC.22) is extremely exact and correct (reliability: 96%). ii) VPP ~90 asks the diagnostic to be corroborated with antibody levels. Description 'hypoechogenous-pseudonodular' does not mean implicitly HT (could be T-ATG, too). iii) VPN 80% (pattern 4), means that, when there is a nodule over 10 mm, then HT is improbable. iv) When there are pattern 6 or 7, normality is almost sure. v) Pattern 5 suggest thyroiditis/ Graves-Basedow disease and thyroid hyperfunction.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

**Table 1** Number of echographies per pattern per disease.

| | HT | T S-N | T-ATG | IM | GBD | Control |
|---|---|---|---|---|---|---|
| Pattern 0 | 9 | 0 | 0 | 5 | 4 | 9 |
| Pattern 1 | 1038 | 1 | 41 | 12 | 4 | 74 |
| Pattern 2 | 175 | 0 | 3 | 34 | 12 | 86 |
| Pattern 3 | 70 | 0 | 3 | 3 | 4 | 127 |
| Pattern 4 | 113 | 4 | 13 | 9 | 9 | 439 |
| Pattern 5 | 181 | 0 | 8 | 0 | 31 | 63 |
| Pattern 6 | 6 | 0 | 0 | 0 | 0 | 43 |
| Pattern 7 | 65 | 0 | 3 | 9 | 11 | 217 |
| Total | 1657 | 5 | 71 | 72 | 75 | 1058 |

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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1680

### The role of color-flow Doppler ultrasonography for differential diagnosis between gestational transient thyrotoxicosis and Graves' disease

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Introduction
The differential diagnosis between Graves' disease (GD) and gestational transient thyrotoxicosis (GTT) is difficult in the absence of history and clinical features of GD. In this study, we aimed to determine the role of peak systolic (PSV) and end-diastolic velocities (EDV) of the right and left inferior thyroid arteries (ITA) as measured by color-flow Doppler ultrasonography (CFDUSG) for discriminating GTT from treatment naive GD.
Methods
ITA-PSV and EDV of 41 patients with GTT, 31 age matched pregnant patients with GD, 24 age and sex-matched non-pregnant patients with GD and 25 age and sex-matched healthy euthyroid subjects were assessed by CFDUSG. Patients were diagnosed based on the history, clinical findings and TSH-receptor antibody (TRAb) levels.
Results
The mean ITA-PSV and EDV in patients with GTT were significantly lower compared to pregnant patients with GD and higher compared to healthy euthyroid subjects. However, the mean ITA-PSV and EDV in pregnant patients with GD were significantly lower compared to non-pregnant patients with GD (Table 1). ITA-PSV and EDV were positively correlated with TRAb levels ($r=0.515$ for right ITA-PSV, $r=0.463$ for left ITA-PSV, $r=0.615$ for right ITA-EDV and $r=0.526$ for left ITA-EDV respectively). However, in spite of the significant difference between mean ITA-blood flow velocities, an overlap was found between ITA blood-flow velocities in a considerable number of patients with GTT and pregnant patients with GD.
Conclusion
Due to the overlap occuring between a substantial number of patients, the measurement of ITA-blood flow velocities by CFDUSG is not a useful diagnostic tool for differential diagnosis between GTT and GD during pregnancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Table 1** ITA-blood flow velocities of the study participants.

| | GTT ($n=41$) | Pregnants with GD ($n=31$) | Non-pregnants with GD ($n=24$) | Healthy euthyroid ($n=25$) | $P$ (ANOVA) |
|---|---|---|---|---|---|
| Right ITA-PSV* | 24.3±7.3 | 37.58±10.89 | 57.03±25.7 | 17.08±4.83 | <0.001 |
| Right ITA-EDV* | 10.39±3.7 | 15.9±6.2 | 24.03±11.87 | 7.78±3.07 | <0.001 |
| Left ITA-PSV* | 24.75±6.15 | 35.19±11.19 | 59.4±22.77 | 17.37±4.81 | <0.001 |
| Left ITA-EDV* | 11.43±3.93 | 15.8±5.05 | 25.97±11.80 | 8.16±2.73 | <0.001 |

*cm/sn.

## P1681

### The prevalence of hypercreatininemia in patients with primary hypothyroidism

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Background and aims
Clinical studies demonstrated that hypothyroidism can be a cause of hypercreatininemia. The aims of the present study were to investigate the prevalence of hypercreatininemia in patients with primary hypothyroidism.
Materials and methods
We retrospectively studied serum creatinine levels in 54 patients with primary iatrogenic hypothyroidism (iatrogenic hypothyroidism result seccondary to radioactive iodine therapy and surgery). Results were compared with measurements in 44 subjects with euthyroid state.
Results
The subjects of study group: 31 women (57.40%) and 23 men (42.59%) were between 44 and 71 years mean age S.D. 62.42±6.34 years. In the hypothyroid state, mean TSH levels, free thyroxine (free $T_4$) and creatinine levels were 16.1 ±2.7 mU/l, respectively 0.3±0.1 ng/dl, 1.1±0.1 mg/dl. Fuve patients (9.25%) had creatinine levels greater than the reference intervals There was significant correlation of TSH levels with creatinine levels ($r=0.661$, $P<0.001$). The prevalence of hypercreatininemia in patients with primary iatrogenic hypothyroidism was 9.25% (five patients – three women and two men). In control group no subjects had a creatinine levels greater than the reference intervals.
Conclusion
We conclude that hypercreatininemia is common, in patients with primary iatrogenic hypothyroidism. The mechanism by which hypothyroidism induces hypercreatininemia is incompletely understood but creatinine levels should be evaluated in any patient with hypothyroidism.
Key words: primary hypothyroidism, creatinine levels, TSH.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1682

### Prevalence of thyroid diseases in patients with colon cancer and benign colon polyps

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Aim
In this study, we aimed to evaluate thyroid functions, thyroid antibody positivity, thyroid ultrasonography (US) findings and prevalence of thyroid cancer in patients with colon cancer and benign colon polyps. Also, we aimed to compare prevalence of thyroid diseases in two groups.
Materials and methods
Fifty-seven patients with colon cancer and 50 patients with colon polyps were included in the study. All patients were evaluated with thyroid US and thyroid fine needle aspiration biopsy (FNAB) was performed in indicated nodules.
Results
There were 57 (53.27%) patients with colon cancer and 50 (46.23%) patients with colon polyps. There was no difference in terms of age and sex distribution between groups ($P=0.622$ and $P=0.529$ respectively). Ultrasonographically, nodular and multinodular goiter was observed in 30 (52.63%) patients with colon cancer and 29 (56%) patients with polyp. FNAB was performed in 23 patients in cancer groups and 14 patients in polyp group. Cytology was reported as malignant in one patient with cancer and in one patient with polyp. In these two patients and in another colon cancer patient with suspicious FNAB result, thyroid cancer was confirmed histopathologically. Presence of thyroid disease defined as thyroid dysfunction or positive antithyroid antibody or presence of nodule or thyroiditis in US was found in 44 (77.19%) patients with cancer and 44 (88.0%) patients with polyp ($P=0.228$).
Conclusion
This study showed that thyroid dysfunctions, thyroid US findings and prevalence of thyroid cancer are similar in patients with colon cancer and colon polyps. Since thyroid pathologies are observed in more than tow or three of patients in both groups, we think laboratory examinations for thyroid functions and thyroid antibodies and thyroid US should be a part of investigation in patients with colon cancer and colon polyps.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## P1683

**'Racing heart': a case of unrecognized hyperthyroidism presenting as monomorphic ventricular tachycardia**

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Ventricular tachycardia may occur in the setting of primary hyperthyroidism despite the absence of typical hyperthyroid symptoms. The case is a 40-year old female coming in for epigastric pain and vomiting who was incidentally found to have monomorphic ventricular tachycardia. Despite this she was in no respiratory distress, and exhibited no symptoms of heart failure. Other than agitation, physical examination showed no signs of hyperthyroidism. Initial management included numerous electric cardio-versions and Amiodarone, which was immediately discontinued after thyroid function tests revealed a low TSH ($<0.005$ mIU/l) and elevated free $T_4$ (35 pmol/l) consistent with primary hyperthyroidism. A neck ultrasound revealed normal sized glands and no increased vascularity. Transthoracic echocardiogram showed normal-sized left ventricle with multi-segmental wall motion abnormalities and depressed overall systolic function (EF = 49% by Teicholz's). After a few months of treatment with Methimazole 20 mg/day and Propranolol 40 mg/day, free thyroxine levels normalized with no recurrence of ventricular tachycardia. Thyroid scan done 6 months after Amiodarone intake and 1 month off anti-thyroid medications showed normal-sized glands and normal 2/24 h uptake values, and a repeat TTE showed normal geometry and improved ejection fraction at 78%. Patient remains functional on her daily activities and remains asymptomatic.

Conclusion

Ventricular tachycardia as an initial presentation of autoimmune hyperthyroidism is rare, the most common arrhythmia being atrial fibrillation. However treatment with anti thyroid medications, β-blockers and close monitoring can result to improved clinical outcomes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1684

**A rare case of periodic hypokalaemic paralysis secondary to Graves' thyrotoxicosis**

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A 42-year-old gentleman of Ghanaian origin presented with a 1 month history of worsening severe proximal myopathy. On presentation his symptoms had progressed to the extent that he was unable to mobilise from his bed. Prior to this illness he had been fit and well. Examination revealed severe proximal myopathy and a uniformly enlarged, smooth thyroid gland. There was no clinical evidence of hyperthyroidism.

Blood tests on admission showed a serum potassium level of 2.1 mmol/l, TSH $<0.05$ mU/l, $FT_3$ 20.3 pmol/l, $FT_4$ 35.0 pmol/l. TSH receptor antibodies were strongly positive at 24.5 μ/ml. A diagnosis of thyrotoxic periodic paralysis (TPP) was made. Within 3 h of commencing intravenous potassium replacement the proximal myopathy resolved entirely.

Following an overnight stay in hospital he was discharged on a combination of Carbimazole 40 mg once daily and Propanolol 40 mg tds. His serum potassium level at discharge was 4.4 nmol/l and remained normal at 6 weeks follow up without additional potassium supplementation. Following normalisation of his thyroid hormone levels he had no further episodes of paralysis.

TPP is a rare, but potentially life-threatening complication of hyperthyroidism due to a sudden intracellular shift of potassium. The electrolyte shift is most probably due to an increased activity of the sodium–potassium adenosine triphosphatase pump (Na/K-ATPase) either directly by the thyroid hormones or indirectly via adrenergic stimulation[1]. It appears likely that TPP patients have a genetic predisposition to activation of the Na/K-ATPase genes[2]. TTP is predominantly seen in patients of Asian descent[3,4]. Here we present a rare example of an Afro-Caribbean man developing hypokalaemic periodic paralysis in the context of Graves' thyrotoxicosis.

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Serum potassium and free thyroxine levels during admission and at 6 weeks follow up.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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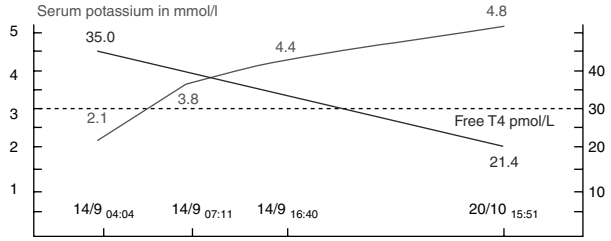


## P1685

**Ultrasonographic aspects in *post-partum* thyroiditis**

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Introduction

*Post-partum* thyroiditis (PPT) is an autoimmune disorder of the thyroid gland, which occurs in the first year after parturition. *Post-partum* thyroiditis occurs in 50% of TPO AB + ve women and is characterised by transient hyperthyroidism followed by transient hypothyroidism during the first 6 months, *post-partum*. A third of the latter group develop permanent hypothyroidism. The aim of this study was to evaluate sonographic characteristics in a group of PPT patients in Sibiu county in 2 years (2009–2011).

Material and method

The study group consisted of 39 PPT patients and 40 normal *post-partum* women as the control group. Physical examination of thyroid, value of TSH, $FT_4$, TPO and ultrasonography were carried out monthly in PPT patients until remission for up to 6 months *post-partum*.

Results

Visible goiter was detected in 19.1% of patients and 6.1% of the control group ($P<0.001$). Hypoechogenecity in thyroid sonography was present in 91.9% of patients and 6% of the control group ($P<0.001$). At 6 months after delivery, only four patients had abnormal thyroid function tests, while three patients with TSH $>10$ mU/l still showed hypoechogenicity in thyroid sonography. In conclusion, sonography may be recommended as an adjuvant to laboratory tests in evaluation of PPT patients.

Conclusion

Prolonged follow-up of the subsequent thyroid function and ultasonographyc characteristics may be needed in women who experience PPTD and/or show a high titre of antithyroid antibody.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1686

**Primary and secondary autoimmune thyroiditis in rheumatoid patients**

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Aim

Studying of features autoimmune thyroiditis associated with rheumatoid arthritis (AIT/RA).

Methods and materials

Twenty-nine women with AIT/RA were examined. Study design included: special protocol of anamnesis, sonography of thyroid gland, evaluation of thyroglobulin antibodies (Tg-Ab), thyreoperoxidase antibodies (TPO-Ab), the

thyroid hormones ($T_3$, $T_4$) and TSH in serum. Two groups of patients have been allocated: I group ($n=21$) – persons at whom RA debuted after debut AIT (primary AIT/RA), II group ($n=8$) – persons at whom debuted the first RA with the subsequent debut AIT (secondary AIT/RA).
Result
The persons with primary AIT/RA were majority – 72%. The age of a debut primary AIT/RA was at $44.3 \pm 1.2$ years, while secondary AIT/RA debut – $55.4 \pm 1.6$ years ($P<0.05$). Thus in II group of 88% of patients had debut of AIT in the postmenopausal period (mean age of a menopause $51.2 \pm 1.2$ years), while the majority of patients of I group – 86% in the menstrual period. Thyroid volume exceeded normal values at 38% of patients of I group and at 25% of patients in II group. Hypothyroidism has been revealed at 90% of patients of I group and at 75% of patients of II group, in most cases was in subclinical grade. Cases of thyrotoxicosis were absent. Tg-Ab level in I group was considerable above, than in II ($507.5 \pm 48$ vs $272.5 \pm 57$ IU/ml accord., $P<0.05$), TPO-Ab level also was above in I group ($P<0.05$).
Conclusions
Primary AIT/RA was observed much more often, than secondary AIT/RA and had debut on a late menstrual phase of ontogeny, while secondary AIT/RA had debut at on early postmenopausal period. Primary AIT/RA had antibodies level in higher concentration in comparison with secondary AIT/RA especially Tg-Ab level and had more often impairments of thyroid function mainly observed as a subclinical hypothyroidism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1687

**The effect of radio-iodine treatment on *Helicobacter pylori* eradication**

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Aim
Radioactive treatment after surgery forms the basis of treatment of differentiated thyroid cancers (DTC) and also used as permanent theraphy of hyperthyroidism. Iodide is being transported into the thyrocytes by the NIS system that located on the thyrocytes' cell membran. NIS system also has been shown at the gastric mucosa. In this study we proposed to analyse the effect of low and high dose radio-iodine treatment on *Helicobacter pylori* eradication.
Material and methods
Total 87 patients included for study. The patients investigated detailed about medications and gastrointestinal symptoms. Patients have neither dyspeptic symptoms nor any usage of $H_2$ receptor bloker, proton pump inhibitor, antiasit or any form of antibiotics. Urea breath test before and 2 months after radio-iodine treatment have been made for detection of *Helicobacter pylori* eradication.
Results
The radio-iodine treatment has been planned to give as permanent threapy to 76 patient (87.4%) with hyperthyroidism and 11 patient (12.6%) with differantial thyroid cancer. The average dose of given radio-active iodine to patients for ablation purposes is $115 \pm 3.3$ and $22.7 \pm 1.4$ mCi. Before RAI treatment, in the group of DTC the urine breath test result of the 44 patients had positive HP and 32 of them had negative HP, and for hyperthyroid patients four of them had positive HP and seven of them had negative HP. All patients having initial positive test results had also positive control test results at the 2nd month of the RAI treatment (100%). Both patient group having initial negative test results, had also negative control test results (100%).
Conclusion
There are some literature studies shows that RAI treatment is effective on HP eradication. Our results indicate that low and high dosage of RAI treatment does not have any effect on HP eradication in early period.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1688

**Hashimoto's (chronic) thyroiditis in perimenopausal women**

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Background
One in 10–30 women in general population has Hashimoto's (chronic) thyroiditis. Development of thyroid dysfunction depends on age. The ratio of woman around 50 with TSH elevation is 10% in general population. Menopause occurs at this age for most women. Many symptoms of hypothyroid state are similar to symptoms of menopause except hot flashes and night sweats. We analyzed the relationship of Hashimoto's disease and menopausal symptoms.
Methods
Subjects are 271 women, new patients range 17–79 years of age (mean: 48.7 years old) at our clinic between January and May, 2010. We performed questionnaires about their menstruation and menopausal symptoms, also measurement of serum F-$T_3$, F-$T_4$, TSH, antithyroglobulin antibodies and anti-thyroid peroxidase (anti-TPO) antibodies at the first visit. Subjects are assorted to four groups depending on menstrual condition. That is pre group (regular menstruation) is 35.7%, peri group (irregular menstruation before menopause) is 24.0%, post group (1–5 years after menopause) is 9.0%, post–post group (over 5 years after menopause) is 31.3%.
Results
Of the total, 28.8% of all subjects has antithyroid antibodies (antithyroglobrin antibodies 21.1%, anti-TPO antibodies 12.7%). The breakdown by groups is pre 20.6%, peri 23.1%, post 45.8%, post–post 37.6%. Hypothyroid state (TSH $>4$ μIU/ml) was observed 11.8% of all. The breakdown by groups is pre 9.9%, peri 22.6%, post 4.2%, post–post 7.1%. Seventy six per cent of all has menopausal symptoms.
Conclusions
Menopausal symptoms in Hashimoto's disease women are characterized by that coldness and fatigability are significantly higher, on the other hand hot flashes, night sweats and irritability are significantly lower than women without Hashimoto's disease. In this study, the frequency of hypothyroid in premeopausal women is higher than postmenopausal women among women with antithyroid antibodies. It is presumably that overt hypothyroid patients tend to visit clinics earlier than menopause.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1689

**Hyperglycaemia in hyperthyroidism predictive factors**

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Glucose abnormalities are frequent in hyperthyroidism; the aim of our study is to determine the predisposing factors of glucose disturbances in hyperthyroidism.
Subjects and methods
It is a retro and prospective study including 263 hyperthyroid subjects, patients having fasting glycaemia $<1$ g/l and/or glycaemia after OGTT $<1.40$ g/l (G1) were compared to patients having fasting glycaemia $\geq 1$ g/l and/or glycaemia after OGTT $\geq 1.40$ g/l (G2).
Results
Seventy-five patients (G2) had glucose abnormalities. Patients of G2 were older than those of G1, underlying hypertension and familial diabetes were more prevalent in G2. There was no difference between the two groups for the other factors studied (Table 1).
Discussion and conclusion
Ageing, underlying hypertension and familial diabetes are risk factors for glucose abnormalities in hyperthyroid subjects whereas the severity of hyperthyroidism is not which suggest that hyperthyroidism may worsen glucose metabolism in patients at risk of type 2 diabetes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1** Predictive factors of hyperglycaemia during hyperthyroïdism.

| | G1 | G2 | P |
|---|---|---|---|
| Number (%) | 188 (71.48%) | 75 (28.52%) | |
| Family Diabetes (%) | 31.38% | 48% | <0.05 |
| Hypertension (%) | 6.91% | 25.33% | <0.05 |
| Age (years) | 40.07±1.22 | 49.27±1.96 | <0.01 |
| BMI (kg/m$^2$) | 22.94±0.33 | 24.05±0.67 | NS |
| FT4 (pmol/l) | 43.55±2.43 | 32.62±3.51 | NS |
| FT3 (pmol/l) | 19.88±1.47 | 16.24±2.05 | NS |

## P1690

### An unusual case of hyperthyroidism

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Introduction
During embryogenesis the descent of the thyroid may not proceed normally and may stop at various sites from the base of the tongue to any site of the thyroglossal duct.
Ectopic thyroid tissue, defined as thyroid tissue not located at its normal site (anterolaterally to the second and fourth tracheal cartilages) is rare. Most often, ectopic tissue is located in the base of the tongue.
Case report
We report a 75-year old female referred to our Department for subclinical hyperthyroidism, diagnosed during investigation of atrial fibrillation and weight loss.
She had negative antithyroid autoantibodies, elevated ESR and normal full blood count.
On Tc-99 thyroid scan there was no uptake. Ultrasound showed an enlarged gland with two nodules 1.6 and 1.2 cm on the right and left lobe respectively. Subacute thyroiditis was diagnosed and no special treatment was administered.
Four months later she came back in a worse clinical condition and clinical hyperthyroidism. Considering the possible diagnosis of an ectopic thyroid gland, a whole body scan with Tc99 was performed. It showed hyperfunctioning thyroid tissue in the base of the tongue, with simultaneous suppression of the eutopic thyroid.
She was prescribed antithyroid drugs.
Macroscopic physical examination of the oral cavity was negative, as well as the MRI of the area.
Conclusion
To our knowledge, it is the first case of hyperfunctioning lingual thyroid with simultaneous existence of the eutopic thyroid gland.
Additionally, in reported cases so far ectopic thyroid tissue is usually diagnosed in childhood or adolescence and is usually accompanied by hypo- and not hyperthyroidism.
In most cases, lingual thyroid is visible as a mass in the base of the tongue, in contrast to our case, where it was invisible.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1691

### Do thyroid hormone levels in critical illness predict outcome? Experience from a medical ICU in north east India

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Alterations in thyroid hormone levels are commonly observed in critical illness with thyroxine replacement remaining a controversial issue. We undertook a prospective observational cohort study in medical intensive care unit of Gauhati Medical College to evaluate whether thyroid hormone levels at admission could be used to predict mortality and compared it to the well established APACHE II scoring system for ICU patients. Serum $T_3$, $T_4$ and TSH were estimated at admission in 100 consecutive adults without prior history of thyroid disease admitted to the medical ICU. An APACHE II score was calculated for each patient on the day of admission. The patients were followed up and were divided subsequently into survivors and non-survivors. The TSH, $T_4$, $T_3$ and APACHE II scores were compared in both groups. Non-survivor group had 46 patients while survivor group had 54 patients. TSH and $T_3$ levels in the non-survivor group ($0.505 \pm 0.515$ mIU/l and $0.684 \pm 0.10$ nmol/l respectively) were significantly lower than that in the survivor group ($1.149 \pm 1.08$ mIU/l and 0.934 $\pm 0.223$ nmol/l) respectively. Total $T_4$ in non-survivor group (65.32 $\pm 40.84$ nmol/l) was also lower than that in the survivor group (75.56 $+34.0$ nmol/l) but this difference was not statistically significant ($P=0.18$). There was a statistically significant inverse correlation between APACHE II score and total $T_3$ and TSH. ROC curve analysis was done for comparing the prognostic value of different parameters. AUC for APACHE II, $T_3$, TSH and $T_4$ were 0.96, 0.85, 0.74 and 0.59 respectively.
TSH and total $T_3$ at admission has prognostic value in patients admitted to the medical intensive care units. Low TSH and $T_3$ correlates with APACHE II score in predicting poorer outcome.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1692

### Vitamin E and selenium have a protective role in apoptosis induced by $H_2O_2$ in human thyrocytes

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Objective
To investigate the influence of vitamin E (VE) and selenium (Se) on apoptosis of human thyrocyte induced by $H_2O_2$.
Methods
The human thyroid epithelium cells (TEC) from normal para-adenoma tissues of patients with thyroid adenoma resection were cultured. In prevention groups, vitamin E (50 μmol/l), selenium (10–7 mol/l) or serum-free medium was respectively added into thyrocytes of monolayer culture before $H_2O_2$ (200–800 μmol/l) with different concentrations added, while in treatment groups $H_2O_2$ (200–800 μmol/l) was added firstly. The survial rates of thyrocyte were detected by MTT stain, and the apoptosis rates by flow cytometry.
Results
When the thyroid epithelium cells exposed to $H_2O_2$ (200–800 μmol/l) only for 24 h, the cell survial rates declined and cell apoptosis rates increased ($P<0.01$) in both prevention and treatment groups, with a relation of dose-effect response. Adding VE (50 μmol/l) and Se (10–7 mol/l) into cell culture for prevention and treatment, the cell survial rates increased and the thyrocyte apoptosis reduced compared to the corresponding simple $H_2O_2$ intervention groups. Above differences were particularly significant ($P<0.05$) when thyrocytes exposed to 400 and 800 μmol/l $H_2O_2$. The decrease of cell apoptosis rates in group with selenium pre-intervention was more significant than that in group with VE pre-intervention ($P=0.018$) when cells exposed to 400 μmol/l $H_2O_2$. While the decline in group with VE was more obvious than that in group with selenium ($P<0.05$) when cells exposed to 200 and 400 μmol/l $H_2O_2$ in treatment groups.
Conclusion
VE and selenium could have protective effect on apoptosis of human thyrocytes induced by $H_2O_2$ in human thyrocytes.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1693

**Exophtalmoses due to a bilateral lacrymal gland MALT lymphoma in first occurrence of Graves'disease**

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Introduction
Bilateral exophtalmoses is common at Graves' disease diagnosis, but context and clinical abnormalities lead sometimes to consider other diagnosis.
Case report
A 52-year-old man had a previous history of pulmonary MALT lymphoma in complete remission and Hashimoto's thyroiditis, which were diagnosed simultaneously 6 years before. He presented recently with hyperthyroidism (TSH $<0.001$ mUI/l; $T_4=25$ pmol/l) after 2 months of L-thyroxine discontinuation, associated with mild goiter, bilateral exophtalmoses and periorbital edema. TSH receptor antibodies (13 UI/l) were positives, which confirmed Graves' disease. Thiamazole were started (5 mg/J) with low dose because of renal failure but led to hypothyroidism in the following 2 weeks. Exophtalmoses worsened with major edema and palpable masses of the orbits despite correction of hypothyroidism. A MRI showed bilateral severe infiltration of both lacrimal glands, without abnormalities of ocular muscles. Biopsy revealed bilateral mucosa-associated lymphoid tissue (MALT) lymphoma (CD 20+). Tumor staging showed no other location. Chemotherapy using weekly Rituximab and chlorambucil with progressive dose during 6 weeks led to an important improvement of the lymphoma. Maintenance treatment is on going.
Conclusion
In this case, the two occurrences of MALT lymphoma were coincident with thyroid auto immunity diseases, which emphasizes the possible relation between these two conditions. To our knowledge, it is the first reported case of bilateral lacrymal gland lymphoma diagnosed silmutaneously with Graves' disease. The presence of palpable lacrymal glands could suggest this differential diagnosis of Graves' ophtalmopathy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1694

**A correlation study of thyroid nodule cytopathology and histopathology**

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Background
Fine-needle aspiration (FNA) biopsies are the cornerstone of preoperative evaluation of thyroid nodules, but FNA diagnostic performance has varied across different studies. We conducted a study to evaluate the effectiveness of ultrasound guided FNA in the thyroid nodule and to review the correlation between cytopathology and histopathology.
Methods
Prospective FNAs were collected from 511 patients who underwent a ultrasound guided FNA between 2008 and 2011. One hundred sixty-two patients underwent thyroidectomy. Cytology and surgical pathology results were matched in this group and sensitivity, specifity, positive predictive value and negative predictive value were calculated.
Results
The mean age was $54.7\pm13.4$ years old (440 women and 71 men). The average size of nodules was $2.2\pm1.2$ cm ($2.75\pm1.45$ in patients undergoing thyroidectomy). Multinodular goiter presented with dominant nodule 406 patients (79.5%) and 105 single nodule (20.5%). The FNA was inconclusive in 7.8% of cases. Benign cytology had 80.4% of patients, 9% indeterminate cytology and 1.8% malignant cytology. One hundred sixty two patients underwent thyroidectomy (58.8%, total thyroidectomy). From this population histopathology malignancy rates for prospective clinic FNAs were 10.7% for cytology indeterminate cases and 88.8% for cytology malignant cases. The FNAs had a sensitivity of 89%, specificity of 99%, positive predictive value of 89% and negative predictive value 99%.
Conclusions
Ultrasound-guided FNA provides important information for the diagnosis and the preoperative evaluation of thyroid nodules. The low rate of false-positive and false-negative results suggests that it might be enough for surgical planning for thyroid nodule.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1695

**Prevalence of thyroid dysfunction in a subgroup of male patients with HIV**

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Introduction
Thyroid dysfunction has been described as a common clinical entity in HIV infected patients, commonly with negative autoimmune tests. The aim of this work was to determine the prevalence of thyroid abnormalities in a cohort of HIV-infected males in a stable clinical state, the effect of exposure to ART on thyroid function and to identify the risk factors.
Materials and methods
This is a cross-sectional, observational study of 90 HIV infected males ($42\pm8.2$ años). Exclusion criteria: HCV co-infection, AIDS-defining active disease, drug abuse or therapeutic non-compliance. Statistical analysis: descriptive, Pearson/ρ Spearman correlation of quantitative variables; Student-$t$ or Mann–Whitney $U$ test for differences of TSH–$FT_4$ on qualitative variables; $\chi^2$ test between thyroid alterations and qualitative data; statistical significance $P<0.05$.
Results
Prevalence of thyroid abnormalities was 6.7% (95% CI 3.1–13.8): 1/90 subclinical hyperthyroidism, 2/90 subclinical hypothyroidism, 2/90 low $FT_4$, 2/90 antiTPO positive. $FT_4$ concentration was associated with CD4 nadir ($r$: 0.221, $P=0.037$), whereas TSH levels were correlated with viral charge (RNA cop./ml; $r$: $-0.26$, $P=0.01$). antiTPO antibodies were correlated both with CD4 concentration ($r=-0.28$, $P=0.008$), and viral charge ($r$ 0.29, $P=0.005$). We did not find any association between HIV treatment and thyroid dysfunction.
Conclusion
Male patients with HIV infection in stable clinical state have similar prevalence of thyroid dysfunction than general population, with lower prevalence of autoimmunity. The mechanism and clinical consequences of low $FT_4$ are not yet known and have to be studied.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1696

**Thyroid concentrations and leptin levels in patients with anorexia nervosa**

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Anorexia nervosa (AN) is known as a state of gonadotrophin and leptin deficiency. Leptin, the product of OB gene, secreted by adipocytes, is proved to play an important role in regulating apetite and energy expenditure. Thyroid hormones are Important regulators of total energy expenditure are thyroid hormones and also are able to increase oxygen consumption by tissues. The aim of the study was to evaluate a possible interaction of thyroid hormones ($T_3$, $T_4$, TSH) with leptin in a patients with AN. We evaluated 19 patients with AN, female age $21\pm1.3$ years who have gained weight (BMI $14.8\pm0.6$–$19.1\pm0.5$ kg/m$^2$). At the moment of diagnosis, serum leptin levels were $2.1\pm0.3$ μg/l while after behavioural therapy and hypercaloric diet for 3–12 months serum leptin levels

rose to 6.5±1.5 μg/l not different from 11 healthy female subjects (age 29±5.2 years, BMI 21.1±0.3 kg/m$^2$) whose serum leptin levels were 9.3±0.7 μg/l ($P>0.05$). Thyroid function was assessed by measuring serum fasting triiodo thyronine ($T_3$), thyroxine ($T_4$) and TSH. None of the subjects was taking any medications. Leptin values correlated well with BMI and the mean serum leptin contrecations in AN subjects was 2.39±0.15 ng/ml as compared to 9.3 ±1.1 ng/ml in control subjects ($P<0.01$). No significant differences in thyroid hormone levels ($T_3$ 1.2±0.3 vs 1.8±0.4 nmol/l, $P>0.05$, $T_4$ 88.3±10.1 vs 110.2±28.3 nmol/l $P>0.05$, TSH 1.5±0.4 vs 1.9±0.9 mUI/l, $P>0.05$) were recorded. Thyroid hormone axis remains normal in secondary leptin deficiency.
Keywords: leptin, anorexia nervosa, thyroid hormones.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1697

### Congenital hypothyroidism as a rare cause of precocious puberty

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Severe hypothyroidism is a rare cause of precocious puberty, because long-standing primary hypothyroidism traditionally leads to both pubertal and growth delay. We report a case of a 4.7 year old girl presented with abdominal/pelvic pain and vaginal bleeding since 1 week. The child was diagnosed as a case with a right ovarian mass and was planned for surgery. She was born at term pregnancy without complication. Physical examination revealed typical features of hypothyroidism. Growth velocity was decreased and contrasted with exaggerated weight gain. She weight 21 kg (weight 90th percentile), height was 92 cm (height <3rd percentile), stature development was 2 year and 9 months, bone age corresponding to 1 year. Breast development was at stage II (S2), no axillary (A0) and no pubic hair (PO). The serum thyroid-stimulating hormone level was enlarged >75 mIU/l (range 0.3–5.0), total thyroxine ($TT_4$) and total triiodothyronine($TT_3$) were undetectable, follicle stimulating (FSH) was 5.1 UI/l (3–8), and luteinizing (LH) 0.2 UI/l (range 1–7), prolactin was elevated, at 29.6 ng/ml (2.7–8.7). Thyroid ultrasound showed absence of thyroid gland in normal location. Scintigraphy with Technetium 99 showed light isotope uptake in the sublingual region and absence of isotope uptake in the normal thyroid position. These results were consistent with the diagnosis of primary hypothyroidism as a result of thyroid agenise. She was treated with L-thyroxin 25 μg/day for the first two weeks than to 50 μg once daily fasting in the morning. Vaginal bleeding was stopped after few days that we start treatment and was not seen again. Breast development, size of uterus and ovaries returned to the prepubertal stage after 6 months of the L-thyroxin therapy.
In conclusion children with precocious puberty having solitary masses on ovary, decreased growth velocity and bone age delay should be assessed for hypothyroidism in order to avoid unnecessary surgery on the ovaries.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1698

### Evaluation of subclinical thyroiditis among Egyptian type 1 diabetic patients by Hesham El-Hefnawy, Atef Bassyouni*, Mohamed Abdel-Kareem**, Nibal Abdel-Rahman***, Mary Aziz****And Ibrahim Emara****

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Autoimmune thyroiditis (AIT) is a group of inflammatory thyroid disorders with either hyperthyroid, euthyroid or hypothyroid state. The aim of this study is to detect the subclinical thyroiditis among the Egyptians type 1 diabetic patients and to study the relation of the thyroid antibodies to different metabolic control indices of diabetes. The study group consisted of 50 type 1 diabetic patients aged from 8 to 18 years. They were selected from the out-patient daily clinics of National Diabetes Institute (NIDE). The control group consisted of 20 healthy subjects comparable for age, sex and socioeconomic classes of study group. Both groups were having no signs or symptoms of thyroid dysfunction. They were subjected to the following: quantitative determination of free $T_3$ and free $T_4$ in serum, quantitative determination of TSH in serum, estimation of thyroid auto-antibodies using ELISA for the semiquantitative detection of thyroglobulin antibodies (TG-AB), and thyroid peroxidase antibodies (TPO-AB) or microsomal autoantibodies. The results of this study revealed that 1.5% of the type 1 diabetic patients were with strong positive of both thyroid auto-antibodies and the same percentage were for diabetic patients with strongly positive T-antibodies, while 22% were positive for M-antibodies. The number of diabetic patients, with weak positive TG-antibodies, were nine patients (18%) and 14 patients (28%) for TPO-Abs. While the weak positive results for both antibodies were six patients (12%). It could be concluded that thyroid antibodies should be done periodically for every type 1 diabetic patient. Patients with positive antibodies should be monitored for TSH elevation at yearly intervals.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1699

### Significant immune associations in chronic thyroiditis

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Objectives
To investigate how the immune associations (IA) may have significance for diagnosis or therapy in thyroiditis.
Material and method
Patients: A. 'Classical' Hashimoto thyroiditis (hyper-ATPO-emia, HT) =1092, B. thyroiditis with isolated hyper-ATG-emia, with normal ATPO (T-ATG) =61, C. thyroiditis 'sero-negative' (normal ATPO and ATG, pathology diagnosis) =5, D. idiopathic myxedema (hypothyroidism, no A, B,C) =70; E. control =998. 2. Statistical analysis: $\chi^2$ test.
Results
1 Total IA: TH =189 (17.31%, $P\ll0.001$); T-ATG =15 (24.59%, $P=0.002$); 'sero-negative' =1 (14.29%); idiopathic mixedema =10 (14.29%); control: 99 (9.46%). 2. Main IA: A. Graves-Basedow disease: TH: 141 (12.91%), T-TAG: 3 (4.92%); B. Vitiligo: TH =35, $P=0.0001$; T-ATG =2; Control =9. C. Dermatitis: TH =16, $P=0.004$. D. Immune ovaritis with precocious menopause: TH =12, $P=0.024$. E. Biermer anemia: only in HT =11; $P=0.001$. F. Drug allergy: TH: 17, $P=0.01$. G. Rheumatoid arthritis: TH =8, but in controls =18; (NS); Mixedema: 2 (NS). 3. Multiple associations: cerebral vasculitis with Sneddon sd, pulmonary fibrosis, cryoglobulinemia, virus C hepatitis (IFN) and sicca sd; Sarcoidosis with drug allergy, scleroderma, adenomegaly and arthritis; IDDM plus widespread vitiligo and hyperthyroidism; bronchial asthma with postpartum trombophilia and antiphospholipidic sd; selective alopecia areata (no eyebrows), ferriprive anemia and miopia; Sharp disease, zona zoster, dispepsia, alopecia areata and trombocytosis.
Conclusions
i) HT and T-ATG has immune associations with increased frequency. ii) The most significant and prevalent association are: Graves-Basedow, vitiligo, Biermer anaemia, drug allergy, early menopause with immune ovaritis. iii) HT, T-ATG and idiopathic mixedema are not significantly associated with other immune conditions: rheumatoid arthritis, IDDM, B/C hepatitis. iv) Multiple immune associations are common. v) Breast cancer are not associated with chronic thyroiditis. vi) Amiodarone administration did not induce TH, T-ATG, significantly.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1700

### On cohorts (over 1000 patients), there are relationships between antithyroperoxydase antibody levels and thyroid function in chronic thyroiditis

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Aim and objectives
To investigate the rapport between the level of thyroid function, through blood level of TSH, both at onset, and in evolution and the level of antithyroperoxydase antibodies (ATPO) and antithyroglobuline antibodies (ATG) in Hashimoto thyroiditis and related diseases.
Material and method
Diagnosis: a. ATPO $>34$ μ/ml = Hashimoto thyroiditis (HT); b. ATPO= normal with high ATG = thyroiditis with only hyper-ATG (T-ATG); c. hypothyroidism without high ATPO/ATG = idiopathic mixedema (IM). 2. Thyroid function: TSH (and $FT_4$) 3. Statistical analysis: linear correlation test.
Results
I. ATPO/ATG at onset: 1. ATPO: A. Number: HT-1092, T-ATG-61, IM-70. B. Average: TH-651,25; SD: 1055 (!); T-ATG-9,1; IM-10. 2. ATG in T-ATG: 539,81; SD: 940 (!).
II. Evolutional types for ATPO/ATG: 1. All ATPO in HT: no.1812, av: 662 μi/ml. 2. ATPO evolution in HT: undulatorious: 142 (41.4%), decreasing: 132 (38,5%), increasing: 69 (20.1%). 3. All ATG in T-ATG: no. 83, av: 494. 4. ATG in T-ATG: undulatorious: 2 (11.76%), decreasing: 9 (52.94%), increasing: 6 (35.29%).
III. TSH. 1. onset HT: av: 9.05 mU/l; S.D. 20.7. 2. onset T-ATG: 4.02 mU/l, S.D. $=4.33$. 3. All HT: av. 7.43.
IV. Linear correlation ATPO-TSH: 1. in HT: a. HT at onset: $r=0.17$, $P<0.001$, slope: 8.31. b. all TH values: $r=0.11$, $P<0.001$; 2. in T-ATG: a. at onset: $r=-0.19$, $P\gg0.1$ (NS), slope: $-35$; b. all T-ATG values: $r=-0.17$, NS; c. MI at onset/and all values, $r=-0.12$, $P\gg0.1$.
V. Linear correlation ATPO-FT4: 1. HT at onset: $r$: $-0.11$, $P<0.001$, slope: $-9.32$. 2. T-ATG at onset: $r=0.001$, NS.
Conclusion
1 A certain correlation is: between thyroid function and only ATPO (not ATG) and only in cohorts (400–1000 probes). 2. ATPO evolve unpredictably: high levels of ATPO occur in hypothyroidism, euthyroidism and hyperthyroidism, too.
Declaration of interest
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## P1701

### How evolves thyroid function in Hashimoto thyroiditis and related disorders

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Aim
Analyzing the evolution of thyroid function in thyroiditis and related disease during 1–15 years.
Materials and methods
A) Diagnostic: Hashimoto thyroiditis (HT): i) antithyroperoxydase antibodies (ATPO) cut-off 34. ii) If ATPO=normal (N), was considered thyroiditis with only antithyroglobuline antibodies (T-ATG); iii) idiopathic mixedema (IM): hypothyroidism, no ATPO, no ATG, no TRAB.
B) Patients: i) HT: 1092; T-ATG: 61; IM: 70. Women/men: HT: 1041/51; T-ATG: 58/3; IM: 59/11 (more men: $P<0.001$).
C) Statistic analysis: test $\chi^2$.
Results
A) At the diagnostic moment. i) HT: euthyroid (EUT): 490 ($\sim$45%), hypothyroid (HOT): 443 ($\sim$40.5%), hyperthyroid (HIT): 159 ($\sim$14.6%) – from these: 141 ($\sim$89%) associated with Graves-Basedow disease (GBD)-(TRAB+; more than in T-ATG).
ii) T-ATG: EUT: 36 (59%, more than in HT), HOT: 19 (31%), HIT: 6 (9.8%) – from these: 3 (50%) associated with GBD-(TRAB+).
iii) IM: (by definition): HOT: 70 (100%).
iv) Significant difference between TH-T-ATG: $P=0.09$ (NS).
B) Follow-up: i) HT: a. 28 (5.71%) with EUT became HOT after 0.2(!)-8 years (av $=2.77$, S.D. $=2.11$). ii) 3 (0.61%) with EUT become HIT (all GBD). iii) 100% HOT remained HOT. iv) 15 (9.43) with HIT become EUT after 1.5–2 years and maintain at least 5 years. v) 4 (2.52%) with HIT become spontaneously HOT (two with GBD).
ii) T-ATG: only two HIT become EUT (33.3%). EUT&HOT remain the same.
iii) IM: all remained HOT, with one exception (man under amiodarone who return spontaneously to EUT after withdrawal amiodarone).
Conclusions
i) Thyroiditis with only hyperATG could be considered different from HT. ii) HT, T-ATG and IM presented differently as hormonal function. iii) H-TAG more than HT (but both) presented more as EUT than HOT. iv) Only 5% EUT-HT become HOT, during first 8 years. v) No EUT-HT after 8 years modified function. vi) Patients with HOT at diagnostic time, either HT, T-ATG or IM, remain HOT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1702

### Influence of thyroxin replacement therapy on cardiovascular system in patients with primary hypothyroidism and arterial hypertension

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Introduction
We investigated short-term influence of thyroxin replacement therapy on blood pressure (BP) and cardiovascular status in patients with first identified primary hypothyroidism (PH) and earlier diagnosed arterial hypertension (AH).
Methods
52 patients with PH and AH were observed twice: prior to replacement therapy and after reaching euthyroid status. All patients underwent daily BP monitoring, echocardiography, and high frequency ultrasound dopplerography of nail ridge arterioles with detection of maximal systolic and end-diastolic velocity and calculation of Purselo index (PI). Results are presented as delta with 95% confidence intervals.
Results
Decrease in systolic (daytime by 8.9 (5.5–12.3), nighttime by 5.0 (2.5–7.5) mmHg) and diastolic BP (daytime by 9.4 (6.4–12.4) and nighttime by 4.5 (2.3–6.7) mmHg), interventricular septum thickness by 0.08 (0.03–0.13) cm, posterior wall thickness by 0.08 (0.04–0.12) cm, left ventricle diastolic diameter by 0.14 (0.05–0.23) cm, left ventricular mass by 24.5 (13.9–35.1) g, maximal systolic velocity by 2.5 (0.8–4.2) cm/s, and PI by 0.13 (0.06–0.20) CU were observed in patients with PH after reaching euthyroid state.
Conclusion
Compensation of thyroid state in patients with PH and AH leads to decrease in BP and improvement of ultrasound left ventricular and peripheral homodynamic parameters.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Dynamics of cardiovascular parameters in patients with PH and AH after achievement of euthyroid status.

| Parameters | Hypothyroid status | Euthyroid status |
|---|---|---|
| Systolic BP (daytime; mmHg) | 133.4 (130.3–136.5) | 124.5 (119.8–129.2)* |
| Systolic BP (nighttime; mmHg) | 118.4 (115.4–121.4) | 113.4 (110.5–116.3)* |
| Diastolic BP (daytime; mmHg) | 85.7 (80.0–91.4) | 76.3 (71.3–80.3)* |
| Diastolic BP (nighttime; mmHg) | 74.5 (70.3–78.7) | 70.0 (66.6–73.4)* |
| PWT (cm) | 1.20 (1.13–1.27) | 1.12 (1.05–1.18)* |
| IVST (cm) | 1.12 (1.06–1.17) | 1.04 (.99–1.09)* |
| LV DD (cm) | 4.78 (4.69–4.88) | 4.64 (4.56–4.72)* |
| LVM (g) | 197.2 (181.6–212.8) | 172.7 (159.8–185.6)* |
| *Vs* (cm/s) | 13.1 (11.2–15.0) | 10.6 (8.9–12.3)* |
| *Vd* (cm/s) | 3.9 (3.0–4.8) | 4.3 (3.6–5.0) |
| PI (CU) | 0.72 (0.67–0.77) | 0.59 (0.55–0.63)* |

PWT, posterior wall thickness; IVST, interventricular septum thickness; LV DD, left ventricular diastolic diameter; *Vs*, maximal systolic velocity; *Vd*, end-diastolic velocity; PI, Purselo index. *$P$ value for $\Delta<0.05$.

## P1703

**Psychosis in hyperthyroidism: a case report**

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Background
The most common psychiatric syndromes in thyrotoxicosis are anxiety and mood disturbances. Psychosis, however, is a rare complication. Here is a report of such a case.
Case report
A twenty-six year old female presented with a 13 year history of palpitations, easy fatigability and anterior neck mass. Patient denies any comorbid conditions. No family history of psychiatric illness. On March 2011, she was admitted due to logorrhea, auditory hallucinations, delusion of persecution and frequent attention calling from family members, and suicidal ideations. Clinically she was thyrotoxic with a diffuse goiter. $FT_4$ 39.6 pmol/l (11–24) and TSH $<$ 0.005 μIU/l (0.3–2.8). There was no evidence of infection. Diagnosis of thyrotoxic psychosis was made, and was started on Propylthiouracil 150 mg q8 and Propranolol 40 mg BID.
After her marked improvement, she was discharged on Propylthiouracil, Propranolol, Olanzapine and Clonazepam. All her psychiatric symptoms gradually resolved and weaned off from Olanzapine and Clonazepam. During her regular follow up, there was note of occasional episodes of agitation. Six months later, after euthyroidism was achieved, patient underwent radioactive ablation, with complete resolution of her psychiatric symptoms.
Conclusion
Thyrotoxicosis may be a precipitant of acute psychosis. This can be promptly controlled with the use of anti-thyroid drugs. Concomitant psychotropic drugs maybe indicated if the symptoms are severe. In cases of treatment failure, a more radical approach such as radioactive iodine ablation and thyroidectomy maybe considered.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1704

**TSH receptor antibody measurement in the diagnosis and management of Graves disease**

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Introduction
Graves' hyperthyroidism is an autoimmune disease sustained by autoantibodies binding to and activating the TSH receptor located on the thyroid follicular cell. The objective of study was to determine the presence of anti-receptor TSH antibodies for the diagnosis of Grave's diseases and the following up after surgery, iodine and treatment with antithyroid drug.
Results
In years 2006–2010 in our clinic in 265 patients for thyroid disorders 77.3% (205) were diagnosed with Grave's diseases (154 female, 51 males with a ratio 3.01:1. Mean age was 44 years old ± 16.2 s.d.); 6% with multinodular toxic goiter; 4.3% with thyroiditis and 12.4% were euthyroid. All the patients were examined for the presence of anti-TSH receptor ab by RIA (Cisbio) and the cut off for positivity was $>$1.0 U/ml. The anti-rec-TSH ab antibodies were positive in 90.7% of patients with Graves' disease before treatment with an average level 8.89 U/l ± 13.71 s.d. with a range 1.1–105, the antimicrosomal antibodies (M ab) were present in 76.5% and thyroglobulin antibodies (Tg ab) in 33%. In those without Graves' disease, anti-rec-TSH ab were positive in 1.6%. In our study we found that anti-rec-TSH ab values before anti thyroid treatment had no any significance in predicting the remission of Graves' disease after 1 and 2 years of anti thyroid drug therapy. In patients with positivity lowered down or became zero during the treatment the remission was very good, while the patient which remained with high level of anti-rec TSH ab the remission is not achieved and they remained hyperthyroid. No differences in patients treated with antithyroid drug and those treated with surgical intervention (total thyroidectomy): the positivity for anti-Rec TSH ab go down gradually in 50–60% of the cases they do remission with lower or disappeared antibodies. In the patients treated with radioiodine those with high titer of anti-rec TSH ab remain still positive after 1 or 2 years.
Conclusion
We conclude according our study that it is important to determine the anti receptor TSH antibodies for the diagnosis of Grave's diseases and to predict the efficacy of the treatment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1705

**Life-threatening amiodarone induced thyrotoxicosis: optimizing the treatment and choosing radical therapy according to clinical course: own experience**

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Amiodarone is a widely used antiarhrythmic agent. Although life-threatening side effects of amiodarone therapy are well known, it's not always possible to replace it with dronedarone. Some clinical trials showed that treatment with dronedarone led to significantly more unfavourable end-points (e.g. in congestive heart failure). Therapy of amiodarone induced thyrotoxicosis still raises many questions concerning optimal treatment choice to achieve euthyreosis as soon as possible. In our experience each case should be considered individually according to clinical course, comorbidities, etc.
Fourty patients (15 females, 25 males, mean age: 61 years) with severe amiodarone induced thyrotoxicosis were hospitalized in our Department between 2001 and 2011. 60% of them had life-threatening cardiological diseases. Mean $FT_3$ and $FT_4$ concentrations were respectively 13.3 pmol/l (*n*: 3.1–6.8) and 62.3 pmol/l – in nine patients $FT_4$ levels were $>$100 pmol/l (*n* 11–22).
Median time of hospitalization was 4 weeks (2–13 weeks), mean time of reaching euthyreosis – 14 weeks. Most common treatment was combined antithyroids and steroids iv. About a half of the patients were finally treated with I-131, after regaining iodine uptake. There were three deaths. One of the patients died because of severe heart arrhythmias. Another one, unresponsive to pharmacological treatment, was disqualified from thyroidectomy because he developed acute respiratory distress syndrome, underwent plasmaphereses, finally died of respiratory failure. The third one died due to massive gastric bleeding caused by hemorrhagic gastritis – complication of steroid treatment. However, in one of the patients, unresponsive to standard therapy, a thyroidectomy was successfully performed.
Conslusions
– Each patient with amiodarone induced thyrotoxicosis requires an individual approach.
– It's substantial to determine the moment of radical treatment.
– Upon reaching euthyreosis it's advisable to treat the patient with I-131 if further treatment with amiodarone is recommended.
Declaration of interest
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## P1706

**Thyroid hemiagenesis and bilateral parathyroid adenoma**

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We present a 66 year old patient with right thyroid hemiagenesis and double parathyroid adenoma one on the side of hemiagenesis and the other on the side of present left lobe. The patient came to our Clinic because of pain in the neck and was diagnosed a primary hyperparathyroidism with elevated parathyroid hormon as well as Hashimoto thyroiditis with high level of Tg and TPO antibodies. Ultrasound and technetium sestamibi scintigraphy determined the absence of the right lobe but not adenoma of parathyroid glands. The patient underwent exploration of the neck which confirmed right thyroid hemiagenesis. Left loboistmectomy was performed with excision of left inferior parathyroid adenoma. Five months after the operation, parathormon level was still increased with calcium values at the upper limit. Sestamibi scintigraphy was performed again which showed increased accumulation of MIBI in the projection of the right

lower parathyroid gland. We performed reexploration of the neck and excision of the right upper parathyroid adenoma wich was located behind cricoid laryngeal cartilage. After surgery we observed a normalization calcium and parathormon value. Thyroid hemiagenesis is a rare anomaly, more common in female (ratio 3:1) and with predominace of left lobe involvment (ratio 4:1). The first to describe this anomaly was Handfield Jones in 1852. From available literature we have not found the case that described double parathyroid adenoma which one on the side of thyroid hemiagenesis.

Hemiagenesis, double parathyroid adenoma.

Declaration of interest
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## P1707

**Laminin and poly-laminin, a polymeric form of laminin assembled at acidic pH, differentially modulate PCCL3 thyroid cell behavior**

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The extracellular matrix protein laminin forms polymers both *in vivo* and *in vitro*. Acidification of pH leads to the formation of an artificial polymer with biomimetical properties, named poly-laminin (polyLM). Follicular cells in the thyroid are in close contact with laminin, but their response to this important extracellular signal is still poorly understood. Thyroid PCCL3 epithelial cells cultivated either on glass, regular laminin (LM) or laminin previously polymerized in acidic pH (polyLM) showed distinct cell morphologies and proliferative rates, as well as differences in the organization of their actin cytoskeleton. While on polyLM cells displayed a typical epithelial morphology and presented actin fibers radially organized, on LM they spread irregularly on the substrate, lost cell contacts and produced thick actin fibers, which expanded to the whole cytoplasm. From a functional standpoint, both substrates of laminin induced a slight decrease in the expression of the sodium iodine symporter (NIS), but a significant decrease in iodine uptake was detected when cells are cultivated on laminin in comparison with cells cultivated on glass. In this study we demonstrated that PCCL3 cells can discriminate between LM and polyLM and that they respond to the latter by better preserving their phenotype as in the thyroid tissue.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1708

**Steroid-responsive encephalopathy associated with Graves' disease**

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A 29-year-old female diagnosed 1.5 years ago with Graves' disease, presented with severe thyrotoxicosis and a large, diffuse goiter after an attempt to taper the anti-thyroid medication. She had also noticed 4 days before presentation right arm mild paresis. The neurological exam confirmed the sensitive and motor deficit. Blood assays confirmed severe thyrotoxicosis (TSH $<0.03$ mUI/l, $fT_4=$ 25.6 pmol/l, $T_3>500$ ng/dl), high levels of anti-thyroid antibodies (TPOAb $>$ 3000 UI/ml; TRAb = 52.8 U/l), without major biochemical abnormalities. She has sinus tachicardia at 130 b.p.m., with features of hyperkinetic heart on ecocardiography but no signs of heart failure. She received increased doses of anti-thyroid medication and β-blocker (Metimazole 20 mg tds and Metoprolol 50 mg tds). Few hours after admission she presented an episode of acute dizziness and lost consciousness for 4–5 min with no seizures. She recovered spontaneously but remained confused, with fluctuating consciousness, severe nausea and headache. The brain CT was normal, excluding a stroke, and the CSF analysis excluded infection. There were nonspecific changes on the EEG, while the brain MRI showed high signal abnormalities in the subcortical white matter of the parieto-occipital areas. In the setting of the high levels of anti-thyroid antibodies, the neurological picture was interpreted as Hashimoto's encephalopathy, and the patient was started on high dose intravenous corticosteroids (500 mg Metilprednisolone daily) with progressive neurological improvement over the following days.

A rare cause of confusion and altered mental status, Hashimoto's encephalopathy is an immune-mediated neurological disorder that occurs in the setting of high levels of anti-thyroid antibodies and responds promptly to corticosteroid therapy. Rarely it can be seen in the setting of hyperthyroidism, which can exert its own neurological effects (tremor, confusion, myopathy), further complicating a rather nonspecific neurological picture. Hashimoto's encephalopathy must always be considered in a female patient with occult neurological manifestations.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1709

**Evaluation of the cytopathologic findings of the thyroid nodules which include macrocalcification**

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Aim
In our study, we aimed to evaluate the cytologic findings of the thyroid nodules, in which macrocalcification is determined with ultrasonography.
Method
We evaluated retrospectively 907 nodules of 269 patients, who have nodular or multinodular goiter and have macrocalcification at least in one nodule. The same patients' nodules that don't have macrocalcification are taken into the control group. Patient's thyroid function tests, ultrasonographic features and cytologic results of the nodules were evaluated. Cytologic findings are classified as benign, non-diagnostic, suspected and malign.
Results
79.9% of the patients were female, 20.1% were male. Macrocalcification was positive in 420 nodules (Group 1A: peripheral macrocalcification ($n=53$), Group 1B: macrocalcification was intranodular ($n=367$). In Group 2 ($n=487$) no macrocalcification was found. Mean nodule size in group 1A, 1B, 2 was respectively $12.94\pm6.26$, $25.50\pm14.27$ and $15.72\pm7.53$ mm ($P<0.001$). Compared the ultrasonographic features of Groups 1 and 2, peripheral irregularity and presence of halo were similar. In group1 microcalcification was positive in the 61.4% of nodules, whereas it was 17.5% in Group 2 ($P<0.001$). Solid nodule ratio was 11.7% in Group 1 and 49.9% in Group 2 ($P<0.001$).
Conclusion
Malign cytology was similar in the nodules, which have macrocalcification or not. In the nodules with peripheral calcification, high ratio of suspected cytology can demonstrate that macrocalcification is associated with malignacy, contrast to the usual opinion. Therefore, we think that, presence of macrocalcification does not always regard as benign nodule.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1710

**Autoimmune and allergic diseases associated with autoimmune thyroid diseases**

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Background
Sometimes, although the serum thyroid hormone levels are in desired ranges, the patient still has complaints. On the other hand, the dose of levothyroxine replacement may be variable in patients with hypothyroidism. These factors directly influencing the clinical practice may be, to some extent, associated with other immunological/allergic diseases that accompany autoimmune thyroid diseases (ATDs).

Aim
To document the other autoimmune/allergic disorders in patients at follow-up for ATDs in a tertiary care university hospital.
Methods
During the study period, consecutive 274 patients diagnosed with and/or at follow-up for Hashimoto's thyroiditis (HT) and 53 with Graves' disease (GD) were included in the study. All the patients were examined and when clinically suspected for other autoimmune/allergic disease, further investigations were applied.
Results
65 (23.8%) patients with HT and 7(13.2%) patients with GD had at least one additional clinical autoimmune/allergic disorder. The distribution of these disorders were as: 28 (10.2%) gastrointestinal (chronic atrophic gastritis, celiac disease, autoimmune pancreatitis, ulcerative colitis, primary biliary cirrhosis), 19 (6.6%) allergic (asthma, chronic urticaria, rhinosinusitis), 12 (4.4%) rheumatological (rheumatoid arthritis, systemic lupus erythematosus, Still disease, Sjögren syndrome, Behçet's disease, ankilosying spondylitis), 10 (3.7%) skin (vitiligo, psoriasis, idiopathic pruritus, total alopecia), 4 (1.5%) endocrinological (hypoparathyroidism, type-1 diabetes mellitus, hypophysitis), 1 (0.4%) hematological (idiopathic thrombocytopenic purpura), 1 (0.4%) renal (crescenteric glomerulonephritis) involvements in HT. In GD, these distributions were similar: 2 (3.8%) gastrointestinal, 2 (3.8%) allergic, 2 (3.8%) skin, 1 (1.9%) rheumatological, 1 (1.9%) hematological. Additionally, 50 (18.2%) patients with HT and 1 (1.9%) with GD found to have vitamin B12 deficient-anemia ($P=0.001$). 28 (10.2%) of HT and 1 (1.9%) of GD patients had dimorphic (both vitamin B12 and iron deficiencies) anemia.
Conclusion
Patients with ATDs are prone to additional autoimmune/allergic diseases. The mostly involved organ system is the gastrointestinal tract in both ATDs. Probably this involvement takes play in exaggaration of some symptoms by leading to anemia caused by both vitamin B12 and iron deficiencies, especially in patients with HT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1711

### L-Thyroxine therapy and depression in subclinical hypothyroidism

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Background and aim
Subclinical hypothyrodism (SH) appears to be associated with mood and changes. The aims of this study were: i) to determine whether depression is more common in SH, and ii) to investigate the effects of L-thyroxine ($LT_4$) therapy on depressive symptoms.
Methods
31 patients with SH and 36 age amd BMI matched healthy controls were included. Beck and Hamilton scales were used to evaluate depression in all participants at baseline and in patients after they have reached euthyroid status by $LT_4$ therapy.
Results
TSH was higher and $FT_4$ was lower in patients with SH ($P<0.0001$ and $P=0.001$ respectively). Scores of depression scales were similar between the patients with SH and controls. $LT_4$ treatment resulted in an increase in $FT_4$ and decrease in TSH, Beck or Hamilton scores did not change significantly after treatment.
Conclusions
Our results suggest that depressive symptoms are more common in patients with SH and that achievement of euthyroid status by $LT_4$ does not have an effect on depression.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1712

### 'Too slow for the heart to go': a case of hypothyroidism-induced cardiomyopathy presenting as acute cardiogenic shock

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Cardiomyopathy can occur due to hypothyroidism or hypocalcemia but often resolves with judicious treatment with levothyroxine and correcting calcium levels to acceptable levels. We discuss a case of a 37-year-old woman presenting with acute cardiogenic shock and a history of total thyroidectomy 6 years prior for papillary thyroid carcinoma stage 1 (T2N1aM0). Patient was non-compliant to levothyroxine treatment and developed easy fatigability and exertional dyspnea 5 months before consult. Patient had no previous co-morbidities, and no family history of heart diseases. Workup was consistent with primary hypothyroidism with an elevated TSH and low free thyroid hormones. Serum calcium was also low and was attributed to post-surgical hypoparathyroidism. Neck ultrasound confirmed absent thyroid glands. Chest radiograph show massive cardiomegaly, which on trans-thoracic echocardiogram (TTE) showed minimal pericardial effusion, global hypokinesia, an ejection fraction of 20%, and a dilated left ventricle. After initial stabilization with inotropes, patient was started on low dose of levothyroxine at 25 μg/day and digoxin. Hypocalcemia was corrected to lower limit of normal with calcitriol 0.5 μg/day and calcium carbonate 2 g/day. She was discharged after a few weeks with gradual up-titration of levothyroxine at 12.5 μg/day depending on clinical tolerability. Euthyroidism was achieved at levothyroxine 125 μg/day and 8 months post-discharge, previous functional capacity was achieved at 4–10 METs with an improved ejection fraction of 62% on repeat TTE.
Keywords: hypothyroidism, hypocalcemia, cardiomyopathy, cardiogenic shock.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1713

### Graves' disease and gene polymorphism of TNF-α, IL2, IL6, IL12, and IFN-γ

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The role of genetic factors in the pathogenesis of Graves' disease (GD) is not clear. The purpose of this study was to investigate the association between single nucleotide polymorphisms in pro-inflammatory cytokine genes and GD in Iranian patients. A case–control hospitalbased study was carried out on 107 GD patients and 140 healthy controls. Cytokine typing was performed by PCR with sequence-specific primers (PCR-SSP) assay. The allele and genotype frequencies of the following cytokine genes were determined: TNF-α (−308A/G, −238A/G), IL2 (−330T/G, ?166G/T), IL6 (−174C/G, A/G nt565), IL12 (−1188A/C), and IFN-γ (UTR 5644A/T). The following alleles and genotypes were significantly overrepresented in patients: TNF-α −308A allele ($P$ 0.01) and AA genotype ($P$ 0.05), IL2 −330G allele ($P$ 0.01) and GG genotype ($P$ 0.01), IL 6 −174C allele ($P$ 0.01) and CC genotype ($P$ 0.01), IL12 −1188C allele ($P$ 0.01) and CC genotype ($P$ 0.01), IFN-γ UTR5644T allele ($P$ 0.01) and TT genotype ($P$ 0.01). In conclusion, this is the first study to show a significant association between GD and IL2 −330G, IL12 −1188C, and IFN-γ UTR 5644T alleles.
Our results support the hypothesis that polymorphism in pro-inflammatory.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1714

### Evaluation of $CD4^+CD161^+CD196^+$ and $CD4^+IL17^+$ Th17 cells in the peripheral blood of young patients with Graves' disease and Hashimoto's thyroiditis

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Up till now, altered balance of Th1 and Th2 immune cells has been postulated to play an important role in the pathogenesis of autoimmune thyroid diseases (AITD). However, recent studies on thyroid diseases suggest a new role for Th17 (T helper 17) cells that have been classified as a new lineage, distinct from Th1, Th2 and Treg cells. Despite wide interest, role of Th17 cells in the pathogenesis of inflammatory and autoimmune diseases is still debated. Th17 cells are involved in immune responses against extracellular pathogens and have the ability to secrete cytokines: IL17, IL17F, IL22 and IL21. Th17 cells can be characetrized by several surface markers, i.e. CCR6 (CD196), IL23R, IL12Rβ2 and CD161.
The aim of the study was to estimate the frequencies of circulating $CD4^+CD161^+CD196^+$ and $CD4^+IL17^+$ Th17 cells in patients with Graves' disease (GD), Hashimoto's thyroiditis (HT) and in healthy controls.
Polychromatic flow cytometry and several fluorochrome-conjugated monoclonal antibodies were applied to delineate Th17 cells with either $CD4^+CD161^+CD196^+$ or $CD4^+IL17^+$ phenotype.
In untreated patients with AITD we observed a tendency to increased frequencies of $CD4^+CD161^+$ $CD196^+$ and $CD4^+IL17^+$ Th17 lymphocytes in comparison to the healthy controls. In cases with HT, positive correlation between percentage of $CD4^+CD161^+CD196^+$T cells and serum level of anti-TPO antibodies was detected.
We conclude that the increase percentage of Th17 cells in children with Graves' disease and Hashimoto's thyroiditis can suggest their role in initiation and development of immune processes in AITD.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1715

### Study thyroid function in patients with acromegaly

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Goal
Study thyroid function in patients with acromegaly. Materials and method: 88 patients with acromegaly, age 54.6 ± 13.2 years, mediana diagnosis acromegaly 4 years, IGF1 528 (265; 701.3 ng/ml, HG 10.4 (3.7; 30.1) μE/ml, only 12 patients had a remission of disease. All patients were taken TSH, $fT_4$, TPO antibodies, revealed anamnesis of thyroid diseases.
Results
19 from 88 patients (21.6%) had hypothyreosis and 2 (2.3%) had subclinical thyrotoxicosis, that higher average prevalence these disease (2 and 1% correspondently). The causes of hypothyreosis were different: eight paitents (9.1%) had central hypothyreosis mainly due surgical ttreatment of acromegaly (seven from eight patients); 12 patients (13.6%) had hypothyreosis due thyroidectomy (six patients) or autoimmune thyroidit (six patients). Thyroidectomy was performed for thyroid cancer in five from six patients. There is interesting combination of different thyroid dysfunction at two patients. One patient 53 years old had acromegaly for 1year. She had hypothyreosis due thyroidectomia 5 years ago and took 75 μg L-thyroxine. But then central hypothyreosis was added after surgical treatment of acromegaly: TSH 0.53 μE/ml, $fT_4$ 8.5 pmol/l on the same dose of L-thyroxine. Doctor has to consider this combination dysfunction because TSH is not informative at patients with central hypothyreosis. The cause of subclinical thyrotoxicosis was toxic polynodular goiter. One patient had combination subclinical thyrotoxicosis with central hypothyreosis. At first, central hypothyreosis was appeared after surgical treatment of acromegaly and the patient took L-thyroxine for 6 years. Then $fT_4$ was increased up to 24 pmol/l, L-thyroxine was stopped but $fT_4$ remained high ($fT_4$ 22 pmol/l, TSH 0.01 μE/ml). Scintigraphy revealed 'hot' nodule. In this case there are two causes of suppressed TSH: central hypothyreosis and subclinical thyreotoxicosis.
Conclusion
The patients with acromegaly have high prevalence of hypothyreosis (21.6%) and subclinical thyrotoxicosis (2.3%). Thyroid dysfunction develops as a result of impact of IGF1 and GH to thyroid and TSH falls after surgical treatment of acromegaly. This simultaneously impact to thyroid function leads to combination of some thyroid diseases and makes the treatment of patients difficult.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1716

### Subclinical hypothyroidism and thyroid autoimmunity during the 1st quarter of pregnancy in oran area

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Introduction
Subclinical hypothyroidism and euthyroid thyroiditis have in common the clinically asymptomatic character. These two diseases can occur during pregnancy with no clinical impact.
Our objectives are:
– Prevalence of subclinical hypothyroidism during the 1st trimester of pregnancy.
– Frequency of autoimmunity in euthyroid pregnant women in 1st quarter.
– Analysis of the association of thyroid autoimmunity with the parity.
Description of methods
Prospective study, made in Oran area, on 270 pregnant women in 1st quarter. Women who smoke (active smoking confessed) and those followed for thyroid disease or taking medications that interfere with the thyroid gland were excluded.
– Study protocol: clinical examination, TSH, free thyroxine ($fT_4$), free triiodothyronine ($fT_3$), thyroid antibodies (anti-peroxidase, anti-thyroglobulin and anti-TSH receptor) and cervical ultrasonography.
– Statistics tests: collection of data on EPI INFO 5.1, bivariate analysis (the $\chi^2$ test, the Pearson $\chi^2$ test, the Yates corrected $\chi^2$ test, the Wilcoxon $\chi^2$ test (log rank sum) or Mann–Whitney, the variance analysis method (ANOVA), the $r$ correlation test, significance level $P<0.05$, multivariate analysis (SPSS: 10 version and MedCalc).
Results
– 23/270 pregnant women (8.5%) had subclinical hypothyroidism (TSH > 2.5 mUI/l, $fT_4$ and $fT_3$ normal) with 7/23 (30.4%) wich had thyroiditis.
– 215/270 pregnant women (79.6%) were euthyroid with 24/215 (11.2%) wich had thyroiditis.
– No association was found between anti-TPO positivity and numbers of pares both in patients with subclinical hypothyroidism than those in euthyroid ($P=0.72$ and 0.45 respectively).
Conclusions
The prevalence of thyroid autoimmunity in the 1st trimester of pregnancy is significant. Only a determination of anti-TPO is able to unmask these euthyroid thyroiditis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1717

### Evaluation of hearing and balance functions in patients with Hashimoto's thyroiditis

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Introduction
It is commonly known that hearing and balance functions are affected in thyroid function disorders. In literature, there are very few studies demonstrating that hearing may be affected due to autoimmunity in euthyroid Hashimoto's

thyroiditis (HT) independent from thyroid functions. In addition, hearing disorders have also been reported in other autoimmune diseases. For this reason, we investigated the functions of the peripheral and central sections of auditory system in patients with euthyroid HT.
Materials and methods
The study included 21 patients with euthyroid HT without any hearing complaints and 21 healthy individuals. Patients with histories of systemic disease, central nervous system pathology, ototoxic drug-substance use, acoustic trauma were excluded. Otoscopic examinations of the patients were normal. Both groups were implemented tympanometry, audiometry, distortion product otoacoustic emission (DPOAE), Brain stem evoked auditory potentials (ABR), vestibular evoked myogenic potentials (VEMP) tests.
Results
Demographic data of patient and control groups were similar ($P>0.05$). A difference was not established between the groups in terms of TSH, free $T_4$, and free $T_3$ values ($P=0.46$, $P=0.39$, $P=0.62$). Thyroid antithyroglobulin and antithyroperoxidase levels were high in the patient group ($P<0.05$). When compared the right and left ear audiometry average results of the groups, hearing threshold was found to be significantly higher at 250 dB in the patient group ($P=0.015$). A significant difference was not found between the two groups in other audiological and vestibular tests. A correlation was not established between thyroid antibody titer and hearing threshold at 250 dB ($P>0.05$).
Conclusion
The fact that low frequency hearing threshold we established in our euthyroid HT patients was higher may be correlated with autoimmune cochlear damage reported in literature. However, since this disorder was not possible to be demonstrated in other tests, we believe that further studies are of necessity.
Keywords: Hashimoto's thyroiditis, hearing loss, autoimmune thyroid disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1718

**Stessful life events and Graves' disease**

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Stress appears to activate the immunoneuroendocrine network and seems to be related to the development of autoimmune diseases. Graves' disease is a multisystem disorder characterized by hyperthyroidism, eye disease and pretibial myxedema. Stress appears also to be related to the development of Graves' disease.
The aim was to describe cases of Graves' disease which developed immediately after a stressful life event.
Patients, ten female, aged 35–56 years old, presented with severe hyperthyroidism after a stressful life event. Within this cohort, three female patients had lost their father, immediately before the development of hyperthyroidism, two female patients had lost their mother immediately before the development of hyperthyroidism, three female patients had lost their husband immediately before the development of hyperthyroidism, one female patient had lost a child and one female patient had lost her husband 1 year earlier and one of her three children immediately before the development of hyperthyroidism.
Hyperthyroidism developed in these female patients in eight of them within a month after the loss of the beloved person and in two within 2 weeks after the loss of the beloved person. Hyperthyroidism was severe and was accompanied by involvement of the eyes only in two of the described cases. TSH receptor antibodies were detected in seven of the described cases. In all cases hyperthyroidism responded to the administration of antithyroid drugs.
In conclusion, stressful life events, in particular the loss of a beloved person, may be an initiating factor for the development of Graves' disease. In these cases hyperthyroidism seems to be severe and to develop shortly after the loss of the beloved person. Thus, it appears that a strong emotional insult may be accompanied by activation of the immunoneuroendocrine axis and the development of a multisystem autoimmune disease, such as Graves' disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1719

**Thyrotoxic storm: clinical challenges**

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Thyrotoxic storm is one of the rare but serious, and sometimes life threatening endocrine emergencies, the mortality rate ranges from 20 to 30%.
We report the case of a 44-year-old female who had an emergency hospital admission with diarrhoea, vomiting and palpitations in September, 2011. She was diagnosed with Graves thyrotoxicosis in 2004 and was commenced on carbimazole. She was non compliant with the treatment and did not attend her hospital appointments regularly. Her past medical history included anxiety, suspected anorexia nervosa in the teenage years and iron deficiency anaemia. Examination revealed bilateral basal crackles and peripheral oedema. Bloods confirmed thyrotoxicosis. The diagnosis of thyrotoxic storm with high output heart failure was made and she was treated with β blockers, propylthiouracil, IV steroids and Lugol's Iodine. Few hours following the admission, she suffered asystolic cardiac arrest and was resuscitated successfully. During the ITU stay, her liver functions deteriorated significantly. It was thought to be either due to propylthiouracil or ischaemic hepatitis secondary to cardiac arrest. Propylthiouracil was swapped with carbimazole and liver functions improved slowly but steadily. On discharge, her thyroid and liver functions had stabilized on Carbimazole and she was referred to ENT team for the consideration of thyroidectomy.
This case poses clinical challenges at multiple steps. Continual non compliance was assumed to be the precipitating factor for the thyrotoxic decompensation. She was lost to follow up due to multiple non attendances and the impact the possible psychiatric history had on her condition was not evaluated further. Propythiouracil use in the acute illness could have contributed to the liver failure. Propylthiouracil is still being used in severe thyrotoxicosis due to its inhibitory effect on $T_4$ conversion to $T_3$. The 2009 FDA warning regarding propylthiouracil and hepatic failure should prompt revision of thyrotoxic storm management guidelines.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1720

**A case of toxic multinoduler goiter coexists in TSH-producing pituitary tumor**

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Introduction
The frequency of toxic multinoduler goiter (TMNG) is different among the countries depending on an intake of iodine, but it is said to be ~1% of adenomatous goiter in Japan. In addition, the frequency of TSH-secreting pituitary adenoma (TSHoma) is rare with 0.5–1.0% of pituitary gland tumor. There is no report that TMNG coexists in TSHoma so far.
Case
A 58 years old female was complained swelling of thyroid and palpitation. We found several nodules which were low echogenic masses of up to 28 mm on both thyroid lobes by cervical sonography. Her blood tests showed hyperthyroidism ($fT_3$ 6.3 pg/ml, $fT_4$ 2.3 ng/dl, TSH 3.20 μIU/ml, TgAb 10 IU/ml, TPOAb 5.1 IU/ml, HTg 167.6 ng/dl, TRAb (ECLIA) 0.3 IU/l). From above data which is syndrome of inappropriate secretion on TSH, we underwent her pituitary gland MRI, then we found the 8 mm mass. A TRH stimulation test: TSH (μIU/ml) was 4.258 (0 min), 5.496 (30 min), 5.303 (60 min). We diagnosed TSHoma and introduced her to a brain surgeon. She underwent a trans-sphenoidal neurosurgery by an endoscope. Though TSH was fallen back to 0.044 μIU/ml, $fT_3$ and $fT_4$ values were continued high even after 3 months of surgery. Therefore she was referred back to us for a close investigation of thyrotoxicosis. As the purpose of thyrotoxicosis differentiation diagnosis, she administered $^{123}I$ for thyroid scintigraphy. There were the hot uptake regions of both lobes and we diagnosed TMNG. After she took 500 MBq of $^{131}I$, her thyroid function returned to normal in a few months.
We also examined the thyroid hormone before treatment of 8 TSHoma cases that we experienced in our hospital. There was no difference of thyroid function between TSHoma alone and this patient.

Conclusion
TSHoma has many invasive cases and there are a lot of recurrence cases after surgery. Because it is difficult to deny complication of TMNG and TSHoma just from thyroid function, we think there might be more patients like this case.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1721

**Pattern of thyroid dysfunction in metabolic syndrome**

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Introduction
Thyroid dysfunction is a common endocrine disorder affecting 300 million people around the world and over half are expected to be unaware of the condition. The number of people suffering from metabolic syndrome is also on the rise and it is estimated that a quarter of the world's adults have metabolic syndrome. Thyroid dysfunction and metabolic syndrome (Met S) are both associated with atherosclerotic cardiovascular disease, hyperlipidemia and low grade inflammation. It is possible that coexistence of these disease entities may substantially increase cardiovascular risk and facts can aid in early treatment that might reduce the risk. Moreover, little is known about the relationship between metabolic syndrome and thyroid function. Thus, the current study was intended to determine the pattern of thyroid dysfunction in metabolic syndrome subjects.
Methods
A total of 128 previously diagnosed subjects with Met S (defined by NCEP-ATP III panel and IDF guidelines) were recruited during a routine health checkup at Dhulikhel Hospital-Kathmandu University Hospital in Dhulikhel, Nepal. The serum TSH, free $T_3$ and free $T_4$ levels were measured to categorize thyroid dysfunction. Statistical analysis was performed using SPSS version 11.5. The results were expressed as percentage to determine the pattern of thyroid dysfunction in Met S.
Results
The overall prevalence of the thyroid dysfunction was 31.25% (40). Of the 128 subjects, 28.90% (37) had subclinical hypothyroidism, 1.55% (2) had overt hyperthyroidism, 0.80% (1) had subclinical hyperthyroidism and 68.75% (88) were euthyroid. Overt hypothyroidism was not present in any of the subject.
Conclusion
Thyroid dysfunction is associated with metabolic syndrome in particular subclinical hypothyroidism and the coexistence of the two will substantially increase cardiovascular risk. Thus, it necessitate the need for evaluating the thyroid status in patients with metabolic syndrome which can help in early treatment that might reduce the risk.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1722

**Alteration of clinical characteristics of subacute thyroiditis in last two decades in our hospital**

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Objective
Subacute thyroiditis (SAT) is a transient inflammatory disease of the thyroid. As we hypothesized that clinical characteristics of SAT were changing recent years, we evaluated data of our patients.
Patients and methods
We retrospectively reviewed the medical records of 192 patients (45 men and 147 women) with SAT who visited our hospital from 1990 to 2009. We analyzed these records dividing into two groups by decade, i.e. the first 10 years (1990–1999) and the following 10 years (2000–2009).
Results
In each decade, female patients were 50/65 (76.9%) vs 97/127 (76.4%), more than 50-year-old patients were 29/65 (44.6%) vs 71/127 (55.1%), CRP higher than 5 mg/dl was observed in 25/60 (41.6%) vs 49/127 (38.6%), and F-$T_4$ higher than 3 ng/dl was observed in 38/65 (58.5%) vs 69/127 (54.3%). Ratio of older patients tended to increase in the second decade ($P=0.13$). We noticed that seasonal distribution of SAT onset changed in two decades. In the first decade, SAT onsets were often seen in spring. On the other hand, in the second decade, the peak in spring became less remarkable and onsets between late summer and autumn were increased. Onsets between August and October in each decade were 16/65 (24.6%) vs 50/127 (39.4%), and significant difference was observed ($P=0.04$).
Conclusion
We observed that seasonal distribution of SAT onset changed. SAT was generally considered to be developed by viral infection, so this alteration may be due to the transition of viral environment.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1723

**Disseminated tuberculosis involving the thyroid: an unlikely scenario**

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Tuberculosis involving the thyroid gland is known to be rare with 76 cases being reported as of 2006, and may vary in their presentation*. We present a case of a previously well 67-year-old male who consulted for decreased sensorium, jaundice and hypotension, all attributed to sepsis. Patient exhibited no hyper-or hypothyroid symptoms and physical examination did not reveal clues to an underlying thyroid disease. Cultures of the blood, urine and sputum were inconclusive and despite empiric antibiotics and inotropic support, patient succumbed to multi-organ failure. Post-mortem examination revealed disseminated tuberculosis involving the thyroid as well as the liver, lungs, spleen, kidneys, pancreas, adrenals and various lymph nodes in the thoracic and abdominal regions.
Discussion
Disseminated tuberculosis by itself is rare even in countries endemic to tuberculosis. Its non-specific symptoms lead to a low detection rate, and despite tuberculous involvement of the thyroid glands, this may be clinically inapparent and may not require treatment. Thyroid function tests may be warranted however in the setting of extensive thyroid involvement.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1724

**Prevalence of goiter and thyroid nodular disease in patients with class III obesity**

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Aim
To evaluate the prevalence of goiter and nodular disease in patients with class III obesity, and also correlate the findings with serum insulin, leptin and HOMA-IR.
Methods
A cross-sectional study with thyroid ultrasound and serum glucose, insulin, leptin and TSH was performed with obese patients and a control group without known thyroid diseases, matched for age and sex, but with BMI of $<25\ kg/m^2$.
Results
Twenty-one patients and 40 controls, $36.7 \pm 8.8$, $35.9 \pm 9.1$ years old, respectively, were included. Median BMI was $50\ kg/m^2$ (patients) and $23\ kg/m^2$ (controls). Median TSH and thyroid volume were 2.2 mUI/ml and $7.9\ cm^3$ (patients) and 1.8 mUI/ml and $7.4\ cm^3$ (controls); $P=0.200$ and $P=0.369$. Medians of serum leptin were 4194 and 762 ng/ml ($P<0.001$).

Medians of HOMA-IR were 5.6 and 1.1 ($P<0.001$). Thyroid volume was correlated with BMI (rs=0.356, $P=0.04$) and HOMA-IR (rs=0.291; $P=0.016$) in the whole group. This last association was stronger in the obese group (rs=0.571, $P=0.01$), but also also present among the controls (rs=0.251, $P=0.05$). This correlation was independent from confounding parameters (TSH, BMI, leptin), as detected in the partial correlation, controlled for BMI (rs=0.349; $P=0.04$). The correlation between BMI and volume was related to the serum levels of leptin and HOMA-IR. Leptin only correlated with volume in obese patients. Normal ultrasound was found in 47.6% of patients and in 75% of controls, $P=0.30$. Nodules were found in 35 and 20%, respectively; $P=0.171$. The nodules were on average higher in patients, but not significantly.
Conclusion
The prevalence of thyroid nodular disease was not significantly
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1725

**Serum nesfatin-1 levels in overt and subclinical hyperthyroidism**

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Objective
Nesfatin-1, was recently discovered in the brain which is derived from nucleobindin2 (NUCB2). Central and peripheral administration of nesfatin-1, inhibits food intake, dose-dependently. Hyperthyroid patients have increased appetite and food intake with a craving for carbohydrate-rich food, at the beginning of disease, but the physiological mechanisms underlying this behavior is not known exactly. In this study, we investigated whether nesfatin-1 is involved in the regulation of appetite and body weight in hyperthyroidism, or not.
Methods
A total of 70 patients with subclinical and overt hyperthyroidism compared with 35 control patients. Serum nesfatin-1 level was measured from all samples by commercial ELISA kit.
Results
Serum free $T_3$ and free $T_4$ levels in the overt hyperthyroidism group was significantly higher and serum TSH level in the overt hyperthyroidism group was significantly lower than subclinical hyperthyroid and control group respectively ($P<0.001$). In addition, the TSH levels in the subclinical hyperthyroid group was significantly lower than control group ($P<0.001$). Serum nesfatin-1 levels were similar between three groups ($P=0.293$). After adjusting for age and body mass index, nesfatin-1 levels in control group was not different from subclinical and overt hyperthyroid group respectively ($P=0.567$ and $P=0.519$). However, there was a significant correlation between nesfatin-1 and platelet, free $T_3$ vs TSH receptor antibody levels ($P<0.05$).
Conclusion
These data showed that serum nesfatin-1 levels do not significant change in overt and subclinical hyperthyroidism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1726

**Spontaneous splenic rupture following plasmapheresis in a patient with refractory amiodarone-induced thyrotoxicosis**

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Introduction
Amiodarone-induced thyrotoxicosis (AIT) is a well-known complication of amiodarone therapy. Type 1 AIT is caused by excessive iodine-induced thyroid hormone synthesis in abnormal thyroid glands and treated with thionamides; type 2 AIT is a drug induced destructive thyroiditis that responds to glucocorticoids. Mixed forms of AIT need a combined treatment of thionamides and glucocorticoids. In refractory cases of AIT plasmapheresis before total thyroidectomy is an efficacious treatment.
Case report
A 56-year-old woman was admitted to the emergency department with thyrotoxic symptoms of poorly controlled AIT that was diagnosed 3 months ago. Medical history revealed a coronary and valvular heart disease. Laboratory findings were TSH 0.04 mU/l (normal range 0.27–4.20), fT4 52.3 pmol/l (12–22), and fT3 5.6 pmol/l (3.1–6.8). Ultrasound of the thyroid gland showed two nodules and hypovascularisation of the parenchyma, therefore mixed AIT was diagnosed. Lithium and potassium perchlorate were added to the treatment with high dose prednisolone and carbimazole, and amiodarone was discontinued. However, within a week's time thyrotoxicosis aggravated to fT4 over 100 pmol/l and fT3 to 7.0 pmol/l. Because of the severe thyrotoxicosis refractory to medical therapy, we decided for plasmapheresis and total thyroidectomy. After the second plasmapheresis sudden pain in the left upper abdomen and hypotension occurred. A CT scan of the abdomen disclosed a splenic rupture and splenectomy was performed after another plasmapheresis, followed by total thyroidectomy.
Conclusions
Spontaneous splenic rupture is an extremely rare complication in plasmapheresis. To date and to our knowledge this is the first case of spontaneous splenic rupture occurring during plasmapheresis in a patient with AIT. In addition to anticoagulation during plasmapheresis hyperdynamic state caused by thyrotoxicosis may have contributed to splenic rupture.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1727

**Does serum inflammatory and cytokine profile influence graves disease severity at diagnosis?**

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Introduction
Autoimmune thyroid disease affects 1% of the population and comprises several disorders, including Graves' disease(GD). Local and serum cytokines are increased in patients with Graves disease, play a central role in co-ordinating immune reactions and have immunolgical and functional effects on thyroid cells.
Aim
To investigate serum inflammatory and cytokines profile in untreated patients with Graves' disease at diagnosis, when the immune response is presumably more specific.
Materials and Methods
We evaluated 118 patients (81.4% women), mean age 47.46 years, 91(77.1%) with GD compared to a control group of 27 with other ethyologyc forms of hyperthyroidism: toxic multinodular goiter, amiodaron induced hyperthyroidism, toxic nodule and subacute thyroiditis.
Results
GD patients were younger, with higher HR, FT 4 and TT3 than the control group. C-reactive protein, TNFα, ESR and fibrinogen levels showed no significant difference when compared to control group. GD patients with personal history of autoimmune diseases had higher TNFα ($P=0.007$) and those with personal history of thyroid diseases had higher CRP ($P=0.003$) and TNFα($P=0.01$) than those without. In the GD group smokers and those with oftalmopathy did not have a modified inflammatory profile. In the GD group: CRP, TNFα, ESR and fibrinogen did not correlate with FT4 or TT3 at diagnosis; CRP positively correlated with age ($P=0.025$); TNFα positively correlated with systolic BP($P=0.031$); ESR positively correlated with age, BMI, trigliyerides and fibrinogen positively correlated with triglycerides.
Conclusion
These results indicate that there may be only a limited role for measurement of CRP, TNFα, ESR and fibrinogen levels in the diagnosis of GD, yet inflammation significantly correlates with other important features.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1728

**TSH according to age and sex. The future of normality**

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Introduction
Reference ranges for classifying a patient as hyper-, normo- and hypothyroidism vary from one laboratory to another, because the techniques used, regional factors (race, iodine..), but there is also the variables of age and sex of the patient are not taken. Article Thyroid Volume 21, Number 1, 2011, 5–11 Boucai et al, published equations to adjust the normal range in terms of these variables. Evaluate these equations and see if there are significant changes to normal.
Materials and Methods
We studied 520 outpatients that have requested TSH from the health center.
Our reference range for TSH is between 0.35 and 4.94 mUI/mL, so adapting the equations described in the article the following equations are obtained to study:
TSH(2.5) = 0.35 + 0.00073*Age − 0.031*Sex
TSH(97.5) = 4.94 + 0.05*Age − 0.223*Sex
Considering age in years and sex 0 for men and 1 for females
Results
With our classification reference range of thyroid function: are 12 hyperthyroid patients (2.3%), normothyroid are 472 (90.8%) and hypothyroid are 36 (6.9%). Applying the equations to study the following results: 13 (2.5%), 498 (95.7%) and 9 (1.8%) respectively.
hyperthyroid patients have antibodies and the patient requested the equation presented discrepant antibody negative. Of the 36 hypothyroid patients, only 14 had requested antibodies, with 28% negative.
patients with negative antibodies with the equation would be reclassified as normothyroid. But of the 10 patients with positive antibodies, 5 would remain hypothyroid and the other 5 would then normothyroid.
Conclusions
These equations to determine new TSH reference ranges are attractive because they allow us to dispense with the verification of results, enlargement of new assays, such as T4, T3 or antibodies. As we have only tested on a working day studies are higher, increasing the size of the sample, obtained by decades of age groups homogenous and making sure that all the patients studied made antibodies present.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1729

**Evaluation of the psychoemotional status of patients after surgical treatment of Graves' disease by catamnesis data**

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Materials and methods
The study included 58 patients (50 female, 8 males) aged from 38 to 60 years, have received surgical treatment for GD with the period of follow-up after treatment for 2 to 10 years. The patients were divided into 2 groups depending on the initial level of TSH. In the first group (33 persons) included patients with low-normal level of TSH (0.4–2.5 mU/ml), the in second group (25 persons) with upper-normal level of TSH (2.6–4.0 mU/ml). An estimation of the psychoemotional status was conducted by means of a scale of depression of Beck and Spielberg-Khanins test.
Results
The groups were comparable for gender, duration after treatment, dose of L-T4. In the first group the median age of patients was 43 years (35;50), the amount of points depression was 10 (2;19), which corresponds to easy depression, the median amount of points situational anxiety 39 (23;50), personal alarm 40 (23;59), which corresponds to a moderate level of anxiety.
In the second group the median age was 56 years (49;64), the amount of points depression was 16 (2;29), which corresponds to mild depression, the median amount of points situational anxiety 44 (25;60), personal alarm 44 (31;67), which corresponds to a moderate level of anxiety.
In the evaluation of mean values of the psychoemotional status in the first and second group, statistically significant differences were observed on all scales ($P < 0.05$).
Conclusions
there are significant differences of the psychoemotional status (presence of anxiety and depression) among patients with upper- and low-normal TSH. It is established that patients with upper-normal TSH had the worst performance, in the course of our study revealed a statistically significant difference between all scales, but most likely due to age.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1730

**Quality of life of patients after surgical treatment of Graves' disease by catamnesis data**

A. Dreval, O. Nechaeva, T. Mamedova, T. Shestakova, I. Chikh & I. Komerdous
Moscow Regional Research Clinical Institute of M.F.Vladimirsky, Moscow, Russian Federation.

Materials and methods
The study included 58 patients (50 female, 8 males) aged from 38 to 60 years, have received surgical treatment for GD with the period of follow-up after treatment for 2 to 10 years. The patients were divided into 2 groups depending on the initial level of TSH. In the first group (33 persons) included patients with low-normal level of TSH (0.4–2.5 mU/ml), the in second group (25 persons) with upper-normal level of TSH (2.6–4.0 mU/ml). Quality of life was assessed with the help of the using the questionnaire Short-Form SF-36.
Results
In the first group the median age of patients was 43 years (35;50),median follow-up after treatment 7 years (5;10), the average level of TSH 1.8 mU/ml (1.55;2.3), a median dose of L-T4 100 mcg (100;125), the median quality of life of 8 scales questionnaire SF -36 ranged from 54 points (vitality) up to 85 (pain).
In the second group the median age was 56 years (49;64), the median follow-up after treatment of 8 years (7;10), the average level of TSH 3.8 mU/ml (3.2;4), a median dose of L-T4 75 mcg (75;100), the median quality of life of 8 scales ranged from 40 points (vitality) to 58 (physical functioning).
In the evaluation of mean values of the quality of life in the first and second group, statistically significant differences were observed on all scales ($P < 0.05$).
Conclusions
The average quality of life of patients were on average and high level, however, there are significant differences in quality of life among patients with upper- and low-normal TSH. It is established that patients with upper-normal TSH had the worst performance, in the course of our study revealed a statistically significant difference between all scales.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1731

**Thyroid dysfunctions in patients with chronic hepatitis c virus infection with and without interferon therapy**

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Objective
to assess the frequency and pattern of thyroid dysfunctions (TD) in Egyptian patients with chronic hepatitis C virus infection (HCV) with and without interferon alpha (IFN) therapy.
Methods
thyroid function as well as thyroid peroxidase antibodies (TPO Ab) were assessed in 40 untreated Chronic HCV patients (HCV group), 30 HCV patients under IFN alpha therapy, for more than three months (IFN group) and 50 healthy age and sex matched controls.

Results
TD, either overt or subclinical, was detected in 12.5% of HCV group, 33.4% of IFN group and 2% of controls. Among HCV group, 5% had overt hypothyroidism, 7.5% subclinical hypothyroidism and no one had hyperthyroidism. While overt hypothyroidism was detected in 10% of IFN group, subclinical hypothyroidism observed in 16.7% and hyperthyroidism reported in 6.7%. Among controls, 2% had subclinical hypothyroidism whereas no one had overt hypothyroidism or hyperthyroidism. TD was more often detected in females (47.8%) compared to males (5.1%). Higher levels of TPOAb were observed among IFN group compared to HCV group and controls ($P<0.01$ & $P<0.01$, respectively). The study showed significant association between thyroid dysfunctions and TPO Ab positivity ($P=0.027$).
Conclusion
TD among Egyptian HCV patients, especially those treated with IFN was more frequent than usually reported. It is likely that HCV and IFN act in synergism to trigger TD in patients. Women appear to be more vulnerable to TD than men. The predominant TD is hypothyroidism. In view of high frequency of TD, routine screening and surveillance of HCV patients, especially after receiving IFN, is recommended.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1732

### Elevated levels of thyroid hormones without TSH suppression in two patients

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Introduction
Resistance to thyroid Hormones (RTH) is a syndrome characterized by reduced tissue responsiveness to thyroid hormones (TH).
This results to constantly elevated levels of TSH despite high levels of free T3 (FT3) and free T4 (FT4).
RTH is due to mutations of the gene encoding the b-isoform of the receptor of TH (THR-b gene), inherited in the autosomal dominant manner.
Clinical expression of RTH varies reflecting different levels of tissue resistance to TH, different mutations and genetic heterogeneity of factors affecting TH action.
Most patients are asymptomatic. Few present with hypo- or hyperthyroidism.
Coexistence of both hypo- and hyperthyroidism symptoms in the same patients is not rare. Goiter is quite common.
Case report
We describe two patients with RTH; a 36-year old female (1st patient) and a 58-year old male (2nd patient).
Both patients had undergone total thyroidectomy for multinodular goiter.
After thyroidectomy, increasing doses of levothyroxine were not enough to lower TSH down to normal, whereas FT3 and FT4 levels were elevated.
Negative pituitary MRI and normal TSH response to TRH stimulation excluded thyrotropinoma (TSH-secreting adenoma) in both patients.
Genetic analysis revealed RTH.
In the first patient analysis of the exon 10 (chromosome 3) showed a C insertion in codon 448, that lengthens the polypeptide chain by two aminoacids.
In the second patient there was a heterozygous replacement of C to T in codon 383 resulting to a change from arginine to cytosine.
In both cases further increase in levothyroxine dose reduced TSH levels to slightly above normal.
Conclusion
Elevated FT3 and FT4 levels without suppression of TSH should raise suspicion of RTH.
Pituitary MRI and TRH stimulation test are necessary to exclude thyrotropinoma.
RTH is confirmed by genetic analysis.
In many patients RTH is 'unmasked' after thyroidectomy, because post-thyroidectomy hypothyroidism is difficult to treat
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1733

### Hyper - and hypothyroidism without symptoms - how much can a patient tolerate ?

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Disturbances of thyroid hormones produce clinical symptoms but hormonal level at which this happens vary from patient to patient. In order to determine how much hormonal change patients can tolerate before developing symptoms we retrospectively collected data of 86 hyperthyroid and 48 hypothyroid patients, 120 females and 14 males aged 28–85 and 23–74 yrs respectively. We included only patients who at some time during treatment had abnormal values of TSH and/or thyroid hormones and/or antibodies but had no disease symptoms at all. We found that patients tolerate TSH values from low $<0.005$ to high 60 and even 105 mIU/L without any complaints. On 94 occasions TSH was below and on 72 above normal levels, majority of elevated values up to 30 mIU/L above upper normal limit. Free thyroxine ranged from $<2.0$ to 75.6 pmol/L(n.v 9.8–16.8) with majority (52) of elevated values up to 20 pmol/L above normal; few patients had higher values (1 up to 30, 5 up to 40, 2 up to 50 and 1 up to 80 pmol/L above upper normal limit) and at that time without any symptoms. Asymptomatic patients had free triiodothyronine from 1.4 to 36.8 pmol/L (n.v.4.6–7.8), majority of elevated values (49) up to 15 pmol/L above upper limit.
46 euthyroid patients with positive thyroid antibodies had no symptoms, 3 patients had symptoms but also other problems. We conclude that many patients have no symptoms at all with hormones slightly, moderately or even extremely elevated or decreased. Our patients do not feel positive thyroid antibodies. The cause for this phenomenon probably involves thyroid hormone receptors and/or sympathoadrenergic system.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1734

### The influence of chronic stimulation of thyrotropin on nodular thyroid goiter

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Introduction
Nodular goiter is clinically recognizable restricted structure changes of the thyroid gland. FNA is the first line diagnostic test for an enlarged thyroid gland. TSH is known as a thyroid growth factor, but the pathogenic role of this hormone in thyroid oncogenesis is unclear.
Objective
The aim of this study is to analyze the relationship between elevated serum TSH concentrations and thyroid malignancies in patients with nodular thyroid goiter.
Material and Methods
We retrospectively reviewed the 637 reports of thyroid FNA cytology of samples obtained by puncture of patients with nodular thyroid goiter, at Department of thyroid gland, Department of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia in the period from October 2007. by January 2010. year. We analyzed the relationship between findings of cytopathological diagnostic categories and serum concentrations of TSH. The data were statistically tested using Kruskal Wallis test of differences using the computer program SPSS 12.0 software package.
Results
Of total 637 patients 3.45% (22/637) had a malignant, and 4.87% patients (31/637) cytopathological indeterminate findings. 91.52% (583/637) patients were with benign findings, and only one puncture failed. The average value of TSH in a group of patients with a diagnosis of malignancy was 9.83 $\pm$17.48 mmol/l with a median 3.31 mmol/l. In all three diagnostic categories (benign, malignant and unspecified) most patients are with the normal concentrations of serum TSH, but there is a relatively large proportion of 27.3% (6/22) of patients with malignant cytopathological findings in the group of patients with elevated TSH concentration. Statistically significant differences between diagnostic categories by group TSH values($P=0.017$).
Conclusion
It is concluded that the risk of developing thyroid malignancy increases in patients with elevated serum TSH concentrations. TSH should be used as an additional diagnostic tool in identifying high-risk patients who require further investigation and / or surgical treatment.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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Keywords: nodular goiter, thyrotropin, fine needle aspiration

## P1735

**Ischemic stroke without cardiac arrhythmia in a middle age female with thyrotoxicosis**

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51 y/o female with past medical history of migraine headache, brought to UR because of hypoactivity, generalized weakness, confusion, lack of balance and dysarthria since one day of evolution. No medications. No Habits. Past history: negative for hypertension, cardiac disease or dyslipidemia. Physical examination: normal vital signs and weight. Alert, awake, and oriented only in person. Palpable nontender left thyroid lobe. Bilateral hyperreflexia.
Labs: CBC, CMP, toxicology, urinalysis, ANA screen, RPR, and Anti-TPO: negative. Sed rate: 40 mmh TSH: 0.159 mIU/mL. TSI: negative. Anti-Thyroglobulin Antibody: positive. Anticardiolipin: negative.
CT scan of head: bilateral basal ganglia infarcts.
24 hours RAIU: 0.4%. Thyroid scan: thyroiditis.
Thyroid Ultrasound: small bilateral nodules. LP: negative. EEG: diffuse cortical dysfunction as seen in encephalophathy states. ACEI, statin and asa were started. Echocardiogram and carotid Doppler: negative. She was discharged after moderate clinical improvement.
Brain SPECT: negative.
MRI/MRA: Vasculitis cannot be excluded.
Hyperthyroidism is associated with atrial fibrillation and cardioembolic stroke. In thyrotoxic patients without cardiac arrhythmia, only 7 cases of acute cerebrovascular ischemic disease have been identified. Hyperthyroidism may be associated with cerebrovascular thrombosis (CVT), Moya-moya disease, antiphospholipid syndrome (APS), giant cell arteritis (GCA). The mechanism contributing to the association between hyperthyroidism and stroke are not fully understood. Possible explanations are: inflammatory factors and metabolic changes, leading to increases in carotid artery intima-medial thickening.
CVT is a very uncommon disease associated with several causes and risk factors, such as inherited thrombophilia, oral contraceptives, pregnancy, and puerperium. APS is a combination of arterial or venous thrombosis, recurrent fetal loss, and lupus anticoagulant or anticardiolipin antibodies. GCA is a chronic vasculitis among individuals $>50$ years of age. Moyamoya is a rare cerebrovascular disorder characterized by bilateral stenosis of the internal carotid which has been ruled-out in view of the MRI findings.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1736

**Middle age female with long standing multinodular goiter, cardiac arrhythmia and thyroid dysfunction: the endocrinology and cardiology connection**

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67 y/o old female, with long history of hyperthyroidism, without treatment and hypertension. Two months prior to admission, she received amiodarone 200 mg daily due to cardiac arrhythmia. One week prior to admission, she developed symptoms of Congestive Heart Failure and weight loss. Family history: hyperthyroidism in two sisters. Physical examination: acutely ill, thin female, in moderate respiratory distress. Alert, oriented x 3.BP: 155/119 mmHg HR: 150.Prominent findings: multinodular goiter. Bilateral crackles up to 2/3 lung fields. Irregular rhythm. Bilateral pitting edema. EKG: A-Fib with fast ventricular response. Echocardiogram: Left/Right atrial enlargement. Diuretics, nitrates, morphine, valsartan, anticoagulants and an Amiodarone drip were started. Laboratory tests: Thyroid panel compatible with hyperthyroidism. Positive Anti - TPO. 24 hours urine iodine: 6428.1 ug/L. TSI: negative.
Thyroid sonogram: hypervascular multinodular goiter. Normal 24 hours thyroid uptake. Thyroid scan: multinodular goiter with functional and non functional nodules. After Endocrinology evaluation, Amiodarone drip was discontinued. Patient improved and was discharged on methimazole 30 mg daily, carvedilol, digoxin and warfarin. At OPD, methimazole was switch to PTU due to body rash. FNAB: negative for malignancy. Three months after discharge patient remained clinically and biochemically hyperthyroid.
Amiodarone is an iodinated compound with 37% iodine by weight. Thyroid dysfunction has been reported to affect 2–24% of amiodarone users. Amiodarone-induced thyrotoxicosis (AIT) is 3%. Excess iodine-induced thyroid hormones synthesis is known as type I AIT, usually occurs in patient with underlying thyroid pathology; whereas destruction of thyroid follicles resulting in a thyroiditis is known as type II AIT. Distinguishing one type from the other may be troublesome and is important because it has a major influence on subsequent management. AIT is associated with a 2.7 fold increased risk of major adverse cardiovascular events. AIT is a condition that is difficult to manage, because of the long half-life of amiodarone.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1737

**Body Mass Index and goiter in school children. Are there any relationship?**

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Introduction
Iodine deficiency is a common problem of public health that usually causes thyroid enlargement called goiter. It is estimated that 750 million people worldwide are at risk of Iodine Deficiency Disorders (IDD). IDD can be presented with a wide variety of clinical manifestations, which induce congenital anomalies, cretinism, deaf mutism, psychomotor defects and severe goiter.
About 17 years after the initiation of salt iodization in Iran, this study considered the prevalence of goiter and possible association between BMI and it in school children in Isfahan, Iran.
Methods and materials
In this cross sectional study, 2293 school children aged 6–13 were enrolled by multi-stage random sampling. The exclusion criteria were: a history of exposure to radioactive iodine, thyroid surgery or significant underlying disease such as cardiopulmonary, liver or renal problems based on available medical records and interviews with parents and teachers. Then goiter grading was done by inspection and palpation of it according to recommended criteria of WHO/UNICEF/IC-CIDD.[Grade 0: No palpable or visible goiter; Grade1: thyroid is palpable but not visible when the neck is in the normal position; Grade 2: A swelling in the neck that is clearly visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated.]. Weight and standing height were measured. Height was recorded to the nearest 0.1 cm and weight was recorded to the nearest 100 g. Body Mass Index (BMI) was calculated using the following formula: BMI = weight (Kg)/height (m) 2.
Results
The mean age $\pm$ SD was $9.39 \pm 1.18$ years for girls and $9.47 \pm 1.12$ years for boys and female to male ratio was 1.6. Overall had goiter (33.1%) that 39.5% of them were boy and 60.5% were girl. Goiter prevalence among girls was 32.4% while 33.7% of boys were goitrous ($P=0.518$).
The mean $\pm$ SD of BMI in the goiterous and non-goiterous children were $15.59 \pm 2.31$ and $16.98 \pm 3.35$ respectively($P=0.00$).
Conclusion
Our study showed goiter is still prevalent between Iranian children and BMI was significantly less in goiterous than in nongoiterous children. Therefore there is an association between BMI and goiter. More researches should be done to distinguish this possible connection.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1738

**The concept 'one thyroid specific immune mechanism - a specific thyroid disease'. Pleading for adoption of a new nomenclature for immune thyroid diseases**

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Aim, Objectives
Conceptualizing new data related to type and level of autoantibodies in thyroiditis Hashimoto, Graves-Basedow disease and releated conditions.
Material
###1. Adoption the integrative concepts of immune network (Nobel Prize, 1984) for immune thyroid diseases. 2. Searching on "www.ncbi.nlm.nih.gov/pubmed" for papers with keywords: thyroid autoantibody, thyroiditis (~2500 papers). 4. New (auto)-antibodies (and antigens/epitopes) found in immune thyroid diseases.
Method
###1. Thyroid diseases pathogenesis is mostly immune. 2. Patients have autoantibodies and the disease is called autoimmune. 3. If there is a pathogenetic antibody, then there must be a pathogenic antigen. 4. Asserting a/one specific immune pathogenic mechanism, there cannot be overlapped disease because there could not be overlapped/more pathogenic mechanisms. 5. Understanding pathogenetic steps means: only one immune mechanism could act. 6. Other antibodies presence (along with the specific one) in patient should be related to upstream and downstream regulation mechanisms of the immune network. 7. Immune thyroid disease name should be linked on a sole mechanism.
Results
1. Medical literature specify 4 clinical situations: Hashimoto thyroiditis, idiopathic mixedema, Graves-Basedow disease, Riedel thyroiditis. 2. Criteria for defining these entities did not allowed overlapping especially for specific auto-antibodies. 3. Thus a disease should be linked to a single immune mechanism and a single auto-antibody. 4. If there are many antibodies in a clinical situation, then there is more than one diseases. 5. Association of many thyroid immune disease in a patient is not a rare situation.
Conclusions
###1. Hashimoto thyroiditis is only ATPO related. 2. Graves-Basedow disease is only TRAB related. 3. Riedel thyroiditis is only IgG4 non-ATPO/ATG/TRAB related. 4. Thyroiditis with only ATG should be name specifically. 5. Idiopathic mixedema is that immune destruction with no yet specific antibody/antigen identify complex. 6. Pathogenesis linked on new immune complexes pendrine/anti-pendrine, NIS/anti-NIS could lead to define new clinical entities.
Declaration of interest
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## P1739

**The metabolic syndrome in thyroid disorders**

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Introduction
The objective of this report is to determine the presence of the metabolic syndrome and its components in people with thyroid disorders.
Methods
112 subjects with a history of thyroid disorders were consecutively enrolled for the study. Clinical data was obtained by interviewing the patients and referring to their Case folders and their prescriptions. The subjects were categorized into three; thyrotoxic, euthyroid, hypothyroid based on clinical parameters and or thyroid function tests. The study subjects were weighed and their anthropometric indices were documented. The laboratory parameters that were analysed for included total cholesterol, high density and low density cholesterol and triglyceride. Statistical analysis included usage of Student's t test, One way Anova test and chi square.
Results
The study subjects were aged between 14–76 years with a mean age of 44.5 (14) years and a female:male ratio of 97:15. The mean age and anthropometric indices were comparable in subjects with thyrotoxicosis, hypothyroidism and euthyroidism. The overall prevalence of the metabolic syndrome was 28% and the frequency of occurrence of the metabolic syndrome in subjects with thyrotoxicosis, hypothyroidism and euthyroidism were 24%, 40% and 42% respectively. The commonest occurring metabolic syndrome defining criterion was dysglycaemia and hypertension and elevated triglyceride were the least documented of the criteria.
Conclusion
The metabolic syndrome occur in one in every four persons with thyroid disorders and as such, routine screening for this cardiovascular risk factor may be of benefit in this group of people.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1740

**The influence of cure of subclinical hyperthyroidism on the level of serum triglycerides and cholesterol fractions**

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Introduce
Subclinical hyperthyroidism (SH) affects 0.2 - 11.8% of world human population and is associated with important cardiovascular risk factors. It remains controversial whether or not to treat patients with low serum TSH levels. Between unwanted potential outcomes of treatment of SH there is an elevation of the level of serum triglycerides and cholesterol which may be responsible for higher risk of cardiac death.
Aim
To estimate an influence of cure of SH on the level of serum triglycerides and cholesterol fractions: total (Tchol), low density (LDL) and high density (HDL).
Method
44 patients (37 women, 7 men) aged mean 45, with 13 month history of only autonomous endogenous SH (mean TSH = 0.16 mIU/l) were examined twice: before and mean 6 months after TSH normalization (obtained mean TSH = 1.32 mIU/l) with radioiodine treatment (mean 12.1 mCi). The average time between examinations was one year.
Results
The cure of SH caused statistically insignificant increase of serum triglycerides from 95.2 to 102.6 mg/dl ($P = 0.273$) and Tchol from 204.6 to 211.1 mg/dl ($P = 0.11$) but significant increase of LDL from 114.3 to 121.9 mg/dl ($P = 0.018$) and HDL from 64.02 to 66.25 mg/dl ($P = 0.041$).
Conclusions
1 Cure of autonomous subclinical hyperthyroidism insignificantly increases levels of serum triglycerides and total cholesterol. 2. Potential deleterious effect of statistically significant increase of serum LDL could be abolished by significant increase of serum HDL. 3. Further investigations are needed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1741

**Increase of Serum Adiponectin And Stable Concentrations of Matrix Metalloproteinases Confirm Safety of Radioiodine Treatment of Thyrotoxicosis**

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Background
Matrix metalloproteinases (MMPs), together with their tissue inhibitors (TIMPs), remodel extracellular matrix under physiological and pathological conditions and are implicated in pathogenesis of cardiovascular diseases, cancer and in chronic inflammation. We have endeavoured to assess whether concentrations of MMPs, TIMPs, proinflammtory thrombospondin and anti-inflammatory adiponectin are altered by administration of radioiodine during treatment of thyrotoxicosis.
Material & Methods
We measured serum concentrations of MMP-2, MMP-9, TIMP-1, TIMP-2, thrombospondin and adiponectin as well as lipids, TSH, fT4, and fT3 before radioiodine administration (RIA), visit 1 (V1), seven days post RIA, visit 2 (V2), and two-three months post RIA, visit 3 (V3) in 20 subjects (2 males), treated for thyrotoxicosis (age (mean ± SD) 52.3 ± 12.4 years).
Results
RIA did not cause any acute change in serum MMP-2, MMP-9, TIMP-1 and TIMP-2, thrombospondin or adiponectin (V1 versus V2). Subsequently, however,

there was a significant increase in serum adiponectin (from 15201 ±8860 ng/mL(V1) to 19373±8657 ng/mL (at V3), $P<0.05$), and TIMP-2 at visit 3 (from 129±45 ng/mL (V1) to 149±38 ng/mL (V3), $P<0.01$). There was no significant change MMP-2, MMP-9, TIMP-1 and thrombospondin between V1 and V3. There was a decrease in fT4 and fT3 from 24.4±15.4 pmol/L (V1) to 14.7±10.6 pmol/L (V3), and from 10.0±5.65 (V1) to 6.1±4.8 pmol/L (V2), $P<0.01$, for fT4 and fT3, respectively. This was accompanied by an increase in total and LDL cholesterol between V1 and V3 ($P<0.01$), however, without significant change in HDL cholesterol ($P=0.23$).

Conclusions

Radioiodine treatment of thyrotoxicosis appears vey safe, as administration of radioiodine does not alter serum MMP-2, MMP-9, TIMP-1 or thrombospondin concentrations either acutely or after about three months of observation. An increase in serum adiponectin might reflect favourable effects of radioiodine administration on cardiovascular risk factors, while an increase in TIMP-2 (principal MMP-2 inhibitor) might lead to a decrease in free MMP-2 concentrations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1742

**Hyperthyroidism and Pregnancy**

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Introduction

Hyperthyroidism affects 2 to 3% of pregnant women. The prevalence of clinical hyperthyroidism during pregnancy is variously appreciated. Hyperthyroidism prior to pregnancy is probably more common than in connection with the gestationnal state as gestational hyperthyroidism usually blocks fertility. Our objectives are the prevalence and the causes of hyperthyroidism in pregnant women in the 1st quarter.

Description of methods

Prospective study, made in Oran area, on 270 pregnant women in 1st quarter. Women who smoke (active smoking confessed) and those followed for thyroid disease or taking medications that interfere with the thyroid gland were excluded.
-Study protocol: clinical examination, thyroid stimulating hormone, free thyroxine, free triiodothyronine, thyroid antibodies (anti-TPO, anti-thyroglobulin and TSI) and cervical ultrasonography.
- Statistics tests: collection of data on EPI INFO 5.1, bivariate analysis (the chi-squared test, the Pearson chi-squared test, the Yates corrected chi-squared test, the Wilcoxon chi-squared test (Log rank sum) or Mann-Whitney, the variance analysis method (ANOVA), the r correlation test, significance level $P<0.05$, multivariate analysis (SPSS: 10 version and MedCalc).

Results

−2/270 Pregnant women in 1st quarter (0.7%) had hyperthyroidism.
-The etiology of thyrotoxicosis was related to gestational transient hyperthyroidism.
- No case of Graves' disease was found.

Conclusion

Hyperthyroidism is a rare disorder during pregnancy but the consequences are serious for both mother and offspring.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1743

**Histopathological results of patients that were undergone thyroid-ectomy due to results of repeated non-diagnostic cytology**

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Objective

In our clinic we aimed to assess ultrasonographic and histopathologic data of thyroid nodules of patients with nodular thyroid disease reported their repeated cytologic results as non-diagnostic.

Methods

Seventy-five patients with nodular thyroid disease whom their TFNAB results were reported 2–3 times non-diagnostically (due to large nodule, ultrasonographically suspicious nodule, high elastosonographic score or strain index, family history of thyroid cancer) have enrolled in this study and their data were assessed. Demographic and ultrasonographic features of these patients and of who their cytologic results were reported malignant features of tumor were also assessed.

Results

40(53.3%) of patients had euthyroid multinodulary goiter (MNG), 8(10.7%) euthyroid nodulary goiter(NG), 17(22.7%) toxic MNG, 6(8%) toxic diffuse MNG, 1(1.3%) toxic diffuse NG, 1(1.3%) relapsed operated MNG and 2(2.7%) relapsed operated NG.. 45 of patients (60%) were euthyroid, 25(33.3%) hyperthyroid and 5(6.7%) hypothyroid. In 41 patients(54.7%) nodul(es) was/were localized at right lobe, in 34(45.3%) at left lobe. Ultrasonographically, microcalcification was found in 30 nodules(40.0%) whereas macrocalcification was found in 20 nodules. 45 nodules(60%) had irregular borders, 28 nodules (37.3%) had hypoechoic halo. Peripheral vascularity was found in 17(22.7%) nodules, peripheral macrocalcification was found in 1 nodul(1.3%). After surgery 57 nodules(76%) were reported benign, 18(24%) malignant. Of malignant nodules 17 nodules (94.4%) had papillary carcinoma whereas 1 nodul had follicular carcinoma. Mean tumour diameter was 0.77±0.63 (range 0.1–2.2) cm. In one patient(5.6%) vascular invasion, in 5 (27.8%) capsular invasion, in 1(5.6%) extracapsular extension and in 6 (33.3%) multicentricity was found.

Conclusion

In our study we found the rate malignancy 24% as high for thyroid nodules which were performed FNA biopsy and reported repeated non-diagnostic cytology results. Therefore, we conclude that when treatment is planned for the patients that have non-diagnostic cytology possibility of high rate of malignancy as we found in this study may be considered.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1744

**Chlorpyrifos induced alterations in serum electrolytes (calcium, phosphate and magnesium) and endocrine glands (calcitonin cells and parathyroid gland) of Wistar rat**

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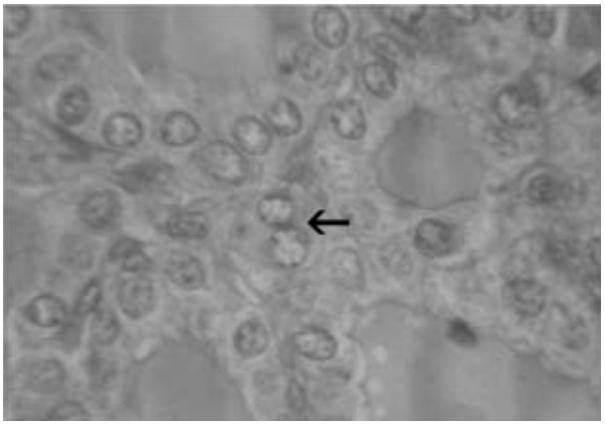
Wistar rats (male) were divided into three groups – group A (GA) served as control, group B (GB) were daily administered orally chlorpyrifos at a dose of 5 mg/kg b wt. and animals in group C (GC) received daily an oral administration of chlorpyrifos at a dose of 10 mg/kg b wt. Rats were sacrificed on 1st, 2nd, 4th, 6th, and 8th week after initiation of the experiment. Changes in serum electrolytes (calcium, phosphate and magnesium) and endocrine glands (calcitonin cells and parathyroid glands) were determined. In chlorpyrifos exposed rats hypocalcemia, hypophosphatemia and hypomagnesemia were recorded. At later intervals an increased levels of serum calcium and phosphate were observed. The parathyroid



glands and calcitonin cells exhibited increased activity which is evident by increased nuclear volume of these cells. This is the first report describing effects of toxicant on serum magnesium level of mammals.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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Keywords: Calcitonin cell, cadmium, phosphate, magnesium, parathyroid

## P1745

### Utility of sonographic features and citology obtained by ultrasound-guided fine needle aspiration in preoperative management of thyroid nodules

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Introduction
Nodular thyroid disease is common in the general population, but only 5% of thyroid nodules are carcinomas. The diagnostic challenge is to identify those nodules that are malignant neoplasias. Some sonographic features of nodules have been correlated with the risk of malignancy, but at present, thyroid cytology obtained by ultrasound-guided FNA is probably the best tool in the preoperative management of nodular disease.
Design
We designed a prospective study that included all patients referred to our department for thyroid nodular disease, with at least one ultrasound-guided FNA. Our aims were to determine the combination of ultrasound variables that have the capacity to discriminate malignancy, and correlate the sonographic and cytologic features with histopathologic analysis after surgical excision.
Results
We present preliminary analysis after the first year of study. A total of 552 nodules were aspirated in 275 patients with ultrasound-guided FNA. The samples were representative in 82.7%. In 45.80% (243) the dominant nodule was located in the left thyroid lobe, 76% of nodules were $\geq$ 1 cm, 74% were solid, 91.32% had regular margins and 76% well defined, 45.47% had no perinodular halo, 67.7% had no calcifications, 47.5% were isoecogenic and 75.10% had only perinodular vascularization. Cytology suggestive of malignancy was significantly correlated with hypoecoechogenicity, and a larger nodular size. 29 patients were referred for surgery, 10 underwent surgery, (of which 6 were thyroid carcinomas), 21 are awaiting thyroid scintigraphy for surgical decision, and the remaining patients are on follow-up.
Conclusions
The hypoechogenicity and increased nodule size were significantly correlated with cytology suggestive of malignancy; therefore, this may be useful in making surgical decisions. These are preliminary results and we need to complete the study
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1746

### Thyroid disorders and lipid abnormalities: An association between primary hypothyroidism and lipid profile pattern

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Introduction
The correlation between lipid profile changes & overt hypothyroidism is well established. Overt hypothyroidism is associated with increased risk for cardiovascular disease (CVD) as indicated by hypertension, elevated lipids levels, hypercholesterolemia, and particularly increased low-density lipoprotein cholesterol (LDL-C) levels. However, lipid profile alterations in primary and subclinical hypothyroidism are controversial. There are hardly any studies from this region that intensify the association of lipids with thyroid hormones. Hence this study aims to determine the pattern of lipid profile abnormalities in patients with primary and subclinical hypothyroidism.
Methods
Forty patients (6 of primary hypothyroidism and 34 of subclinical hypothyroidism), diagnosed in the Department of Clinical Biochemistry (ELISA, Lab Life ELISA 2007, RFCL, India) were subjected for lipid profile testing (Erba Chem 5 Plus, Germany) from August to October 2010 at Dhulikhel Hospital-Kathmandu University Hospital, Nepal. The results of this prospective study were statistically analyzed by SPSS 14.0.
Results
Majority of the patients were female (90%) of the age 11 to 66 years (38.625 $\pm$13.93). Of all 40 patients enrolled, 35% (14) had mean serum cholesterol $>$200 mg/dl, 30% (12) had LDL-C $>$150 mg/dl, 17.5% (7) had triacylglycerol (TAG) $>$150 mg% and 42.5% (17) had HDL cholesterol (HDL-C) $<$40 mg/dl. The evidences for primary and subclinical hypothyroidism showed a strong correlation between elevated TSH and total cholesterol, HDL-C, TAG ($r=0.388$, $P<0.05$). However, there is a weak correlation between TSH and LDL-C ($r=0.201$, $P<0.214$).
Conclusion
Total cholesterol, TAG and HDL-C showed statistically significant higher values. This association of two separate states of disorders might be helpful in the treatment and management of hypothyroidism.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1747

### Clinical experience in a high-resolution thyroid nodule clinic

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Background and objectives
The finding of a thyroid nodule is a very common occurrence in clinical practice. Management of thyroid nodules requires a multidisciplinary approach that may be eased by a high-resolution thyroid nodule clinic. We report our clinical experience and outcomes in a high-resolution thyroid nodule clinic.
Patients and methods
All patients referred to Virgen de la Victoria Hospital (Málaga) from 2005 to 2007 were evaluated following thyroid nodule guidelines. Statistical analysis was performed using SPSS software.
Results
In the study period, 658 patients (mean age 48.6 years, 89.8% females) were seen at the thyroid nodule clinic. Thyroid nodules were discovered in 85.5% of patients. Mean nodule diameter was 1.96 cm. Of these nodules, 74.2% were solid, 55.8% hypoechogenic, and 4.7% showed microcalcifications. Fine needle aspiration (FNA) was performed in 475 patients (76.5% of all cytological samples were benign, 19.1% had suspected malignancy, 2.1% were malignant, and 2.3% inadequate). Referral for surgery was required in 23.3% of patients (mainly because of nodule size or suspected malignancy). Malignancy was confirmed in 24.9% of nodules. Sensitivity and specificity of cytology (considering biopsy as gold standard) were 81.8% and 94.7% respectively.
Conclusions
Implementation of a high-resolution thyroid nodule clinic decreases delay in diagnosis and optimizes available resources, thus providing for satisfactory clinical outcomes
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1748

**Hypothyroxinemia and pregnancy**

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Introduction
The maternal hypothyroxinemia would be an adaptive process of the thyroid gland to iodine deficiency, regardless of the TSH. Our objectives are the prevalence and pathophysiological approach of hypothyroxinemia in pregnant women in the 1st quarter.
Description of methods
Labor-looking statements, made in Oran area, on 270 pregnant women in 1st quarter. Women who smoke (active smoking confessed) and those followed for thyroid disease or taking medications that interfere with the thyroid gland were excluded.
Study protocol: clinical examination, TSH, FT4, FT3, anti-TPO, anti-thyroglobulin, TSI and urinary iodine. Statistics tests: collection of data on EPI INFO 5.1, bivariate analysis (the chi-squared test, the Pearson chi-squared test, the Yates corrected chi-squared test, the Wilcoxon chi-squared test (Log rank sum) or Mann-Whitney, the variance analysis method (ANOVA), the r correlation test, significance level $P<0.05$, multivariate analysis (SPSS: 10 version and MedCalc).
Results
-22/270 pregnant women in 1st quarter (8.1%) had a hypothyroxinemia which 21/22 had benefited from a determination of iodide.
−06/21 cases (28.6%) had an inadequate iodine intake (urinary iodine $<150$ µg/l).
-09/21 cases (42.9%) had correct urinary iodine (between 150 and 250 µg/l).
−06/21 cases (28.6%) had a more than adequate iodine intake (urinary iodine $\geq 250$ µg/l).
Conclusion
Although the pathophysiological mechanisms of hypothyroxinemia during pregnancy are not yet well understood, the knowledge of the disease is nevertheless appropriate given its harmful consequences.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1749

**A case of subacute thyroiditis in a patient on Adalimumab for treatment of refractory palmo-plantar pustular psoriasis**

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We describe a case of subacute thyroiditis in a psoriatic patient treated with Adalimumab, with a very good clinical evolution with nonsteroidal anti-inflamatory medication.
Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine produced by a variety of cells, comprehending T lymphocytes. TNF-α mediates its effects through two receptors, known as p55 (TNF-R1), and p75 (TNF-R2). TNF-α plays a primary role in both the induction and the maintenance of inflammation in autoimmune reactions: as soon as the inflammation begins, TNF-α acts to activate T-cells and macrophages, up-regulating the expression of endothelial adhesion molecules, and pro-inflammatory cytokines.
Adalimumab is a TNF-alfa human monoclonal antibody blocking agent used in severe psoriasis, with an increased risk of infections. Adalimumab tightly binds to human TNF-α, which is a naturally occurring cytokine involved in the acute phase of inflammatory immune responses.
As the use of biologic drugs becomes more widespread over years,clinicians from a variety of disciplines are increasingly likely to encounter cutaneous side effects of this treatment.Liason between dermatologists and, in this case, endocrinologists, will help to determine the prevalence of these reactions and to provide insights into the very complex mecanisms of both diseases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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# Thyroid cancer

## P1750

**Indoleamine 2,3-dioxygenase 1 (IDO1) and thyroid carcinoma: Ret/PTC appears as a strong genetic determinant for IDO1 expression**

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Indoleamine 2,3-dioxygenase 1 (IDO1) is a single chain oxidoreductase that catalyzes tryptophan degradation to kynurenine. In cancer, it appears to exert an immunosuppressive function as part of an acquired mechanism of immune escape. In view of this notion, IDO1 has started to be considered a novel target in cancer therapy. Objective of this study was to evaluate IDO1 expression in papillary thyroid carcinoma (PTC) and to correlate its expression with genetic determinants of thyroid carcinogenesis. IDO1 expression was evaluated by QPCR in 105 PTCs and by immunohistochemistry in a subgroup of 55. IDO1 expression was also analyzed in 5 human thyroid carcinoma-derived cell lines and in PCCL3 cells characterized either by the doxycycline-inducible or stable expression of BRAFV600E and ret/PTC3. IDO1 expression resulted significantly higher in PTC than in normal thyroid. In basal growing conditions, IDO1 was overexpressed in FTC133 and B-cpap cells, but after stimulation with γ-interferon, all analyzed cell lines (including also TPC-1, 8505c and C643) showed up-regulation of the enzyme. Correlation of IDO1 expression with genetic background in PTC demonstrated a close to statistical significant association between higher IDO1 mRNA expression and BRAFWT status, which was lost at immunohistochemistry. However, while BRAFV600E-expressing PCCL3 cells did not show IDO1 overexpression, ret/PTC3-expressing ones demonstrated an increase of the enzyme. Ret/PTC3-induced activation of STAT1-IRF1 pathway appeared as the signaling cascade involved in IDO1 overexpression in the studied cell line. This study shows for the first time that papillary thyroid carcinoma overexpresses the immunomodulating protein IDO1 and that this event is correlated either to genetic determinants as ret/PTC or to the responsiveness of genetic programs to stimuli coming from the tumor microenvironment. Because the metabolite of IDO1 enzymatic reaction, kynurenine, has been shown to promote tumor cell growth and motility and to inhibit immune cell proliferation and function, these data put forward the possible importance of testing novel targeted therapies against IDO1 for the treatment of papillary thyroid carcinoma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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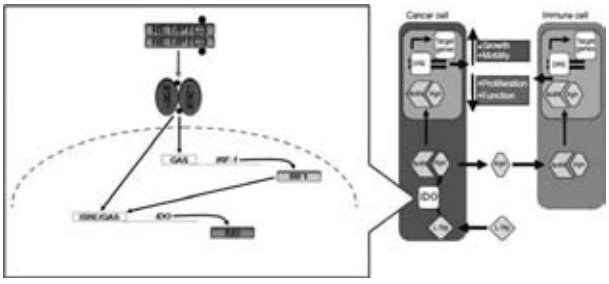


## P1751

**Androgen receptor expression in human thyroid cancer tissues: a potential mechanism underlying the gender bias in the incidence of thyroid cancers**

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Gender bias in the incidence of thyroid cancer is well known, however, the underlying mechanism is largely unknown. The current study determines variations in the molecular characteristics of thyroid cancers between men and women. Normal and cancerous thyroid tissues were collected from a total of 125 men and women who underwent surgical removal of the thyroid gland. Elevated

levels of testosterone in serum and thyroid cancer tissues were observed in women while it decreased in men compared to respective control groups; whereas, ligand binding activity was increased in men and decreased in women. Androgen receptor (AR) mRNA expression increased in a majority of men while it decreased in a majority of women except women with follicular thyroid carcinoma (FTC). In thyroid cancers of women, Pearson's correlation analysis showed a positive correlation of AR mRNA with AR protein, CBP and Sp1, whereas AR mRNA showed a negative correlation with p53. In case of men, AR mRNA showed a positive correlation with AR and cyclin D1 protein in papillary thyroid carcinoma (PTC); and CBP and Sp1 in follicular thyroid adenoma (FTA), whereas AR mRNA showed a positive correlation with p53. Our study identified for the first time that AR is posttranscriptionally regulated by miR-124a. Further, our *in vitro* studies with a PTC cell line (NPA-87-1) showed miR-124a as the potent inhibitor of AR that inhibited NPA cell proliferation even in the presence of testosterone. Thus, the current study suggests that: i) the varying pattern of testosterone level and AR status in thyroid tissues of men and women may predispose to the gender-specific incidence of thyroid tumors ii) miR-124a plays a significant role in determining the AR gene expression pattern and thus, androgen mediated thyroid tumor growth.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1752

### cAMP analogs as potential therapeutic agents for poorly differentiated thyroid cancer

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Thyroid cancer is the most common endocrine malignancy characterized by a good prognosis. However, the subgroup of poorly differentiated thyroid carcinomas (PDTCs) includes neoplasia highly aggressive and scarcely responsive to currently available therapies.

Site-selective cAMP analogs are able to inhibit the growth of poorly differentiated solid tumors and may represent valuable candidates for the therapy of PDTCs.

We evaluated the effects of a PKA I-selective cAMP analogs association and another analog (8-Cl-cAMP) on a panel of 6 cell lines from papillary, follicular, poorly differentiated and anaplastic thyroid cancer. The targets of our pharmacological study were modifications of cell-cycle, apoptosis and principal signaling pathways involved in cell proliferation.

8-Cl-cAMP had a significant anti-proliferative effect on all the cell lines tested, with EC50 values between 1.0 and 16.8 μM; PKA I-selective analogs showed a preferential cytostatic action on PDTCs (8505c, HTC-C3 and SW579 cell lines) with an EC50 between 37.2 and 44.5 μM.

In particular, 8-Cl-cAMP was able:

1) to influence cell cycle progression as evidenced by a reduction in the G0/G1 fraction and an accumulation in S phase;

2) to induce apoptosis, as determined by FACS and Annexin V/propidium iodide-staining.

Otherwise, PKA I-selective analogs caused a slight increase in the G0/G1 cell fraction without apoptosis induction. Furthermore, the analysis of intracellular pathways showed that both treatments were able to reduce Akt activation, one of the principal regulator of cell proliferation. Moreover PKA I-selective analogs significantly reduced ERK1/2 activation in SW579 cell line.

In conclusion, 8-Cl-cAMP may represent a powerful broad-spectrum, pro-apoptotic anticancer drug, while PKA I-selective analogs seem to preferentially inhibit undifferentiated cancer cell growth. These studies prompt further *in vivo* experimentation to test the efficacy and safety of these agents.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1753

### The cross-talk between estrogen receptor and peroxisome proliferator-activated receptor gamma in thyroid cancer cells

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Both of peroxisome proliferator-activated receptor gamma (PPARγ) and estrogen receptor (ER) contribute to the development of thyroid cancer. Previously, the activation of ERα was found to promote the proliferation of thyroid cancer cells, whereas activation of either ERβ or PPARγ resulted in apoptosis of thyroid cancer cells. Furthermore, a transactivation between ER and PPARγ was reported in estrogen-responsive malignancy, such as breast cancer. However, the relationship between ER and PPARγ in thyroid cancer remains unknown. In this study, we used thyroid papillary carcinoma cells (K1) and anaplastic carcinoma cells (FRO) to examine the interaction between ER and PPARγ. We found that PPARγ protein expression and activity was reduced by over-expression of either ERα or ERβ. PPARγ agonist rosiglitazone (RTZ) could overcome the effects of ERα or ERβ over-expression on PPARγ. The application of RTZ also reduced the expression of ERα and ERβ proteins. Furthermore, knockdown of PPARγ increased ERα and ERβ expression, as well as ER activity. The results of MTT and wound healing assays showed that increment of ERα and ERβ had opposite effects on cell proliferation and migration. While the enhancement of PPARγ by RTZ lessened the proliferative effect of ERα but raised the inhibitory impact of ERβ, especially in K1 cells. Finally, the interaction between ER and PPARγ altered levels of cytoplasmic fractions of apoptosis-inducing factor (AIF), cytochrome *c* (cyto *c*), caspase 3 and mitochondrial Bax, indicating involvement of the non-genomic apoptosis pathway. Collectively, these findings provide evidence of cross-talk between ER and PPARγ and a cooperatively inhibitory function of ERβ and PPARγ in thyroid cell proliferation and migration. These findings support the cross-talk between ER and PPARγ contributes to thyroid carcinogenesis and they may provide some clues for the development of novel therapeutic strategy against thyroid cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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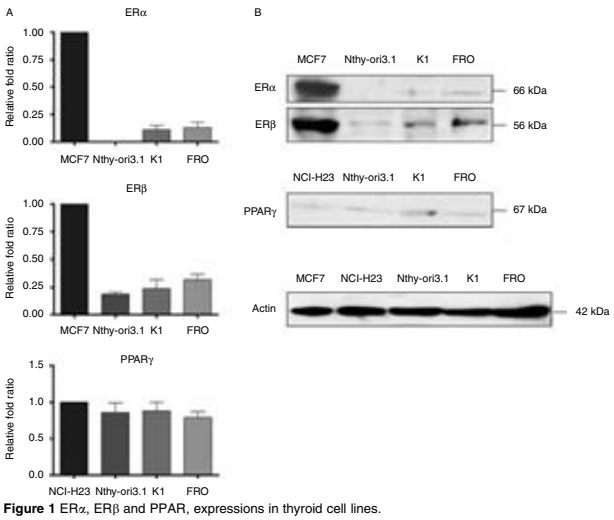


**Figure 1** ERα, ERβ and PPAR, expressions in thyroid cell lines.

## P1754

### Thyroid autoimmunity and risk of malignancy in thyroid nodules submitted to fine-needle aspiration cytology

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Background

It is a controversial issue whether the risk of cancer for a thyroid nodule is different in patients with Hashimoto's thyroiditis (HT) and in patients without

thyroid autoimmunity. Some studies have reported an increased risk of malignancy in thyroid nodules associated with HT while only limited data based on fine-needle aspiration cytology (FNAC) are available.
Methods
Between May 2005 and April 2011, 3279 ultrasonography-guided FNACs were performed on 2255 patients. In all patients serum $FT_4$, $FT_3$, TSH, CT, anti-thyroglobulin (TgAb) and anti-thyroperoxidase (TPOAb) antibody were determined.
Results
Patients with suspicious nodules had lower age ($44.00 \pm 16.24$ vs $55.56 \pm 13.33$ years; $P < 0.001$) and smaller maximum lesion diameter ($13.23 \pm 7.2$ vs $15.69 \pm 7.93$ mm; $P = 0.004$). Patients with TgAb positivity had suspicious nodules more frequently than patients without TgAb (8.5 vs 5.1%; $P = 0.03$), independently from TSH levels. No significant difference was recorded between benign and suspicious nodules in gender ratio, in the rate of positive TPOAb or in the rate of both autoantibody positivity. Risk factors for thyroid cancer suspicious cytology were: younger age (OR 0.94; $P < 0.001$), smaller maximum diameter (OR 0.95; $P = 0.004$), single lesion (OR 1.99; $P = 0.007$), microcalcifications (OR 3.80; $P < 0.001$), and TgAb positivity (OR 1.73; $P = 0.04$). Mixed content of the lesion resulted as a protective factor (OR 0.30; $P < 0.001$). All these factors being included into a multivariate logistic regression analysis model, only age, mixed content and microcalcification confirmed significance. By computing FNAC report classes separately, no significant difference results between TPOAb positive or negative groups and between TgAb positive or negative groups.
Conclusions
Thyroid nodules in patients with HT are not more frequently cancerous than those in patients without thyroiditis. Positive TgAb is – however - an independent predictor for suspicious cytology in thyroid nodules, independently from TSH levels.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1755

### The TRHR gene is associated to hypothalamo-pituitary sensitivity to levothyroxine in thyroidectomized patients

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Background
Patients thyroidectomized for thyroid cancer need variable doses of levothyroxine ($LT_4$) to obtain TSH suppression. A predetermined thyroid function set-point for each individual has been hypothesized, suggesting a genetic influence in the regulation of pituitary-thyroid axis. We hypothesized of the TRHR gene could be associated with a different hypothalamo-pituitary sensitivity to the negative feedback of the thyroid hormones.
Methods
We performed a case–control association study, enrolling 107 thyroidectomized patients, in follow-up for differentiated thyroid cancer, and 99 volunteer controls. Patients were evaluated first when TSH levels were suppressed ($< 0.1$ mIU/l), by the lowest effective $LT_4$ dose, and then when TSH was subsuppressed ($0.1 < \text{TSH} < 0.5$ mIU/l). We selected two SNPs of TRHR gene, rs3134105 and rs3110040, identified as informative markers, using the online database 'HapMap'. We performed a frequency analysis of the mapped SNPs, followed by a linkage analysis using the HaploView software. Genotyping was performed using the High Resolution Melting technology.
Results
The selected SNPs were in linkage disequilibrium. A significant difference between the three possible genotypes for rs3134105 was found for $fT_4$/TSH ratio ($P = 0.03$). Moreover, despite similar serum concentrations of fT3 and fT4 obtained by similar levothyroxine doses, carriers of at least one A allele of rs3134105 had significantly lower serum TSH levels ($P = 0.04$) as well as higher fT3/TSH ($P = 0.05$) and fT4/TSH ratios ($P = 0.02$).
Conclusions
We demonstrated an association between TSH and discrete alleles of the TRHR gene identified by the markers SNPs rs3134105 and rs3110040 in totally thyroidectomized patients with diagnosis of thyroid cancer under subsuppressive $LT_4$ therapy. The TRHR gene is a determinant of hypothalamo-pituitary sensitivity to levothyroxine in such patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1756

### Role of cAMP analogs in the therapy of medullary thyroid cancer

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Medullary thyroid carcinoma (MTC) is a neuroendocrine tumor highly resistant to chemo-and radiotherapy. Since several reports showed that cAMP has an antiproliferative effect on different types of solid tumors both *in vitro* and *in vivo*, we evaluated the potential anti-neoplastic activity of cAMP analogues (8-Cl-cAMP and the equimolar combination of 8-PIP-cAMP and 8-HA-cAMP) in two MTC cell lines (TT and MZ-CRC-1 that harbor C634W and M918T RET mutations, respectively).
Both 8-Cl-cAMP and 8-PIP-cAMP/8-HA-cAMP significantly inhibited MTC cells in a dose-dependent manner, evaluated by MTT proliferation assay, after 6 days of treatment. Interestingly, the antiproliferative effects for both compounds were more prominent in TT (8-Cl-cAMP: IC50 = 4.4 μM, maximal inhibition = −75%; 8-PIP-cAMP/8-HA-cAMP: IC50 = 9.7 μM, maximal inhibition = −70%) than in MZ-CRC-1 cells (8-Cl-cAMP: IC50 = 8.8 μM, maximal inhibition = −47%; 8-PIP-cAMP/8-HA-cAMP: IC50 = 12.8 μM, maximal inhibition = −63%).
After 6 days of incubation, 8-Cl-cAMP (25 μM in TT and 50 μM in MZCRC-1) significantly decreased the population of TT and MZCRC-1 cells in S phase, as suggestive for a delay in G0/G1-S phase transit, while there was no significant effect on the cell cycle during incubation with 8-PIP-cAMP/8-HA-cAMP in both cell lines. In addition, 8-Cl-cAMP induced a potent stimulation of apoptosis in TT and MZCRC-1 cells, as determined by Annexin V/propidium iodide staining and flow-cytometric analysis. While, a moderate increase of apoptotic MTC cells was observed during incubation with 8-PIP-cAMP/8-HA-cAMP.
These studies uncover a novel potential role for cAMP analogs in the treatment of MTC, thus prompting further *in vivo* experimentation on the efficacy and safety of these drugs.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1757

### Optimal prophylactic and definitive therapy for bicalutamide induced gynaecomastia: results of meta-analysis

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Objective
Bicalutamide is approved as an adjuvant to primary treatments (radical prostatectomy or radiotherapy) or as monotherapy in men with locally advanced, nonmetastatic prostate cancer (PCa); however, this treatment induces gynaecomastia in most of patients, which results in treatment withdrawal. Optimal therapy for these breast events is not known so far. We conducted a meta-analysis to assess the efficacy of different treatment options for bicalutamide induced gynaecomastia.
Patients and methods
The Medline, CANCERLIT, Cochrane library database and Google search engine were searched to identify prospective and retrospective controlled studies published in English from January 2000 to December 2010 comparing prophylactic or curative treatment options with control group (no treatment) for PCa patients who developed bicalutamide induced gynaecomastia. Further, radiotherapy induced cardiotoxicity was evaluated.

Results
Nine controlled trials with a total patient population of 1573 were identified. Pooled results from prophylactic trials showed a significant reduction of gynaecomastia in PCa patients treated with prophylactic tamoxifen 20 mg daily dose (odds ratio 0.06 95% CI 0.05–0.09 ($P$ 0.09)) and pooled results from treatment trials showed significant response in gynaecomastia with definitive radiotherapy (odds ratio 0.06 95% CI 0.01–0.24 ($P<0.0001$)). Aromatase inhibitors and weekly tamoxifen were not found effective as prophylactic and curative options. For the radiotherapy, skin to heart distance (SHD) was found important risk factor cardiotoxicity ($P$ 0.006). The resultant funnel plot of meta-analysis showed significant heterogeneity (Egger test $P<0.00001$) due to low sample size.
Conclusion
Our meta-analysis suggests use of prophylactic tamoxifen 20mg daily as first line preventive measure for bicalutamide induced gynaecomastia and radiotherapy as first line treatment option shall be considered for patients who are not candidates for tamoxifen. The aromatase inhibitors and weekly tamoxifen are not recommended.
Keywords
Meta-analysis, bicalutamide induced gynaecomastia, prostate cancer.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1758

### BRAFV600E mutation is an indipendent predictive prognostic factor for persistent/recurrent disease in low risk differentiated thyroid cancer patients: a 5 year follow up study

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BRAFV600E mutation is the most frequent genetic alteration (29–83%) of papillary thyroid carcinoma (PTC). Many authors have demonstrated that the presence of the mutation is associated with a more advanced tumor stage at diagnosis and a worse outcome but anyone assessed if BRAFV600E mutation could be useful prognostic marker in low risk PTC patients (T1-T2N0M0, 7th TNM classification).
Aim of this study was to evaluate if the presence of BRAFV600E mutation in the primary tumor could be a predictor of persistent/recurrent disease in this subgroup of patients.
We retrospectively analyzed the clinicopathological features of 431 consecutive PTC patients treated with total/near total thyroidectomy and iodine-131 (when appropriate) and we selected 319 low risk PTC patients.
Genomic DNA was purified from paraffin-embedded tumoral tissue. A PCR SSCP analysis of exon 15 of BRAF was performed and direct genomic sequencing of SSCP positive cases was made.
The mutation was present in 106/319 patients (33.2%) and was significantly associated to the absence or invasion of tumoral capsule ($P<0.0001$), aggressive histological variant ($P=0.0001$) and multifocality ($P=0.02$). After 5 years of follow up, 24 (8.5%) patients had persistent disease and 295 (85.3%) were free of disease. BRAFV600E mutation was present in 89 patients (89/295 = 30.2%) free of disease and in 17 (17/24 = 70.8%) patients with persistent disease. At univariate analysis persistent disease was associated only with the presence of BRAFV600E mutation in the primary tumoral tissue ($P<0.0001$).
In conclusion, our results show the correlation of BRAFV600E mutation with aggressive features also in low risk PTC patients and indicate that the presence of BRAFV600E mutation is a bad prognostic factor for persistent/recurrent disease also in low risk PTC patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1759

### Is elevated preoperative TSH level predictive for differentiated thyroid cancer

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Introduction
Although numerous noninvasive markers are being used in order to determine preoperative thyroid cancer risk in patients with multi-nodular goiter, none of them are optimal. The objective of this study was to compare retrospectively the TSH level between two groups of subjects who underwent total thyroidectomy for a nodule and whose final histology was benign or malignant.
Methods
Our study included 208 patients that underwent thyroidectomy due to nodular goiter in our hospital between 2008 and 2010. While differentiated thyroid cancer (DTC) was established in 151 patients, pathology of 57 patients was detected to be benign. Patients were evaluated in terms of age, gender, and preoperative TSH levels.
Results
There was no significant difference between the two groups in terms of age, sex, family history of thyroid disease. Preoperative TSH level was established as 1.63 in the group with DTC and as 1.61 in the control group ($P$:0.923). A correlation was not established between the tumor size and TSH ($P$:0.79).
Conclusion
There are studies in literature that established positive correlation between elevated preoperative TSH and DTC. In our study, a correlation was not found between preoperative TSH level and DTC with tumor diameter. The reason for this may be the fact that patients with subclinical hypothyroidism and subclinical hyperthyroidism were included in the study.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1760

### Remarkable enhance in CYP24A1 expression in human thyroid cancer tissue

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Background
It is well established that 1,25-dihydroxyvitamin D3 (1,25-D3) inhibits cell growth and induces apoptosis in numerous tumors. Increased expression of 1,25-D3 inactivating enzyme, (24-hydroxylase - CYP24A1), has been observed in numerous thyroid cancer cell lines after calcitriol treatment *in vitro*. We examined the expression of CYP24A1 as well as the activating 1-α-hydroxylase (CYP27B1) gene in human thyroid cancer tissue.
Methods
The gene expression analyses of thyroid carcinoma samples were carried out by Taqman probe-based quantitative real-time RT-PCR. Total RNA was isolated from each sample with Roche High Pure Total RNA Isolation kit.
Results
CYP24A1 mRNA expression was markedly increased in all but one papillary cancers compared to that of normal thyroid tissue, reaching sometimes 300-fold elevation. No significant alteration was seen in CYP27B1 gene activity between neoplastic and normal tissues.
Conclusions
If the observed increase in CYP24A1 expression proves to be true on a larger samples size of human thyroid cancers, the use of higher doses of vitamin D3 and/or the development of CYP24A1 inhibitors or 24-hydroxylase-resistant vitamin D analogues could open a new approach to the effective treatment of thyroid cancers.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1761

### Differential proteomics in human normal or papillary thyroid carcinoma cell cultures

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Up to now there are few studies about differential proteomics in thyroid. Our group has developed a method for standard human primary thyroid culture maintaining the phenotype through passages. Thus, we believe of great interest the search for biomarkers in these cultures by 2D-PAGE coupled to MALDI-TOF/TOF-MS.

Aims

i) To standardize the conditions for sample extraction and controls. ii) To compare and identify the differential proteomic pattern between normal or benign thyroid (Pendred's Sd. Hyperplasia) pathologies against thyroid papillary carcinomas, combining (2D) gel electrophoresis, image analysis (PDQuest) and mass spectrometry (MALDI-TOF/TOF-MS).

Results

Soluble proteins of the human thyroid culture samples (NT, T-PS, T-PC), were suspended in a lysis buffer containing 7 M Urea, 2 M Thiourea, 4% CHAPS and 40 mM DTT. 50 µg proteins were rehydrated in 7 M Urea, 2 M Thiourea, 5% CHAPS, 1.2% DeStreak, 0.5% of ampholytes 3–10, loaded in 11 cm pH gradient strips (IPG 3–10 l, Amersham) and focused for 6000 V. Second-dimension separation was performed in 15% SDS-PAGE. Spots were visualized by silver staining. Three replicate gels per sample were scanned with a GS800.

Spot detection, pattern evaluation and normalization were performed using the PDQuest software (vs 8.0.1, Bio-Rad).

The individual protein spot quantity was normalized to the total quantity of all valid spots and is expressed as parts per million (PPM).

Individual protein quantities were evaluated by the Student's *t*-test within the PDQuest analysis in order to compare the two groups and identify sets of proteins that showed a statistically significant difference with a confidence level of 0.05.

Twenty two spots were statistically different in tumoral cultures (T-PC) vs normal thyrocytes (NT) and benign pathologies (T-PS). Of them, 12 were specifically increased and eight decreased in T-PC.

MALDI-TOF/TOF-MS identified some of the last spots. One of the most interesting is a low molecular weight variant protein-spot 8002.

We are performing several functional studies in order to validate this protein.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1762

### Lymphocytic chronic thyroiditis and papillary thyroid carcinoma: relation with prognostic factors

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Introduction

Recent studies have shown that chronic lymphocytic thyroiditis is associated with increased risk of developing papillary thyroid carcinoma (PTC). Although still controversial, these carcinomas appear to have a better prognosis. This study aims to evaluate the presence of thyroiditis in thyroids with PTC and the association with histological variables of prognostic value, Tg and evidence of metastasis in $^{131}$I scan.

Methods

Descriptive study of all PTC submitted to thyroid surgery during 24 months ($n=192$). Information on demographics, histology, Tg at the time of ablative therapy with $^{131}$I ($^{131}$IAT) and fixation in the post-therapy scan ($n=116$) was obtained. The association between variables was assessed using chi-square tests, multivariate analysis was done using binary logistic regression.

Results

The mean age at diagnosis was $46.9 \pm 15.7$ years, 82.3% were female patients. Most (77.1%) underwent total thyroidectomy, the average size of the largest tumor focus was $1.4 \pm 1.2$ cm. The prevalence of thyroiditis was 42.1%. Thyroiditis was significantly more frequent in females ($P=0.042$), classic variant of the PTC ($P=0.002$), presence of capsule invasion ($P=0.022$) and in patients with undetectable Tg ($P<0.001$). There were no significant associations between thyroiditis and tumor size, encapsulation, presence of necrosis, multifocality, perineural or vascular invasion, extra-thyroid extension, involvement of surgical margins, lymph node metastasis and evidence of distant metastases in $^{131}$I scan. Multivariate analysis in the total group of patients showed that only female gender and classic variant of PTC were associated with higher prevalence of thyroiditis, while in the group of patients that underwent $^{131}$IAT (in which the variable Tg was included), only absence of detectable Tg and classic variant maintained statistical significance.

Conclusions

Thyroiditis was associated with female gender, classic variant of PTC and absence of detectable Tg, which seems to be in favor of a better PTC prognosis when associated with thyroiditis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1763

### Ultrasound-guided fine-needle aspiration biopsy (US-FNAB) of thyroid nodules: effects of the operator's experience on adequacy of sampling: the MoCyThy (Modena's Cytology of the Thyroid) DATABASE

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Introduction

Thyroid FNAB is the gold standard to discriminate malignant from benign thyroid nodules. However, inadequate cytology ranges from 1 to 20% (up to 33.6%) in different settings and could depend on several factors, including the operator's skills.

Aim

To evaluate the effect of operator's experience on US-FNAB diagnostic adequacy we reviewed 7029 US-FNABs performed from January 2006 to March 2009.

Materials and Methods

US-FNABs were performed by 15 different operators; of them four had more than 5 years of experience, and 11 < 2 years of experience. All clinical data of the patients were collected and analyzed using the MoCyThy DATABASE, which is the part of the institutional database ENDOBASE (based on the MyQ16L open source technology) devoted to store data of all institutional US-FNABs.

Results

Five hundred twenty one (7.41%) of 7029 US-FNABs resulted inadequate. The inadequate sample rate was higher in the group of less experienced (<2 years) than in the group of more experienced (>5 years) physicians (9.05 and 6.98% respectively; $P=0.009$ at $\chi^2$ test). Furthermore, US-FNABS in the hands of less experienced operators resulted in a higher number of slides in comparison with the more experienced operators ($11 \pm 0.081$ vs $8 \pm 0.048$; $P<0.001$ at Kruskal–Wallis test). The number of slides, however, was not significantly related to sample inadequacy ($P=0.059$ at Kruskal–Wallis).

Conclusions

The experience of the operators is determinant for a better outcome in terms of lower inadequate sample rates and a lower number of slides needed to obtain a diagnostic outcome. In clinical practice, these results point out the impact of the operator's experience on US-FNAB procedure, suggesting that US-FNABS is cost-effective and less time-consuming in skilled hands.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1764

**Value of repeated US-guided fine-needle aspirations (US-FNAB) in the follow-up of thyroid nodules: the MoCyThy (Modena's Cytology of the Thyroid) DATABASE**

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Introduction
There is no consensus about the usefulness of repeating the US-FNAB during the follow-up of nodules when a benign (Thy2) or indeterminate (Thy3) report is obtained at first US-FNAB.
Aim of the study
To investigate the clinical value of repeating US-FNAB after a previous adequate Thy2 or Thy3 US-FNAB.
Methods
We reviewed the US-FNABs performed from 2006 to 2009. All clinical data of the patients were collected and analyzed using the MoCyThy DATABASE, which is the part of the institutional database ENDOBASE (based on the MyQ1L open source technology) devoted to store data of all institutional US-FNABs. Among 7983 records, we searched out 288 patients (327 nodules) undergoing at least two consecutive adequate US-FNABs for a total of 686 US-FNABs. We compared the first US-FNAB (Thy2 or Thy3) at baseline with the results of the following US-FNABs (2nd or 3rd US-FNAB).
Results
Of the 327 baseline US-FNABs, 58% were Thy2 and 42% Thy3. Of the 189 Thy2 at baseline, 157 (83%) were confirmed as Thy2 at follow-up, while 32 (17%) did not confirm the first diagnosis: 29 (15%) of them were Thy3 and 3 (2%) Thy4. No modifications of volume or US-features were recorded in these Thy4 from baseline. Of the Thy3 at baseline, 55 (40%) were confirmed as Thy3 at the follow-up, 84 (60%) did not confirm the first diagnosis: of them 6 (4%) were Thy4/5 and 78 (56%) Thy2.
Conclusions
The outcome of a subsequent US-FNAB is often discordant compared with the first cytological diagnosis. A first cytological diagnosis of Thy2 does not completely exclude a malignant (Thy4) or an indeterminate (Thy3) lesion. A second US-FNAB after 6–12 months may be useful, in clinical practice, to definitively confirm benign lesions or to reduce the rate of malignant tumor or of follicular lesions unrecognized by the first US-FNAB.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1765

**The Association between thyroid carcinoma and Hashimoto's thyroiditis: is really Hashimoto's thyroiditis increase the risk of thyroid cancer?**

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Aim
The aim of the study was to determine the association between Hashimoto's thyroiditis (HT) and differentiated thyroid carcinoma (DTC).
Patients and methods
Seven hundred seventy two patients with thyroid nodular goiter who underwent fine-needle aspiration cytology (FNAC), followed up at Endocrinology and Metabolism out-patient clinic of Cerrahpasa Medical School, University of Istanbul between January 2000 and December 2010 were included retrospectively in this study. All patients were evaluated for the presence of HT diagnosis by measuring thyroid autoantibodies. If a patient had at least one positive thyroid autoantibody, then the patient was defined as HT with thyroid nodules. Demographic features, ultrasonography (US) findings and cytology results of the patients were evaluated.
Results
Three hundred ninety three patients (39 male and 354 female, mean age 46.11 $\pm$12.53) with thyroid nodules associated with HT (HT group), 379 patients (53 male and 326 female, mean age 47.5 $\pm$12.6) with thyroid nodules without HT (control group) were determined. The prevalance of DTC in the patients with HT was 6.6%. In contrast, it was 12.9% in the control group ($P=0.03$). US findings were similar in both groups. When the whole population is considered in terms of autoimmunity, positive anti-TPO rate was found significantly higher in benign nodules ($P=0.008$).
Conclusion
The malignancy rate in the patients without HT was twice more than the patients with HT. Many of the US features of benign thyroid nodules are similar in patients with and patients without HT.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1766

**Clinicopathological characteristics of patients with thyroid papillary microcarcinoma; preliminary results**

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Background and aim
The clinical significance of papillary thyroid microcarcinoma (PTMC) is debatable. The purpose of this study is to analyse the clinicopathological characteristics of patients with PTMC and document the risk factors for poor prognosis.
Materials and methods
Eighty-eight patients (18 males, 70 females) were included in the study. Clinical and laboratory parameters were recorded.
Results
The mean age of the patients were 47.6 $\pm$ 11.4. The most common presenting symptom was swelling in the neck but half of them (45 patients) were incidentally diagnosed. 4 patients had a family history of thyroid cancer. 78 (88.6%) patients were operated for suspicion of malignancy after fine needle aspiration biopsy. One had Graves' disease, one had hyperparathyroidism and rest of them were operated for large nodule size $\geq$ 3 cm. 48 patients had tumor size $<$5 mm, 40 had 6–10 mm tumors. Lymph node invasion was present in seven patients and three patients had capsule invasion. Only tumor size was an independent risk factor for lymph node metastasis at diagnosis.
Conclusion
Increased tumor size increases the risk for poor prognosis. Tumor size should be considered in the follow-up for these patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1767

**Differentiated carcinoma in dysembriogenetic thyroid lesions**

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The prevalence of differentiated thyroid carcinoma (DTC) in lingual thyroid (LT) and thyroglossal duct cysts (TDC) is around 1%. Nowadays, almost 200 cases of DTC were reported in TDC and $<$60 cases in LT. Here we report four cases of neoplasia in LT (1/4) and TDC (3/4) in a consecutive series of 950 DTC patients (0.4%).
Case 1
D.F. 63 year-old woman with thyroid follicular carcinoma in ectopic gland located at her tongue's basis. March 2009: the lesion, infiltrating surrounding tissues, presented insular-like areas (T3N1Mx). July 2009 and 2010: two radiometabolic treatment (cumulative dose of 224 mCi of $^{131}$I) with no evidence of local/distant metastases and undetectable serum thyroglobulin (Tg), following the last radioiodine administration.

Case 2
B.B. 37 year-old woman with TDC. October 2001: exeresis of a thyroid papillary carcinoma (PC) in TDC infiltrating soft local tissues (T3NxMx). December 2001: Total thyroidectomy (tTx) with histologic finding of benign adenomatous goiter. 2002–2008: five radiometabolic treatment (cumulative dose of 500 mCi of $^{13}$1I) due to iodine uptaking lung metastases. September 2009: persistence of elevated Tg levels (56.1 ng/ml) following recombinant human TSH stimulation. Proposal of further radiometabolic treatments refused.
Case 3
B.S. 42 year-old man. November 2001: PC of thyroglossal duct infiltrating the surrounding fibro-adipose tissue with lymphatic and intravascular diffusion. March 2002: tTx with evidence of two foci of PC (T3bN0Mx). July 2002: 100 mCi of $^{13}$1I, Tg undetectable and WBS negative.
Case 4
D.B. 21 year-old woman with a PC follicular variant in TDC. July 2010: tTx with histologic evidence of benign macrofollicular goiter.
Conclusions
The higher prevalence of DTC (also in form of aggressive variants) in dysembrogenetic than in eutopic thyroid tissue (0.4 vs 0.004%) argues in favor of a close monitoring of all the dysembriogenetic thyroid lesions.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1768

**Nursing assistance increases the efficiency of ultrasound-guided fine-needle aspiration biopsy (US-FNAB) in the management of thyroid nodules: the MoCyThy (Modena's Cytology of the Thyroid) DATABASE**

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Introduction
US-FNAB is the most cost/effective and accurate diagnostic procedure for the evaluation of thyroid nodules. In the clinic, the nursing assistance is not always available for the US-FNAB procedure in all endocrinological centers and its value remains to be established.
Aim of the Study
To demonstrate the role of nursing assistance in US-FNAB procedures in improving the efficiency of this procedure.
Methods
All clinical data of the patients were collected and analyzed using the MoCyThy DATABASE, which is the part of the institutional database ENDOBASE (based on the MyQ5L open source technology) devoted to store data of all institutional US-FNABs. Of the 7377 US-FNAB performed at the Unit of Endocrinology of Modena from 2006 to 2009, we compared 4831 US-FNAB performed with nursing assistance with 2546 US-FNAB performed by the same medical team, but without nursing assistance.
Results
The number of US-FNAB performed for every work session ($7.57 \pm 3.94$ vs $6.59 \pm 3.03$), the number of slides assessed for every work session ($77.55 \pm 42.93$ vs $41.61 \pm 31.81$), the number of slides prepared for each FNA ($10.23 \pm 3.2$ vs $6.31 \pm 2.89$) were all significantly higher in the sessions with nursing assistance than in those without nursing assistance ($P < 0.001$ at Mann–Whitney Rank Sum Test).
Conclusions
The support of nursing assistance has a relevant impact on the efficiency of the US-FNAB procedure in terms of the number of US-FNAB performed in each session and of number of slides prepared for each session and for each US-FNAB. In clinical practice, nursing assistance may improve the outcome of US-FNAB procedures and is cost-effective.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1769

**Multiplicity as a prognostic factor of papillary thyroid carcinoma**

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Backgrounds
Mutiplicity of papillary thyroid carcinoma (PTC) are not an unusual finding, although the origin of these foci is unclear. Either intraglandular metastases from a single dominant tumor or unrelated neoplastic clones were definitively proven as the means by which multicentric PTC form. In addition, there is insufficient clinical information concerning multicentric PTC presentation, prognosis, and long-term follow-up studies after treatment. Multiplicity of PTC has not been considered as an independent prognostic factor from a variety of tumor staging systems.
Aims
To evaluate whether that the presence of mulciplicity would be associated with tumor recurrence in PTC patients.
Methods
A total 249 PTC patients at a single institution who underwent total thyroidectomy and node dissection were retrospectively reviewed; the mean follow-up period was 2.8 years. Postoperative radioactive iodide ablation for thyroid remnant was performed after surgery for most patients.
Results
Of all the PTC cases reviewed, 85 cases (34%) were categorized as multicentric PTC. Compared with patients with unifocal PTC, multicentric PTC patients demonstrated higher cervical lymph node metastasis and tumor recurrence. Multiplicity was also significantly associated with tumor recurrence; 6 vs 1% with and without multiplicity, respectively ($P = 0.022$ by log-rank test). However, this association was lost on multivariate analysis adjusting for conventional clinicopathological predictors of recurrence.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1770

**Frequency and predictive factors of malignancy in residual thyroid tissue after partial thyroidectomy for differentiated thyroid cancer**

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Background
The main objective of this study is to establish the rate of malignancy in residual thyroid tissue in patients with DTC, and whether the serum thyroglobulin (Tg) level before complementary thyroidectomy and histopathologic characteristics of the tumor would be able to foresee malignancy in residual tissue.
Methods
Our study included 58 patients with DTC that underwent complementary thyroidectomy that results were analyzed retrospectively. Patients were then divided into two groups as patients that were established to have tumor in residual tissue (group 1) and not to have tumor in residual tissue (group 2) based on the pathology findings of residual tissue following complementary thyroidectomy. Both groups were compared in terms of serum Tg levels before complementary thyroidectomy and histopathologic characteristics of tumor.
Results
Fifty three patients were found to have papillary thyroid cancer and five had follicular thyroid cancer. Median tumor diameter was 0.8 cm (0.1–5.5 cm), 16 patients (27.6%) was found to have multifocality, 4 patients (6.9%) had perithyroidal invasion, 16 patients (27.6%) had capsular invasion, and 7 patients (12.1%) was established to have vascular invasion. Following the complementary thyroidectomy, 13 patients (22.4%) out of 58 patients with DTC were found to have malignancy in residual tissue. A statistically significant difference was not observed between the two groups in terms of gender, age, serum Tg level before complementary thyroidectomy, type of tumor pathology, tumor size, bilaterality multifocality, arterial invasion, capsular invasion, and extrathyroidal invasion presence ($P > 0.05$).
Conclusions
Factors that enable foreseeing malignancy in residual thyroid tissue are not completely known. In our study, we established that serum Tg level before complementary thyroidectomy and histopathologic characteristics of tumor does

not have a predictive value in foreseeing malignancy in residual thyroid tissue; however, other studies containing more patients are of necessity to clarify the issue.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1771

### Ultrasound (US) features of thyroid nodules with cytology suspicious for malignancy

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Introduction
Several studies in literature have shown that some features of thyroid nodules at US are associated with malignancy. However, previous studies were focused mainly on subjects affected by multinodular goiter (about 70%) rather than subjects with thyroid cancer (about 30%). Furthermore, the main limitation of previous studies was the lack of thyroidectomy in all subjects.
Aim of the study
To evaluate the diagnostic value of US features in a selected sample of patients with thyroid nodules cytologically suspected for malignancy (THY4–THY5) by comparing US features of each nodule with the results of histological analysis after thyroidectomy.
Methods
In this prospective study, we enrolled 54 patients with cytological result suspicious of malignancy. All subjects underwent thyroid ultrasound before thyroidectomy. We evaluated the following US features: size, content, shape, margins, echogenicity, calcification, halo sign, vascular pattern, for all the nodules (those citologically suspected and those not suspected). All enrolled patients underwent total thyroidectomy, therefore all benign and malignant nodules previously assessed at US received histological verification.
Results
In all the 54 patients a diagnosis of differentiated thyroid cancer was confirmed. Each of the following features: microcalcifications, macrocalcifications, irregular margins and hypoechogenicity at US correlate with malignancy at histology by using chi-square ($P<0.001$).These features have high specificity but low sensitivity (microcalcifications 93.9–40.4%, macrocalcifications 98–22.8%, hypoechogenicity 96–21% respectively). Irregular margins is the feature with the best pair of sensitivity (65%) and specificity (65%).
Conclusions
These results confirm and reinforce previous studies that showed a correlation among microcalcification, irregular margins, hypoechogenicity and malignancy in a highly selected sample of patients undergoing thyroidectomy. Furthermore, in contrast with literature, we found a strong correlation also between macrocalcification and malignancy. US is a valid tool to select which nodules require FNA evaluation according to sonographic features closely related to malignancy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1772

### Incidence of ultrasound thyroid scan anomalies in healthy volunteers in modena, italy

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Introduction
We assessed the incidence of ultrasound (US) thyroid scan anomalies in adult volunteers in the district of Modena
Methods
From December 2010 to October 2011 we performed US thyroid scan (Siemens Acuson Antares, 10 Mega Hertz-Linear scanner- B mode) in a cohort of 201 volunteers, recruited by local advertisement, women ($n=135$) and men ($n=66$), mean age $46\pm10.7$. All participants were unaware of any thyroid disease and at their first thyroid US scan. Fine needle aspiration cytology (FNA) was performed in 13 subjects.
Results
US thyroid scan anomalies were found in 101 subjects (50.3%): 93 nodular goiters (95%) and 13 subjects with ultrasound features of thyroiditis (12.8%), 11 of them confirmed by positive anti Tg and/or anti TPO antibodies. Positive family history was present in 30% of subjects affected by thyroid US anomalies. In all subjects with nodules serum calcitonin was normal. 13 subjects (6.5%) with nodular goiter underwent FNA with the following citology: 10 patients THY 2 (77%), 1 patient THY 3 (7.7%), 2 patients had THY 4 (15%) followed by histological confirmation of thyroid papillary carcinoma after total thyroidectomy (both women aged 48)
Conclusions
The incidence of thyroid anomalies, mainly nodular goiter, is very high in subjects unaware of any thyroid disease in the district of Modena, Italy. Thyroid cancer was found in 1% of all subjects, 2% of those affected by nodular goiter. Among subjects who underwent FNA the prevalence of cancer was 15%. Compared to other well-established screening programs like breast and colorectal cancer providing a yearly detection rate of about 0.45% and 0.27% respectively, the incidence of thyroid cancer seems to be much higher: thyroid US mass screening could allow the detection of asymptomatic cancer at a very early stage with a high cost-benefit ratio.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1773

### Surgical treatment of locally advanced thyroid carcinoma with larynx infiltration: case report

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Introduction
Infiltration of larynx by thyroid cancer represents fourth stage of the disease and is threatening disease. Lethal outcome of advanced thyroid cancer, which invades trachea and larynx, is usually associated with airway obstruction.
Patient and method
We are presenting 58 year old woman operated due to the advanced papillary carcinoma Patient underwent total thyroidectomy, central neck dissection, modified radical dissection of the right side, selective dissection on the left side, auto-transplantation of left parathyroid gland, partial vertical laryngectomy and reconstruction of defects with epiglottis and surgical tracheotomy. After surgical treatment she received a dose of 5.5 GBq J 131st. Postoperative stenosis of the larynx was treated twice with laser surgery.
Results
Traheostomy has been closed and the phonatory and respiratory functions were preserved after treatment. One year of follow up has passed with no signs of relapse.
Conclusion
Decision of resectability of the tumor with reconstruction of the defect in the larynx is the most commonly intraoperative decision. Radical surgery is a logical and rational therapeutic approach for thyroid cancer in the fourth stage.
The goal of radical surgery in locally advanced thyroid cancer is to prevent lethal outcome but can also be curative form of therapy with good quality of life.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1774

**Role of intensity modulated radiation therapy in patients with non-anaplastic thyroid carcinoma: king fahad medical city experience**

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Background
To evaluate outcomes and toxicities in patients with non-anaplastic thyroid cancer treated with intensity modulated radiation therapy (IMRT).
Materials and methods
This was a single institution retrospective review of 18 patients with non-anaplastic thyroid cancer treated with IMRT at King Fahad Medical City between April 2005 and December 2010. The median age was 69 years. Among the 18 patients, fifteen had papillary carcinoma, two had T3 and 12 had T4 disease, twelve had N1 disease, and one had distant metastases. Radioactive iodine was given to all patients, while the median radiotherapy (RT) dose was 60 Gy (range; 60–66Gy).
Results
Median follow-up was 30 months (range: 6-to-64 months). The Kaplan-Meier estimates of 3-year local control, locoregional control, and overall survival rates 90, 90, and 80% respectively. Nine patients (50%) had grade 2 skin toxicity, nine had grade two mucositis (pharyngitis, esophagitis), 1(11.1%) had grade G4 mucositis, 2 (22.2%) had grade 2 laryngitis, and 1(11.1%) had grade 3 laryngitis. Few grade 1 late toxicities were observed. Multivariate analysis showed poor prognostic factors for local and overall survival were; age above 45, postoperative gross residual disease, distant metastasis (DM) and no radioactive iodine (RAI) treatment. In patients with no DM and no postoperative LR disease, adjuvant RAI ablation reduced both LR failure (relative risk (RR) 0.29) and DM (RR 0.2).
Conclusion
IMRT is effective in the postoperative setting for high risk patients of thyroid cancer to reduce locoregional recurrences with acceptable morbidity. Long-term follow-up is still needed to assess the incidence of late toxicities.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1775

**Urine neopterin levels in differentiated thyroid cancer**

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Aim
Neopterin is a mareker of inflammation secreted from monocytes and macrophages. It is found to be increased in malignant diseases. The aim this study was to evaluate the urine neopterin levels in thyroid cancer.
Materials and methods
Sixty nine patients with thyroid cancer, 76 patients with benign thyroid pathology and 33 healthy subjects were evaluated. First morning urine samples were collected from the patients and the normal subjects for neopterin and creatinin measurment and stored at −20 °C until analyzed.
Results
Neopterin levels were 149.3 (15.2–1602.2) μmol/mol creatinin in the malignant group, 32.0 (5.2–275.6) μmol/mol creatinin in the benign group and 9.2 (2.7–78.7) μmol/mol creatinin in normal subjects. Urinary neopterin levels were significantly higher in patients with throid cancer than patients with benign thyroid pathologies and normal subjects. There was 22 (%31.9) patients with chronic thyroiditis and 47 (%68.1) patients without chronic thyoiditis in the malignant. Urinary levels of neopterin didn't differ in both groups (168.6 (21.3–716.8) μmol/mol creatinin and 135.3 (15.2–1602.2) μmol/mol creatinin respectively, $P=0.381$). Patients who had capsul invasion, vascular invasion and lymph node invasion had a higher urine neopterin level although not statistically significant.
Conclusion
Urinary neopterin levels are high in thyroid cancer and this result is not affected from the presence of chronic thyroiditis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1776

**Hyperthyroidism and thyroid cancer-a prospective study**

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Introduction
The effect of hyperthyroidism on the development of differentiated thyroid cancer (DTC) has been controversial in the international bibliography. The aim of our study was to find the possibility of thyroid cancer appearance in patients with hyperthyroidism who underwent total thyroidectomy in comparison with patients without thyroid hyperfunction.
Material-Methodology
Between 2005–2010 228 patients (182 females/46 males) underwent total thyroidectomy for a variety of functional and non-functional thyroid disorders and the specimens were analysed in the Pathology Department of our Hospital.
Results
34 patients with single non-functional thyroid nodule (14.9%),152 with non-toxic multinodular goiter (66.7%),26 with toxic multinodular goiter (11.4%), 6 with toxic adenoma (2.6%) και 10 with Graves disease (4.4%) were operated.In the two first groups we found respectively 11 people (32.4% of the patients with single nodule) and 21 (13.8% of the people with non-toxic multinodular goiter) with differentiated thyroid cancer. Conclusively 17.2% of the people with non-functional thyroid disorders were diagnosed with DTC. On the contrary from the 42 patients of the other three groups with hyperthyroidism only one young male with papillary carcinoma and Graves disease was detected (10% of the patients with Graves or 2.4% of the total hyperthyroid patients) ($P=0.014$, odds ratio 8.5).
Conclusion
The presence of hyperthyroidism in the material of our clinic possibly reduces significantly the potential of differentiated thyroid cancer appearance in comparison with the cases of single non-functional nodule and non-toxic multinodular goiter.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1777

**Coexistence of chronic lymphocytic thyroiditis and well differentiated thyroid cancer-a prospective study**

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Introduction
The coexistence of Hashimoto thyroiditis with welll differentiated thyroid carcinoma (DTC) has been discussed as far as the follow-up and prognosis of patients with chronic autoimmune thyroiditis (CAT) are concerned. The aim of our study was to find the coincident appearance of the two disorders in histologic specimens from patients who underwent total thyreoidectomy.
Material-Methodology
Between 2005–2010 228 patients (182 women/46 men) were operated with total thyroidectomy in our clinic. The surgical specimens were studied in the Pathology Department of our hospital.
Results
In 228 surgical specimens thyroid cancer was found in 33 (14.5%), from them 31 were DTC (13.6%),while elements of chronic lymphocytic thyroiditis were found in 56 (24.6%).Coexistence of Hashimoto and DTC was detected in 6 patients (19.3% of the DTC diagnosed patients, females/males 5/1). In the population of patients with autoimmune thyroiditis the cancer prevalence was 10.7%. There was no statistically significant difference as far as the age is concerned ($P=0.677$).

Conclusion
Further analytical clinical studies are required in order to establish a possible association between Hashimoto thyroiditis and DTC.Our results show that a careful examination and follow-up of the patients and mainly the women with the frequent diagnosis of the autoimmune thyroiditis is essential.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1778

**Thyroid papillary carcinoma, 30 years of experience**
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Papillary carcinoma is the most common histological type of thyroid cancer. The optimal treatment remains controversial, particularly with regard to the papillary microcarcinoma ($\leq$ 10 mm).
The aim of this study is the characterization of patients with this condition undergoing surgery in our hospital over a period of 30 years, evaluating treatment outcomes.
Between January 1, 1980 and December 31, 2010, a total of 564 patients underwent surgical treatment for thyroid disease, with histological confirmation of papillary carcinoma.
Of these, 461 patients were female (81.7%) and 103 males (18.3%), aged between 12 and 86 years.
The presence of papillary thyroid microcarcinoma was found in 209 patients (37%).
The morbidity and survival in our series were similar on the published literature.
Keywords, Papillary, Microcarcinoma, Thyroid
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1779

**Thyroid cancer in multinodular toxic goiter: A report of two cases in the philippines**
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Synopsis
Thyroid cancer with concomitant thyrotoxicosis is variably reported to be rare. Malignancy occurs as postoperative incidental finding of a small malignant focus in Graves' disease, as part of a toxic multinodular or a toxic adenoma. We report two cases of large toxic multinodular goiters with metastasis diagnosed preoperatively as follicular neoplasm and confirmed postoperatively as differentiated thyroid cancer. Review of literature, pathophysiology and treatment strategies will be discussed.
Case 1
A 49 year old female presented with twelve year history of enlarging goiter and mass in the sternal notch which developed over one year. Examination revealed a large multinodular goiter measuring 12$\times$12 cm with 7$\times$7 cm mass in the sternal notch. Investigations revealed follicular neoplasm on fine needle aspiration biopsy and T3 toxicosis. A 1.6$\times$1.8 cm functioning nodule in the superolateral right lobe was demonstrated on thyroid scintigraphy. She underwent total thyroidectomy and excision of the manubrial mass. Histopathology showed follicular variant of papillary cancer with metastasis to the manubrium.
Case 2
A 71 year old female presented with recurrent thyroid nodules of 21 year duration. She had three thyroid surgeries in the past which revealed follicular cancer. She was lost to follow-up after each surgery and received no subsequent treatment. She again consulted for recurrence of the thyroid masses but no symptoms of hypo/hyperthyroidism, obstruction or voice changes. Examination revealed a large multinodular goiter measuring 10$\times$6 cm which yielded follicular neoplasm on fine needle aspiration biopsy. TSH was suppressed at 0.008 mIU/l (NV: 0.3–3.8). Free T4 was elevated at 36.2 pmol/l (NV: 11–24). Thyroid scintigraphy showed functioning thyroid tissues with a conglomerate size of 8$\times$5.6 cm. Chest imaging revealed lung metastasis. She is presently being treated with Methimazole 40 mg daily in preparation for thyroidectomy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1780

**CLM94, a novel cyclic amide with anti-VEGFR-2 and antiangiogenic properties, is active against primary anaplastic thyroid cancer *in vitro* and *in vivo***
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Introduction
The antitumor activity of a novel cyclic amide, CLM94, with anti-VEGFR-2 and antiangiogenic activity, in primary anaplastic thyroid cancer (ATC) cells *in vitro* and *in vivo*, has been studied.
Design and Methods
CLM94 was tested: i) in two human cell lines (HMVEC-d, dermal microvascular endothelial cells; 8305C, undifferentiated thyroid cancer) at 0.001–100 mcM; ii) in ATC cells at the concentrations of 10, 30, 50 mcM; iii) in a ATC-cell line (AF) in CD nu/nu mice.
Results
CLM94 significantly inhibited VEGFR-2 and EGFR phosphorylation in HMVEC-d, and proliferation in HMVEC-d and 8305C cell. A significant reduction of proliferation with CLM94 in ATC cells ($P<0.01$, ANOVA) and a slight but significant reduction of proliferation with CLM94 30 and 50 mcM in normal thyroid follicular cells ($P<0.01$, ANOVA) were shown. CLM94 increased the percentage of apoptotic ATC cells dose-dependently ($P<0.001$, ANOVA) and inhibited migration ($P<0.01$) and invasion ($P<0.001$). AF-cell line was injected sc in CD nu/nu mice and tumor masses became detectable 25 days after. CLM94 (40 mg/kg/die) inhibited significantly tumor growth (starting 10 days after the beginning of treatment). CLM94 significantly decreased the VEGF-A gene expression in the AF cell line and the VEGF-A protein and microvessel density in AF tumour tissues.
Conclusions
The antitumor and antiangiogenic activity of a new 'cyclic amide' compound CLM94 is very promising in anaplastic thyroid cancer, opening the way to a future clinical evaluation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1781

**PI3K/HIF and ATM signalling in radio-resistance of thyroid-carcinoma: new therapeutic implications?**
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Background
Anaplastic thyroid-carcinomas (ATC) and a subset of papillary (PTC) and follicular (FTC) thyroid carcinomas behave aggressively showing metastatic spread and radio-resistance. Both the PI3K and HIF pathways are associated with aggressiveness and metastasis in thyroid carcinoma.

Aims
To assess if the PI3K/HIF pathways contribute to radio-resistance and assess the effect of PI3K/HIF inhibition on radio-sensitivity of thyroid-carcinoma cells (8505c, FTC133, WRO, BcPAP) *in vitro* and *in vivo*.
Methods
PI3K was inhibited pharmacologically (via PI-103/GDC-0941) and genetically (via PTEN rescue in PTEN-null FTC133 cells). HIF-1 activity was inhibited using a dominant-negative variant of HIF-1α (dnHIF). Cells were irradiated with 0, 2, 4 and 6 Gy with/without PI-103/GDC-0941 under normoxia/hypoxia (1% oxygen) /anoxia. *In vitro* DNA double-stranded breaks (DSBs) were assessed by gamma-H2AX expression, HIF-1α activity by use of luciferase reporter assays and migration by use of the scratch-wound migration method. Activity of PI3K, ATM and DNA-PK (proteins critically involved in the DNA damage response) were analysed by assessing pAKT/pATM/pDNA-PK expression. *In vivo*, mice bearing FTC-xenografts were exposed to 5×2 Gy with/without GDC-0941 (orally), tumour pAKT, pATM and pDNA-PK expression and volumes were assessed. Spontaneous-lung metastasis was quantified by clonogenic assay.
Results
GDC-0941, dnHIF and PTEN rescue increased and prolonged radiation-induced DNA-DSBs under normoxia/anoxia in carcinoma cells and had no effect on immortalised 'normal' thyroid cells. Mechanistically this was via inhibition of pATM, with the degree of inhibition being dependent on oxygen environment and cell-type. Radiation-induced HIF-1alpha activity/expression in normoxic/anoxia FTC133 s. GDC-0941 reduced HIF-1alpha activity and clonogenicity in irradiated FTC133 and 8505c cells. Radiation increased FTC migration, which has important therapeutic implications. PI-103/GDC-041 and PTEN rescue inhibited migration in irradiated cells. GDC-0941 increased growth delay of irradiated tumours and reduced radiation-induced pAKT and pDNA-PK in FTC-xenografted mice.
Conclusions
These data link PI3K inhibitors combined with radiotherapy may improve therapeutic response.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1782

### Functional and molecular characterization of cancer stem cells in anaplastic thyroid carcinoma

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Anaplastic Thyroid Carcinoma (ATC) is the most aggressive thyroid gland malignancy characterized by undifferentiated morphology. It has been suggested that cancer stem cells (CSCs) might play a central role in ATC. Previously on the basis of CD133 positivity, our group suggested that CD133+ cancer stem cells (CSCs) might play a central role in ATC. Here we analyzed a panel of different stem cell markers in order to identify a peculiar CSC pattern in ATC.
Aim
i) to characterize CSCs from ex *vivo* ATC specimens; ii) to evaluate in a well characterized ATC cell line (SW1736) the percentage of CSCs and the influence of transcription factor SOX2 on Oct-4 and Nanog expression and drug resistance.
Methods
Ex *vivo*: Eight formalin-fixed, paraffin-embedded ATC specimens were analyzed by RT and qRT-PCR, immunohistochemistry and immunofluorescence for pluripotent CSC markers (CD133, SSEA4, Thy1, ABCG2, c-kit, SOX2, Oct-4, Nanog, Lin28, Klf4 and c-Myc). *In vitro*: CSC marker expression was evaluated in SW1736 cells by qRT-PCR, flow cytometry and immunofluorescence; the SOX2 effect on Oct-4 Nanog and ABCG2 expression and chemosensitivity were evaluated after SOX2 silencing by siRNA method.
Results
Stem cell markers expression in ATCs and SW1736 cell line proved to be as follows in the table.
SOX2 plays a pivotal role in this model: SOX2 silencing down-regulated Oct-4 (67.6%±2.0 $P<0.05$) and Nanog (85.5%±5.4 $P<0.01$) expression. SOX2 silencing sensitized SW1736 cells causing a significant mortality increase of 1.8 fold with 10 μM cisplatin and 1.4 ($P<0.01$) fold with 1.5 μM doxorubicin in comparison to control cells. Conclusions: The analysis of multiple pluripotent stem cell markers is useful to identify CSCs in ATC. In particular, SOX2 exerts a fundamental role in regulating Oct-4 and Nanog expression. Our data suggest that SOX2 switch-off may be essential for overcoming CSC chemotherapy resistance in ATC.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Stem Cell Markers

| | CD133 | SSEA4 | Thy1 | ABCG2 | c-kit | SOX2 | Oct-4 | Nanog | Lin28 | Klf4 | c-Myc |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | – | – | – | – | – | – | – | – | – | – | – |
| ATCs | +(4 out of 8) | +(4 out of 8) | + | + | + | + | + | – | – | + | + |
| SW1736 | – | + | – | + | – | + | + | + | – | – | – |

## P1783

### Regulation of hPTTG expression and phosphorylation: autocrine interactions with growth factors in thyroid cells

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Introduction
The human Pituitary Tumor Transforming Gene (hPTTG) is a phosphorylated proto-oncogene induced in multiple tumour types. hPTTG phosphorylation is mediated by cyclin-dependent kinase 2 (CDC2) and expression is regulated by specificity protein 1 (SP1). In thyroid cancer, hPTTG induces genetic instability and propagates growth through induction of growth factors (GFs).
Methods
The interplay between hPTTG phosphorylation, SP1 regulation and GF induction was evaluated in thyroid cells *in vitro* and in mouse models of PTTG over- and underexpression *in vivo*.
Results
EGF(5nM), TGF-α(5 nM) and IGF1(10 ng/l) induced hPTTG protein in K1(twofold, 3.6-fold and 2.3-fold, $P=<0.01$) and TPC-1 papillary thyroid carcinoma cells(twofold, 4-fold and 2.6-fold, $P=<0.01$) and in human primary thyrocytes(threefold, 2.4-fold and 2-fold, $P=<0.01$). These effects were associated with activation of mitogen activated protein kinase (MAPK) and phosphoinositide three-kinase (PI3K), but not with increased cellular proliferation rates. GF treatment of TPC-1 cells following siRNA knockdown of CDC2 or SP1 confirmed that GFs induce hPTTG independently of these hPTTG regulators. CDC2 depletion in TPC-1 cells resulted in enhanced expression and phosphorylation of hPTTG and reduced cellular proliferation. Transient transfection with hPTTG induced EGF (1.7-fold, $n=4$, $P=0.004$), IGF1 (1.6-fold, $n=5$, $P=0.002$) and TGF-α mRNA (1.6-fold, $n=3$, $P=0.024$) expression. Treatment of human primary thyrocytes with conditioned media from hPTTG transfected cells resulted in autocrine upregulation of hPTTG protein, which was ameliorated by GF depletion or GF receptor inhibitors. *In vivo* evaluation of our transgenic mouse model with thyroid-targeted hPTTG overexpression confirmed increased mEGF (2.7-fold, $n=3$, $P=0.012$) and mIGF1 (2.0-fold, $n=3$, $P=0.02$) mRNA, compared with WT mice. Further, mEGF mRNA expression was downregulated in the thyroids of $PTTG^{-/-}$ knockout mice (0.4-fold, $n=4$, $P=0.001$), consistent with reduced EGF mRNA expression in TPC-1 cells transfected with hPTTG siRNA (0.68-fold, $n=4$, $P<0.05$, compared to scrambled controls).
Conclusion
Together, our results indicate that hPTTG is involved in autocrine signalling mechanisms with GFs in the thyroid, independently of CDC2 and SP1, and that aberrant control of these pathways may enhance transformed cell growth.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1784

**Mass spectrometry allows accurate measurement of serum thyroglobulin (Tg) in the presence of anti-thyroglobulin auto-antibodies (TgAB)**

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Background
Serum thyroglobulin (Tg) measurements are one of the main pillars of thyroid cancer follow-up, because of the high organ and disease specificity of Tg in athyrotic patients. However, 20–25% of patients have detectable serum anti-Tg auto-antibodies (TgAB), which might cause false low Tg measurements in immunometric assays (IMA). This can lead to significant challenges in patient management. Measurement of Tg by liquid chromatography-tandem mass spectrometry (LC–MS/MS) after tryptic digestion can in theory overcome this problem, as TgAB and Tg are both digested equally, and Tg-specific tryptic fragments can be detected selectively. Proof of concept for such a LC–MS/MS Tg assay was shown for TgAB-negative patients in 2008 (Clin Chem 54:1796–1804). We extended these observations to TgAB-positive patients.
Methods
Samples were depleted of albumin and other low/mid molecular weight proteins, trypsin digested, and purified by solid phase extraction after addition of synthetic non-radioactive isotopic peptide internal standard (IS). The extracts were analyzed by LC–MS/MS for a highly ionizable proteotypic Tg fragment and its corresponding IS. Purified Tg, matched to the international Tg reference preparation, was used for calibration.
Results
The assay's limit of detection was 2 ng/mL. Inter-assay imprecision (CV) was 4–12% (Tg range: 3.8–150 ng/ml). Method agreement with the Beckman Coulter Tg IMA in 119 TgAB-negative patients with detectable Tg showed a slope of 0.75, intercept: +3.8, R-squared: 0.87. By contrast, in 20 TgAB-positive patients with detectable Tg, the slope was 1.77, consistent with under-recovery of Tg in the IMA. Consistent with these observations, a comparison of Tg recovery between LC–MS/MS and IMA, following addition of high TgAB concentrations to Tg samples, showed under-recovery in the IMA.
Conclusion
Tryptic digest-based LC–MS/MS assays can measure Tg accurately in the presence of TgAB that lead to false low IMA measurements.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1785

**Dual specificity phosphatase 5 (DUSP5), a specific negative feedback regulator of ERK signaling, is controlled by serum response factor (SRF) and Elk-1 transcription factors**

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Mitogen-activated protein kinase (MAPK) pathway abnormalities, specifically rearrangements (RET/PTC) or activating mutations (RAS or BRAF), are highly prevalent in papillary thyroid carcinomas (PTCs). Constitutive activation of this signaling cascade causes sustained phosphorylation of extracellular signal-regulated kinase (ERK). DUSP5, which is positively regulated by ERK signaling, acts as a negative regulator of its activity. We have previously shown that DUSP5 is overexpressed in PTCs. We sought to characterize the regulation of DUSP5 expression.
We demonstrated, using pharmacological inhibitors in NIH3T3 (murine) and PC12 (rat) cell lines, that DUSP5 is an early response gene regulated by the MAPK pathway, mostly at the transcriptional level. We next focused on DUSP5 transcriptional regulation. Analysis of DUSP5 promoter region allowed us to identify two contiguous CArG Boxes, binding SRF, and several Ets responsive elements (EBS), binding Elk-1, which is positively regulated by ERK signaling. We studied in NIH3T3 the implication of these responsive elements by transient transfection of luciferase reporter genes corresponding to wild type or mutated responsive elements DUSP5 promoter (i.e. mutation of CArG Box and/or EBS). These experiments demonstrated a role for SRF and Elk-1 in the transcriptional regulation of DUSP5, confirmed by EMSA and CHIP assay. Our data indicate that the CArG Boxes play a key regulatory role in DUSP5 expression.
DUSP5 expression tracks in tandem with MAPK pathway activation. DUSP5 is regulated mainly at the transcriptional level by the transcription factors Elk1, a known target of ERK signaling and SRF. These factors can form a ternary complex (Elk1-SRF-DNA) on the DUSP5 promoter, thereby providing a link to the ERK signaling pathway. These data highlight an important feedback loop that allows a tight regulation of pERK levels.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1786

**Is thyroid cancer recurrence risk increased after transplantation?**

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Context
An increased mortality by cancer is reported in grafted patients.
Objective
This multicentric study aimed to investigate whether the prognosis of thyroid cancer was modified by transplantation
Results
Sixty nine patients (35 M/34 F; median age 41 years) with a history of both thyroid cancer and transplantation were recruited via specialized networks (TUTHYREF and DIVAT). Evolution and outcome were retrospectively analyzed after a median follow-up of 13 years. Ninety one per cent were papillary and 9% of the follicular type.
Thyroid cancer had been diagnosed before transplantation in 33/69 patients from whom 36.4% had a high risk cancer. At the time of the graft, 31 were in remission, four of them after a recurrence. Two patients recurred after transplantation and a remission was obtained after a complementary treatment. In a young female patient who presented before transplantation lung metastases, a 2 years complete remission was observed after bilateral lung transplantation for cystic fibrosis.
In 36 patients thyroid cancer was diagnosed after transplantation from whom 47% were at high risk of recurrence. After a median follow-up of 14 years, 31 patients were in remission, four had persistent disease, one deceased from progression of thyroid cancer. In nine cases, a second transplantation was performed 6.5 years after thyroid cancer diagnosis. During follow-up, a remission after two local recurrences occurred in one patient, persistent disease remained stable in one and there was no recurrence in the seven other patients.
As a whole, 90% of patients were in remission at the time of the study. Seven per cent of patients experienced a recurrence and all of them were N1.
Conclusion
The prognosis of thyroid cancer does not seem to be hampered by transplantation. This suggests that thyroid cancer should not be considered as a contra indication to transplantation, even in patients with persistent disease.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1787

**Simultaneous occurrence of BRAF mutation and RET/PTC rearrangement is frequent in papillary thyroid carcinoma**

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Context
Initial studies reported that RET rearrangements, RAS mutations and BRAF mutation are mutually exclusive genetic events in papillary thyroid carcinoma (PTC). Subsequently, simultaneous occurrence of BRAF mutation and RET/PTC or H4-PTEN has been described in few PTC cases, indicating that these genetic alterations might coexist in PTC in the same cell or in different cells, at least sporadically. In light of the recent finding that frequently BRAF mutation is present only in a subset of cells in PTC, we analyzed more extensively the possibility that BRAFT1799A and RET/PTC can coexist in the same tumor.
Study design
Total RNA extracted from 75 FNABs classified as PTC at histology, were analyzed by Southern blot on RT-PCR for the presence of RET/PTC-1 and RET/PTC-3 rearrangements. Genomic DNA extracted from the same samples was analyzed by pyrosequencing for the presence of the BRAFT1799A mutation.
Results
RET rearrangements were present in 28 over 75 PTC (37.3%, 9 RET/PTC-1 and 19 RET/PTC-3). In one sample, both RET rearrangements coexisted. BRAFT1799A was present in 42 (56%) in the range 44.7–5.1% of total BRAF mutated alleles. In 14 samples, both a RET rearrangement and BRAF mutation were present (14.7%). In these tumors, BRAF mutation was always as a subclonal or oligoclonal occurrence in the range 37.5–6%.
Conclusions
These data demonstrate that RET rearrangements and BRAF mutation are not mutually exclusive but rather are frequently co-expressed in the same PTC. The data, do not exclude the hypothesis of a coexistence of the two oncogenes in different cells within the tumor.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1788

**Immunohistochemical expression of βIII-tubulin and cell-cell adhesion proteins in papillary thyroid carcinoma: a preliminary report**

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Introduction
Tubulin is a multifunctional cytoskeletal protein involved in key cellular processes, including cell movement, intracellular transport and mitosis. An increased expression of βIII-tubulin (TUBB3) has been observed in several cancer types and associated with aggressive and drug refractory disease.
Expression of claudins (CLDNs), the main component of tight junctions, is frequently altered in human cancers, including papillary thyroid carcinoma (PTC). E-cadherin (E-CD) plays a critical role in the maintenance of epithelial phenotype. Loss of E-CD expression is considered as the hallmark of epithelial-to-mesenchymal-transition.
Aim
To analyze the presence of TUBB3 protein in PTC samples in relation to expression of molecules with cell-cell adhesive role (E-CD, CLDN1 and CLDN7).
Methods
The study included 70 PTCs, 17 lymph node metastases, seven follicular adenomas and five nodular hyperplasias.
Results
No positivity was observed in follicular epithelium, nodular goiter and follicular adenoma. In PTC samples, the reactivity was heterogeneous and demonstrated strong cytoplasmic staining in widely infiltrating PTCs associated with fibrous stroma, particularly at invasive front of tumour, or in moderately differentiated PTCs with loss of cellular polarity/cohesiveness. In these areas, CLDN1/7 expression were decreased or less intense in comparison with the center of neoplasia. Decreased E-CD staining was also observed at invasive front of tumour. Conversely, PTCs with expanding margins or well-developed follicles were constantly negative.
The majority of lymph node metastases showed strong TUBB3 immunoreactivity.
Conclusions
We report for the first time TUBB3 expression in thyroid tissue.
In analogy with carcinomas of other sites, TUBB3 expression appears to be increased in PTCs with 'aggressive' histological features, thus suggesting that changes in tubulin isotype composition could modulate the invasive and metastatic potential of cancer cells.
The decreased CLDNs and E-CD expression in combination with the TUBB3 positivity, at invasive front of tumour, could be a possible morphological indicator of epithelial-to-mesenchymal-transition in PTC.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1789

**The occurrence of mutations in the CHEK2 gene in patients with papillary thyroid carcinoma and its impact on the course of the disease and the coexistence of other neoplasms**

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Introduction
CHEK2 is a gene encoding a protein involved in the mechanism of repairing damaged DNA. Mutations in the CHEK2 gene are associated with an increased risk of various neoplasms. The most common mutations in the Polish population are: I157T, less frequently IVS2+1G> A and 1100delC.
Materials
The study consisted of 277 patients with papillary thyroid carcinoma: 252 female, 25 male, aged 15 to 76 years.
Method
Patients underwent testing of the CHEK2 gene using PCR-HRM and sequencing of DNA isolated from peripheral blood. Age, severity of disease at diagnosis and the incidence of other neoplasms were assessed.
Results
Mutations in the CHEK2 gene were confirmed in a total of 47 patients, of which 38 had I157T mutation, and 9 had IVS2+1 G> A mutation.
The average age of the patients at the moment of making the diagnosis was 48 years old, both in the groups with or without the mutation.
Stage of disease progression in carriers of CHEK2 mutations at time of diagnosis ($n=47$): I-72%, II-11%, III-11%, IVA-6%.
Stage of disease progression in patients without mutations at time of diagnosis ($n=230$): I-77.4%, II-6.5%, III-12.6%, IVA-3%, IVC-0.5%. The reported differences were not statistically significant ($P=0.5727$).
Occurrence of other neoplasms were found in 28% (13/47) of patients with the mutation compared to 5.2% (12/230) of patients without mutations in the CHEK2 gene. The reported difference was statistically significant ($P<0.0001$).
Conclusions
i) Mutation of the CHEK2 gene are present in 17% of patients with papillary thyroid carcinoma.
ii) The occurrence of mutations in the CHEK2 gene are associated with a higher risk for other neoplasms, most often breast cancer especially in the case of I157T mutation (21%).
iii) There was no observed influence of mutations in the CHEK2 gene on the degree of advancement of neoplasm at the time of diagnosis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1790

**Risk of malignancy in thyroid incidentalomas detected by $^{18}$F-fluorodeoxyglucose positron emission tomography. A systematic review**

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Background
The expanding use of $^{18}$F-fluoro-deoxy-glucose positron emission tomography ($^{18}$F-FDG PET) has led to the identification of increasing numbers of patients with an incidentaloma in the thyroid gland. The aim of this study was to review the proportion of incidental thyroid cancers found by $^{18}$F-FDG PET or PET/CT imaging.
Methods
Studies evaluating thyroid carcinomas discovered incidentally in patients or healthy volunteers by $^{18}$F-FDG PET were systematically searched in the PubMed database from 2000 up to 2011. The main exclusion criteria were known thyroid disease, lack of assigned diagnoses, investigation of diffuse uptake only, or investigation of patients with head and neck cancer, or cancer in the upper part of the thorax.
Results
Twenty-two studies met our criteria comprising a total of 125754 subjects. 1994 (1.6%) had an unexpected focal hypermetabolic activity, while 999 of 48 644 individuals (2.1%) had an unexpected diffuse hypermetabolic activity in the thyroid gland. A diagnosis was assigned in 1051 (271 had surgical confirmation) of the 1994 patients with a focal uptake (366, corresponding to 34.8%, were malignant), and in 168 of 999 patients with diffuse uptake (7, corresponding to 4.4%, were malignant). In the eight studies reporting individual maximum standard uptake values ($SUV_{max}$), the mean $SUV_{max}$ value was 4.8 (SD 3.1) and 6.9 (SD 4.7) in benign and malignant lesions, respectively, ($P < 0.001$).
Conclusions
Incidentally found thyroid nodules, using $^{18}$F-FDG PET, are at high risk of harboring malignancy if uptake is focal. SUV-values are significantly higher in malignant than in benign nodules. The pronounced inhomogeneity and other shortcomings of the studies are discussed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1791

**Desmoplastic stromal reaction and extrathyroidal invasion predict lymph node involvement in sporadic macro medullary thyroid cancer**

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Background
Locoregional nodal metastases are the first step of tumor spread in MTC and have been shown to be a prognostic factor for survival. Neither palpation nor neck ultrasound can estimate nodal staging accurately.
Aim
To identify predictive factors for LNI in MTC through a retrospective study on 142 consecutive patients operated in Lille University Hospital between 1995 and 2010.
Methods
Analyses were carried on 54 patients (median age = 52 (41–61), M/F ratio = 0.54; mean tumor size = 25 mm (15–35), median calcitonin (CT) value = 1177 pg/ml (304–3470)) after exclusion of familial MTC ($n = 38$) and microMTC ($n = 27$). Total thyroidectomy plus LN dissection was performed in 98%. Pathologic slides were reviewed. Pre and postoperative CT (pre/postCT) were all available.
Results
Twenty-six (48%) patients with a sporadic macroCMT had LNI, 6 (23%) of whom had a curative resection confirmed by undetectable postCT. All LN free cases had accordingly undetectable postCT. Thirty three percent of MTC with preCT > 2000 pg/ml were unexpectedly LN free with undetectable postCT. Under univariate analysis, age and tumor size did not influence LN status. Male patients ($P = 0.027$) and higher preCT level ($P = 0.001$) were more likely associated with LNI. Positive correlation was observed between LNI and DSR ($P = 0.001$), no peritumoral capsule ($P = 0.001$), peritumoral invasion ($P = 0.00004$), vascular invasion ($P = 0.0004$) and ETI ($P = 0.003$). Multivariate analysis showed both DSR (OR = 131.67; $P < 0.0001$) and ETI (OR = 255.03; $P = 0.01$) to be independent factors for LNI. The rate of RET somatic mutation found in 45% of 29 analyzed tumors was not significantly higher in MTC with LNI.
Conclusion
At surgery, 52% of sporadic macro CMT was LN free. Though higher preCT may be indicative of LNI no threshold can be set. Since no preoperative factor can ascertain nodal status, a systematic LN dissection is still requiered. ETI and DSR are strong predictors of LNI: their impact should be addressed on further survival studies.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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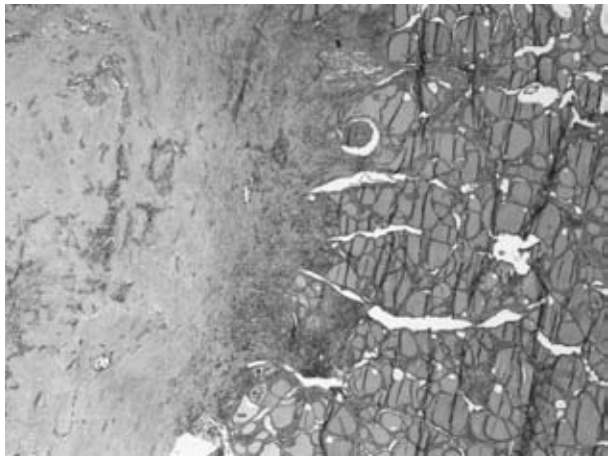

**Figure 1** Invasive MTC with an important DSR.

## P1792

**Circulating BRAFV600E in the diagnosis and follow up of differentiated thyroid cancer**

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Introduction
Although overall accuracy of fine needle aspiration (FNA) in identifying thyroid cancer is considered excellent, 20–30% of aspirates do not allow definitive diagnosis of malignancy. Proto-oncogene somatic mutations such as BRAFV600E in FNA strongly suggest the presence of malignancy. However, only few studies reported the use of circulating BRAFV600E-mutated alleles in plasma as an useful marker for non invasive diagnosis and follow up of this disease.
Methods
We propose an allele specific Taqman-based real-time PCR assay to measure plasma-circulating BRAFV600E concentration in patients affected by thyroid nodules ($n = 100$) and healthy subjects ($n = 33$) used as control group.
Results
Plasma-circulating BRAFV600E concentration (ng/ml) was significantly higher in the cytological categories of follicular lesions (Thy 3; $n = 47$, mean ± S.E.M: 2.2 ± 0.9, $P = 0.04$) and suspicious plus diagnostic for malignancy (Thy 4 + Thy 5; $n = 29$ mean ± S.E.M: 1.7 ± 0.5, $P = 0.01$) than in control group (mean ± S.E.M: 0.3 ± 0.1). Plasma-circulating BRAFV600E levels in patients with negative for malignancy cytology (Thy 2 group; $n = 27$ mean ± S.E.M: 0.8 ± 0.4, $P = 0.6$) were not significantly different compared to control subjects.
In 18 subjects affected by differentiated thyroid carcinoma the level of circulating BRAFV600E decreased significantly after surgery (from 3.1 ± 0.8 to 0.8 ± 0.3 ng/ml, $P = 0.02$).
ROC curve analysis indicated that BRAFV600E absolute concentration has the maximal diagnostic relevance with 76% sensitivity and 82% specificity.
At present all the patients with Thy 3 cytology are submitted to surgery. In this group, the comparison of the BRAFV600E status with the histological examination demonstrated a negative predictive value of 73%.

Conclusions
The present results suggest that circulating BRAFV600E might be used in the diagnosis of differentiated thyroid cancer. It may be also helpful in the follow-up of these type of cancer, especially in the cases in which thyroglobulin (Tg) is not informative, i.e when antiTg antibodies are present, preoperatively Tg is undetectable and patients were not subjected to radioablation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1793

**Screening for thyroid diseases in people from former Soviet Union emigrated in USA after Chernobyl accident**

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Research objective
After the Chernobyl nuclear reactor accident, there was a dramatically increase in thyroid cancer incidence in children and adolescents in Belarus, Ukraine and the Russian Federation. The increasing incidence of thyroid cancer in last 10 years not only in former Soviet Union but also in the United States, is real medical problem. 'Project Chernobyl' has been established in New-York for early diagnostics of radio induced thyroid cancer among people that were irradiated during Chernobyl accident and later emigrated in USA.
The study was aimed at the investigation of prevalence of thyroid diseases by screening in people who have emigrated in USA after Chernobyl accident.
Patients and Methods
A two cohorts of people (total 6870 subjects are living now in New-York area) why have emigrated from former Soviet Union underwent ultrasonography screening of thyroid and examination of thyroid function on the basis of TSH, FT4, Ab-TPO, Ab-Tg levels in serum. First cohort consisted of 2550 subjects (mean age $59 \pm 7$), exposed to fallout from Chernobyl. Second cohort consisted of 4320 subjects (mean age $53 \pm 2$) that have not been exposed to the radiation.
Results
Prevalence of nodular goiter was more higher in irradiated subjects, comparing to the not-irradiated-cohort (43.0 vs 17.5%). Thyroid cancer (8.6 vs 4.0%) and different type of thyroid dysfunction (19.0 vs 6.0%) are two times more common among of the subjects, exposed to the radiation. The patients from the cohort of the radiation exposed people have required more aggressive type of treatment. The frequency of the total thyroidectomy was more higher (95 vs 73%) in the cohort of the patients, exposed to radiation in the result of the Chernobyl accident.
Conclusions
Significantly higher frequency of serious thyroid disorders among the people exposed to radiation during Chernobyl accident and emigrated in USA was diagnosed by screening investigation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1794

**About the reproducibility of a Thyroglobulin assay**

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Thyroglobulin (Tg) is a tumoral marker of differentiated thyroid cancer (DTC). Iterative Tg assays are then mandatory for their follow-up. The maximum imprecision of any Tg measurement should be $<5\%$. However, it is unlikely that current Tg assays can maintain such tight precision over the 6–12 months time period used for monitoring patients with DTC. This precision can be overcome by measuring archived frozen samples from the patient in the same run as the current specimen. This aims to minimise inter-assay variability and to avoid inappropriate therapeutic action.
We retrospectively investigated the systematic re-assay of archived serum samples from the last 10 year: 2992 out of a total of 16689 sera. From the re-assayed sera, we analysed only those $<2.5$ ng/ml ($n=2063$). The same kit was always used: thyroglobulin IRMA, CIS bio international. Intra-assay CV were 6.4 and 3.5% at 11.6 and 37.1 ng/ml, respectively. Inter-assay CV was 4.6 (1.1–10.2)% and 4.5 (0.8–8.6)% for the last 2 year for the two controls of the kit (about 11 and 36 ng/ml).
One thousand seven hundred seventy four results were $\leq 1$ ng/ml. The median (2.5th-97.5th percentile) coefficient of variation between the two assays was 0.0 (0.0–2.2)%. When reassayed, 1748 results were $\leq 1$ ng/ml. The 26 'discordant' results ranged from 1.01 to 1.52 ng/ml. When the recommended threshold (1.5 ng/ml) for analytical variability was considered there was 1 'discordant' result (1.52 ng/ml).
Two hundred eighty nine results were $>1$ and $<2.5$ ng/ml The coefficient of variation between the two assays was 8.7 (0.4–36.7)%. When reassayed, 64 results were "discordant": 23 were $\leq 1$ ng/ml and 41 were $\geq 2.5$ ng/ml (range (2.5–4.1) ng/ml). When analytical variability was considered, this amounted to 3 'discordant' results (4.02, 4.07, 4.1 ng/ml).
Although some physicians do not draw clinical conclusion from Tg values $<1$–2 ng/ml, the assay precision is excellent throughout this measuring range questioning the opportunity of systematic retesting prior archived samples at each individual run.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1795

**Ret somatic mutations are not an early event in the tumoral transformation of sporadic medullary thyroid cancer**

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The reported prevalence of RET somatic mutations in sporadic MTC is about 40–50% and the most frequent somatic mutation is Met918Thr in exon 16. MTC harboring a somatic RET mutation have been demonstrated to have a more advanced stage at diagnosis and a worse outcome. Although RET mutations are believed to be driving events in the MTC tumorigenesis only the finding of somatic mutations in microMTC can confirm this hypothesis.
Aim of the present work was to search for RET somatic mutations in sporadic microMTC ($<1$ cm) and to compare their prevalence between microMTC and MTC of bigger size.
We selected a group of 148 MTC cases in which RET exon 16 point mutation was analyzed by direct sequencing. Tumors were classified according to the size of the nodule as follows: group A, $<1$ cm; group B, $>1$ and $<2$ cm; group C, $>2$ and $<3$ cm; group D, $>3$ cm.
The overall prevalence of RET mutation was 18.24% (27/148). RET mutations were differently distributed in the four groups. In particular it was 4.6% (2/43) in group A, 12.5% (8/64) in group B, 40% (8/20) in group C and 42.8% (9/21) in group D, thus showing an increasing rate according to the increase of the tumor size. Furthermore, when comparing the prevalence of mutations in the four groups we found a lower prevalence in microMTC ($P<0.0001$).
In conclusion these data indicate that: i) the overall prevalence of RET somatic mutations is lower than expected; ii) the prevalence of RET somatic mutations is very low (4.5%) in microMTC suggesting that they are not an early event in MTC tumorigenesis. As an alternative to this hypothesis we have to suppose that microMTC could be caused by other oncogene(s) with a lower transforming activity.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1796

**A proposal of a new clinical, ultrasonographic and cytological scoring system for thyroid nodules: the 'cut' score**

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Aim
To develop a cost-effective instrument to evaluate the preoperative malignancy risk of thyroid nodules (TNs).
Patients&Methods
A meta-analysis of the clinical (C) and ultrasonographic (U) features associated with an increased risk of malignancy was conducted searching in the PubMed database. For each of them we computed the odds ratio and we consequently assigned a score. The resulted C + U score along with the 5-tiered score of fine-needle aspiration (FNA) result (T = 1–5) designed the CUT score of the TN.
Moreover we enrolled 673 consecutive patients (514W/159M) with 689 TNs, we submitted them to clinical evaluation, ultrasonography and US-guided FNA. Hence we applied the CUT score and we correlated it with the histopathological diagnosis of the 79 TNs that underwent surgery.
Results
Features associated with a higher risk of malignancy resulted: male sex, family history of thyroid cancer, single nodule, size $>4$ cm, taller-than-wide shape, solid structure, hypoechogenicity, irregular margins, absent halo, microcalcifications and intranodular vascularization. A score was assigned to each of them.
Out of the 35 TNs with histopathological diagnosis of benign nodule, the average C + U score was 3.7 for the 3 TIR1 TNs, 2.5 for the 16 TIR2 TNs, 3.7 for the 11 TIR3 TNs and 4.1 for the 5 TIR4 TNs. Nevertheless out of the 44 TNs with histopathological diagnosis of malignancy, the average C + U score was 5.7 for the 8 TIR3 TNs, 7.6 for the 10 TIR4 TNs and 7.7 for the 26 TIR5 TNs. Sensitivity and specificity of the C + U score were respectively 96 and 56% with a cut-off of 3.5 and 67 and 94% with a cut-off of 6.5.
Conclusion
The CUT score can be a useful and cost-effective instrument in the preoperative management of TNs, especially for those with indeterminate or repetitively non-diagnostic FNA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1797

**Papillary thyroid carcinoma with high percentage of BRAFV600E alleles have a higher recurrence rate**

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Context
Although unexplained conflicting results are present in the literature, most of studies report the association of BRAFV600E in papillary thyroid carcinoma (PTC) with a more advanced disease and with a worst prognosis. We recently demonstrated that in most of the cases PTC consists of a mixture of tumour cells with wild-type and mutant BRAF. Hence, we examined the association of percentage of BRAFV600E alleles with clinicopathologic parameters at diagnosis and disease outcome in a large series of PTC.
Study design
Tumour genotyping for BRAFV600E was performed by Big Dye Terminator and pyrosequencing in 168 patients with PTC. The association between clinico-pathologic characteristics including disease recurrence at follow-up (median = 5.1 years) and the percentage of BRAF mutant alleles was determined.
Results
The prevalence of BRAFV600E was higher by pyrosequencing then by Big Dye Terminator (53.6 vs 36.9%). In the PTC positive for BRAFV600E, the percentage of mutant alleles ranged 5.1–44.7% of total BRAF alleles, with a median of 20.6%. The presence or the percentage of BRAFV600E alleles did not correlate significantly with gender, multicentricity, lymph node metastasis, and tumour stage. The percentage of BRAFV600E alleles directly correlated with age at diagnosis and tumour volume ($R^2 = 0.223$, $P = 0.039$ and $R^2 = 0.166$, $P < 0.001$ respectively). The percentage of BRAFV600E alleles ($P = 0.014$), tumour volume ($P = 0.012$) and lymph node metastasis ($P = 0.008$) predicted the disease outcome. The odds ratio of recurrence in PTC with BRAFV600E alleles $\geq 30\%$ was a 5.31-fold higher ($P = 0.002$) in comparison with PTC with BRAFV600E alleles $< 30\%$.
Conclusions
High percentage of BRAFV600E alleles defines a PTC molecular subtype with a poorer disease outcome. Analysis of BRAF mutation by pyrosequencing is useful to refine the risk stratification of patients with PTC.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1798

**Sentinel lymph node biopsy in differentiated thyroid carcinoma and decision for selective modified radical neck dissection**

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Background
The accuracy of sentinel lymph node biopsy (SLNb) in decisions for surgical management of lymph nodes in differentiated thyroid ccarcinoma (DTC) was demonstarted in a few previous studies.
Aim
To determine whether SLN biopsy of first draining node/s in jugulo-carotid chain is accurate technique to select patients with true positive LN for selective modified radical neck dissection (MRND).
Patients and methods
We have performed SLN biopsy in 172 patients with DTC. Before mobilization of the thyroid gland, 0.2 ml of 1% solution of methylen blue dye was injected peritumorally. After 10 min the dissection was continued around omohyoid muscle, towards the internal jugular vein and carotid artery until blue stained LN were found and sent for frozen-section examination. An extended dissection of level III/IV was done consecutively. All LN were examined by frozen section and conventional (HE) histopathology examination. If positive, MRND was performed after total thyroidectomy and routine dissection of central neck compartment.
Results
Identification rate of SLN was 93.5%. Specificity and sensitivity of the method were 100 and 80% respectively. Negative and positive predictive values were 94.7 and 100%. Overall accuracy of the method was 95.6%. Conclusions: According to previous data, status of lower jugulo-carotid LN significantly predicts the status in upper two thirds. Our results imply that SLNb in the jugulo-carotid chain using methylene blue dye mapping, is feasible and accurate method for estimating LN status in the lateral neck compartment. The method may support a decision to perform selective MRND in patients with DTC.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1799

**Therapeutic outcome and prognosis in young patients with papillary and follicular thyroid cancer**

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The aims of this study are to assess the clinical characteristics of papillary and follicular thyroid cancer in young patients. We performed a retrospective analysis of 116 patient aged 20 years who underwent thyroidectomy and a mean follow-up of $11.1 \pm 0.6$ years. There were 28 (24.1%) patients were classified into the residual cancer or relapse groups. Two of the 28 patients died of thyroid cancer. Thirteen patients who showed relapsed underwent 131I whole body scan; six of the 13 patients were diagnosed with distant metastases. Among the young patients, the 5- and 10-year progression-free survival rates were 79.1 and 73.4%, respectively. In conclusion, the progression-free survival in young patients with papillary and follicular thyroid cancer was lower than the patients of age 20–45 years; otherwise, cancer survival higher than age group over or equal 45 year-old.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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**Table 1** Characteristics of papillary and follicular thyroid carcinoma patients in different age groups.

| Group | ≤20 years ($n$=116) | >20 and <45 years ($n$=1.308) | ≥ 45 years ($n$=1.119) | *P* value |
|---|---|---|---|---|
| Age (years) (S.D.) | 17.1 (3.3) | 33.8 (6.3) | 56.6 (9.1) | a,b,c |
| Gender (female/-male) (ratio) | 92/24 (3.8) | 1,091/217 (5.0) | 841/278 (3.0) | 0.0001 |
| Histology (papillar-y/follicular) (ratio) | 98/18 (5.4) | 1,201/107 (11.2) | 956/163 (5.9) | 0.0001 |
| TNM stage (I/II/III/IV) | 109/7/0/0 | 1,267/41/0/0 | 361/202/163/393 | 0.0001 |
| Microcarcinoma (%) | 6 (5.6%) | 226 (18.4%) | 204 (19.9%) | 0.0014 |
| Tumor size (cm) | 3.0 (0.1) | 2.3 (0.1) | 2.7 (0.1) | a, c |
| Multicentric (%) | 11 (9.5%) | 191 (14.6%) | 259 (23.1%) | 0.0001 |
| Surgical method* (less aggressive) | 28 (24.1%) | 260 (19.9%) | 222 (19.8%) | 0.5315 |
| Status at diagnosis | | | | |
| Local neck (%) | 36 (31.0%) | 369 (28.3%) | 364 (32.5%) | |
| Distant metastasis (%) | 7 (6.0%) | 41 (3.1%) | 128 (11.4%) | |
| Post-op 131I uptake (%) | 10.3 (1.5) | 7.9 (0.4) | 7.4 (0.4) | b |
| Post-op 131I abla-tion (%) | 102 (87.9%) | 1,102 (84.3%) | 912 (81.5%) | 0.0742 |
| Postoperative Tg∧ (ng/ml) | 182 (93) | 112 (35) | 389 (90) | c |
| External radiation | 9 (7.8%) | 41 (3.1%) | 125 (11.2%) | 0.0001 |
| Relapse / residual | 13/15 | 78/63 | 113/179 | 0.0083 |
| Disease free | 44 (37.9%) | 486 (37.2%) | 293 (26.2%) | 0.0001 |
| 2nd primary cancer | 3 (2.6%) | 46 (3.5%) | 114 (0.2%) | 0.0001 |
| Follow-up period (years) | 11.1 (0.6) | 9.6 (0.2) | 7.3 (0.2) | a, b, c |
| Thyroid cancer mortality (%) | 2 (1.7%) | 27 (2.1%) | 159 (14.2%) | 0.0001 |
| Total mortality (%) | 3 (2.6%) | 40 (3.1%) | 256 (23.0%) | 0.0001 |

Data are mean (SD) unless otherwise stated. Surgical method*, less aggressive than total thyroidectomy. Postoperative Tg∧, serum thyroglobulin level 4–6 weeks after thyroid operation. a: ≤20 years vs >20 and <45 years; b: ≤20 years vs ≥45 years; c: >20 and <45 years vs ≥45 years, $P<0.001$.

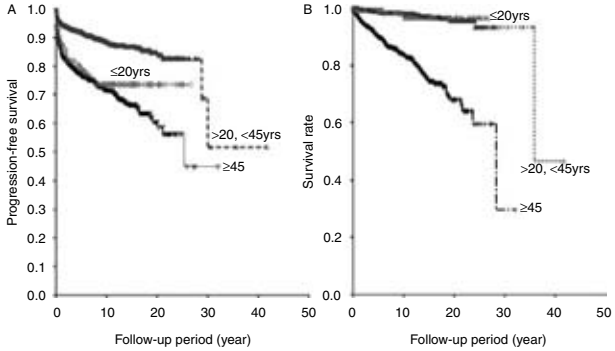


**Figure 1** Progression-free survival (A) and thyroid cancer-specific survival (B) curves of the thyroid cancer patients in the different age groups. (FIG 2A: <20 years vs >20 years to <45 years, $P=0.0001$; <20 years vs >40 years, $P=0.2369$; >20 years to <45 years vs >40 years, $P=0.0001$). (FIG. 2 B: <20 years vs >20 years to <45 years, $P=0.7119$; <20 years vs >40 years, $P=0.0001$; >20 years to <45 years vs >40 years, $P=0.0001$).

## P1800

### High prevalence of BRAFV600E mutation in the papillary thyroid carcinomas in Korea by peptide nucleic acid clamp real-time PCR

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Introduction
Activating somatic mutation of the BRAF gene (V600E) has been identified as the most common genetic event in papillary thyroid carcinoma (PTC) with a variable frequency (32–87%) in various races by different methods.
In this study, we report the genetic analysis of BRAF mutation in a Korean cohort of 211 PTC by peptide nucleic acid clamp real-time PCR.
Methods
PNA clamp real-time PCR was applied for BRAF analysis in 211 PTC tissues. Statistical analyses for clinicopathological findings were obtained by means of Fisher's exact test or Student *t*-test.
Results
The BRAF gene was mutated in 90% of Korean TPC. The BRAF mutation occurred at a significantly higher frequency in male patients than in female patients ($P=0.002$). Age, tumor size and tumor stages were not correlated with BRAF mutation, while lymph node metastasis ($P=0.004$) and tumor multi focality ($P=0.03$) were correlated with BRAF mutation. Extra thyroidal invasion had tendency to correlate with BRAF mutation ($P=0.058$). BRAF mutation was highly occurred in all variants of PTC.
Conclusions
The present study on the BRAF mutation in Korean PTC by PNA clamp real-time PCR shows highest prevalence ever reported, and we can confirmed that this mutation is the key role in tumorigenesis of PTC. The PNA clamp real-time PCR method for the BRAF mutation detection is very sensitive test. So it can be applicable in clinical setting as with minute amount of cells by FNA.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1801

### Role of realtime elastography in diagnostic of thyroid cancer

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Background
The most important aspect in the diagnostic approach of thyroid nodule is identifying the malignancy. Elastography is a newly technique that measures the elasticity of tissue, after applying a external force, standardized control. US elastography is currently used in differentiation of malignant from benign lesions.
Material, Method
This prospective study included 69 patients, mean age 50.08±12.43 years, 67 females and 2 men, with thyroid nodules on conventional US, with a volume higher than 0.20 ml: a total of 107 nodules. Conventional, Doppler ultrasound and real time sonoelastography was performed with a HITACHI EUB 7500 HV machine with 6–13 MHz variable frequency linear probe, with Doppler and elastography software, with recording of frames of all lesions prospectively on color elastography color map 1 (red-yellow-green-blue color map); Hitachi Medical System, Tokyo, Japan. All patients underwent surgery after complete evaluation. Extemporaneous and postsurgical histopathological exam was performed in all cases. Tissue stiffness was scored from one (greatest elasticity) to 5 (no strain), according to the UENO scale.
Results
###27 nodules had score 1 on US elastography and 37 nodules had score 2. All nodules were benign on histopathological exam. Score 3 was found in 33 cases, 32 benign and one papillary carcinoma. Score 4 as found in 10 cases, all carcinomas. ES score of 4 is highly predictive for malignancy (sensitivity of 90.9%, specificity of 98.96%, positive predictive value of 100%, negative predictive value if 98.60%). The diagnostic quality of elastography was independent of the nodule volume.
Conclusion
US elastography has a good potential in diagnosing thyroid malignancy, independent of nodule volume, allowing the study of small nodules, less than 0.5 ml.
Predictive value of us elastography in patients with histopathological diagnostic
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** PREDICTIVE VALUE OF US ELASTOGRAPHY IN PATIENTS WITH HISTOPATHOLOGICAL DIAGNOSTIC

| ES Score | sensitivity | specificity | positive predictive value | negative predictive value |
|---|---|---|---|---|
| ES 1 for benign condition | 28.12 | 100 | 1.0 | 0.137 |
| ES 2 for benign condition | 66.66 | 100 | 1.0 | 0.25 |
| ES 1-3 for benign condition | 100 | 90.9 | 1.0 | 0.90 |
| ES 3 for cancer | 9.09 | 66.66 | 0.03 | 0.864 |
| ES 4 for cancer | 90.9 | 98.6 | 1.0 | 0.986 |

## P1802

**Coexistence of multiple lipomatosis and differentiated thyroid cancer: more than a coincidence?**

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Subcutaneous lipomas (SL) are the most common benign mesenchymal tumors with an estimated prevalence of 1%. They usually appear single, between 50–60 years of age, without gender differences. However, multiple lipomatosis (ML) occurs in 5–10% of patients, more frequently in men and almost 30% of them have family history of ML. Cytogenetic abnormalities are found in 50–80% of cases (at 12q15) and the presence of an underlying mitochondrial dysfunction has been suggested. Up to this date, there are no reports of SL/ML prevalence in patients with differentiated thyroid cancer (DTC). We have reviewed 240 patients with DTC, identified those with SL/ML and compared their features with those of the whole group. Among 194 women and 46 men (19%) with DTC, being 5% Hürthle cell carcinomas (HCC), we found 15 patients, 9 women and 6 men (40%), with ML mainly in arms, back and legs. Twelve of them (80%) referred appearance of ML in several members of their families at an early age. In the ML group there were 5 HCC (33%) (4 mixed with areas of multicentric papillary thyroid microcarcinoma), 1 multicentric mixed papillary-follicular carcinoma and 9 papillary (1 diffuse sclerosing variant, 2 microcarcinomas and 4 multicentric). Four men had suffered nonthyroid cancer (NTC): 1 seminoma, 2 melanomas and 1 colon cancer. Eleven patients had first-degree relatives with NTC mostly lung, breast and liver. Only 2 cases remained free of DTC. In our series the prevalence of ML is significantly higher than that of the general population with an increased frequency of familiar forms. When compared to the whole DTC group, ML is significantly associated with a higher rate of men, HCC, mixed forms, multicentricity and less probability of remaining free of disease (all $P<0.001$). Based on our findings ML could be considered a new bad prognostic marker in DTC.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1803

**Thyroid follicular lesion of undetermined significance - the risk of malignancy in postendemic area**

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The introduction of the new classification of the thyroid FNAB results which stratifies the risk of malignancy of thyroid follicular lesions is of special interest in endemic and postendemic areas. In such regions patients with non-neoplastic nodular goitre predominate and nodules diagnosed previously as 'follicular neoplasm' showed to be thyroid cancer less frequently than in iodine-rich areas. The aim of the study was to evaluate the frequency of "follicular lesion of undetermined significance" (FLUS) diagnosis and to assess the risk of malignancy in this category in postendemic areas.

In 2010 our Department adopted six-category diagnostic approach in reporting thyroid FNAB results that was based on National Cancer Institute (NCI) recommendations. In this system aspirates from follicular lesions are classified into 2 categories: FLUS and SFN (suspicious for a follicular neoplasm). The study included 5480 patients in whom 8877 nodules were biopsied between May 2010 and November 2011.

There were 218 nodules diagnosed as FLUS (2.5%) in 203 patients, including 4 cases of the oxyphilic type. In 92 cases the follow-up was determined: 51 patients underwent control FNAB and 48 patients were treated surgically (7 patients were operated after control FNAB). The results of control FNAB showed: papillary cancers in 2 cases, lesion suspicious for papillary cancer - 1, SFN - 1, FLUS - 19 and benign lesions - 24. In 4 patients control FNAB was non-diagnostic. Histopathological examination showed papillary cancer in 4 patients (in 1 patient it was microcancer in the contralateral lobe), follicular adenoma in 4 patients, chronic thyroiditis in 3 patients and hyperplastic nodule in 37 patients. In total the thyroid cancer was found in 4.3% (4/92) of verified patients with FLUS.

In postendemic areas the incidence of thyroid cancer in the nodules diagnosed as FLUS is lower than 5–15% reported by the NCI. It suggests that such lesions should be followed up clinically without prompt surgical intervention.

Declaration of interest
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## P1804

**TSH levels do not associate with the risk of papillary thyroid microcarcinoma in Korean patients**

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Background
Thyroid cancer has been increasing worldwide and became one of the most popular cancer. Recent reports have shown that an elevated thyrotropin(thyroid stimulating hormone, TSH) level is associated with risk of thyroid cancer. Nevertheless, the association between TSH level and thyroid cancer risk is not yet known in patients diagnosed with papillary thyroid microcarcinoma (PTMC).
Methods
We collected cases of 212 patients who underwent thyroid surgery and were diagnosed with PTMC between 2009 and 2011. We also investigated data from control group patients who were diagnosed with benign nodules $\leq$ 1 cm in size by US-guided fine needle aspiration. Patients who have multinodular disease, were not euthyroid or took thyroid medication were excluded.
Results
The mean age of all patients was $52.5 \pm 16.0$ years and 70.8% were women. The mean age of those with PTMC was significantly lower than that of the control group. The mean TSH level was $1.47 \pm 0.93$ mIU/L, and the mean free T4 level was $15.96 \pm 2.32$ pmol/L. There was no difference in TSH level between the PTMC and control groups ($1.49 \pm 0.93$ mIU/L vs. $1.46 \pm 0.76$ mIU/L, $P=0.93$). After adjusting for age, TSH level was not correlated with tumor size ($r=0.02$, $P=0.77$) in the PTMC group.
Conclusion
TSH levels did not differ between PTMC patients and control group. Therefore, further studies are needed to use serum TSH as a tumor marker for thyroid nodule in euthyroid patients.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1805

**Differentiated thyroid cancer and hemodialysis: Long-term outcomes and safety after radioiodine use.**

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Introduction
The use of radioiodine is a common treatment for differentiated thyroid cancer. However, special care should be exercised in treating patients who are on hemodialysis, due to the renal elimination of the radionuclide, which implies several technical difficulties and safety concerns. We have previously described the procedure and the short-term outcome and safety in three patients (Jimenez et al, Thyroid 2001; 11(11):1031–1034). Our results showed that the radioactive iodine therapy was a safe procedure in these patients. Nevertheless, that report was limited to the short-term outcome.
Objective
To determine the long-term safety and outcome in patients on hemodialysis treated with radioiodine
Methods
We conducted a retrospective descriptive study, searching our clinical record database from 2000 to 2010. Inclusion criteria: Differentiated thyroid cancer and radioiodine treatment on hemodialysis. Exclusion criteria: anaplasic thyroid cancer, medullary thyroid cancer, multiple endocrine neoplasia, peritoneal dialysis. Final sample $n=9$.

Results
All time periods are expressed as median values. Baseline characteristics: $n=5$ males, age 48 years; $n=8$ papillary thyroid cancer, $n=1$ follicular thyroid cancer; $n=5$ nodal invasion; $n=1$ metastatic disease. After 7.5 years since radioiodine treatment on hemodialysis, $n=7$ patients were deemed free of thyroid disease, while only one persisted with non-localized disease. 3.25 years after radioiodine treatment, $n=4$ patients underwent renal transplantation without complications related to the radioisotope use. The other four patients were not eligible for transplantation due to causes not related to their thyroid disease or the I131 treatment, and no changes in the evolution of their renal disease were recorded. One patient died from cardiac arrest, free of thyroid disease.
Conclusions
The radioiodine treatment in patients on hemodialysis with differentiated thyroid cancer is a safe and effective procedure at medium-long term, and allows renal transplantation without developing a higher risk of complications compared to patients which are not on hemodialysis.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1806

**Utility of repeated recombinant human TSH (rhTSH)-stimulated tiroglobulin (Tg) test in patients with differentiated thyroid carcinoma (DTC) without evidence of disease at their initial rh-TSH-stimulation test**

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Current guidelines recommend measurement of rhTSH-stimulated Tg, with neck ultrasound 6–12 months after the initial therapy for DTC (Total thyroidectomy plus 100 mCiI131 ablation of the thyroid bed). Uncertainty persists regarding whether the subsequent follow up should be based on measurement of basal serum Tg or whether rhTSH-stimulated Tg should be performed and at what frequency. The aim of our study was to evaluate the utility of repeated rhTSH-stimulated Tg. This was a retrospective study of 40 patients; 30 female; mean age was 45.5 years (25–74); Histology: 32 Papillary, (3 follicular variant and 1 tall cell variant); 7 Follicular. Initial disease stage I $n=28$; II, $n=3$; III, $n=7$; IV, $n=2$. Mean tumor size was 2.61 cm. Pre-I131 ablation Tg levels were 7.4 ng/mL (0–29.2)(functional sensitivity 0.9 ng/mL). All of them had an undetectable rhTSH-stimulated Tg with undetectable Tg antibodies at initial evaluation. The number of repeated tests performed varied from 1 to 3 during a follow-up period of 48–132 months. (1 in $n=40$; 2 in $n=34$; 3 in $n=12$).
Tg was undetectable in all patients in the first test. In three patients Tg become detectable in the second or third: 1 patient false positive, 1 patient showed supraclavicular nodal disease (basal Tg was also detectable) and other developed lung metastases. Both patients were older than 65 years old and had at the time of diagnosis a stage III disease with extrathyroidal extension of the tumor and unfavorable histology (columnar cell).
In our patients with DTC, the first rhTSH-stimulated Tg is an excellent predictor of remission independent of clinical stage at presentation. In our experience repeated rhTSH stimulating testing was of limited value although a second negative test performed 5 years after initial treatment might assure clinical remission. Most of the patients can be followed up with basal Tg and neck ultrasound.
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## P1807

**Epidemiology of refractory thyroid cancer**

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Introduction
Thyroid carcinomas (TC) are rare; the incidence in the French Marne-Ardennnes thyroid registry is 10/100.000 in 2010. The 10 year survival rate is greater than 90%. Nevertheless some TC are refractory to conventional therapy.
Aim of the study
To describe frequency and clinical features of refractory TC (RTC).
Patients and methods
Retrospectiv study from the database of the registry. 1427 patients with TC were diagnosed between 01/01/1983 and 31/12/2005. We used AJCC, 6th edition. RTC included metastatic differentiated TC (DTC) refractory to radioiodine therapy, metastatic or locally advanced medullary TC (MTC), anaplastic TC (ATC).
Results
RTC represented 99/1427 (7%).
34 DTC: 2.5% of all DTC; 23 women, 11 men, mean age at diagnosis 63.5 years, mean tumor size 52.5 mm. 28/34 (82.3%) were stage pT3 or pT4 and 18/34 (52.9%) M1 at diagnosis. Classification has RTC occured after 40.8 months, mean survival was 7.8 years. 23.5% were papillary (2/18 papillary NOS, 1/18 follicular variant, 4/18 diffuse sclerosing, 1/18 columnar cell carcinoma); 53%, were follicular (1/18 minimally invasive; 5/18 widely invasive; 12/18 oxyphilic cell type) and 23.5% insular (8/34)
19 MTC: 22.6% of all MTC; 9 women, 10 men, mean age at diagnosis 59.3 years. 11/19 sporadic and 1 familial case, no genetic results for 7/19, mean tumor size 30 mm. 17/19 were stage IV. Mean survival was 4 years when they were M1 at diagnosis, 11 years for M0.
46 ATC: 33 women, 13 men, mean age at diagnosis 72.6 years, mean tumor size was 73.7 mm, 16/46 M1 at diagnosis. Mean survival was 7 month.
Conclusion
In this retrospectiv study 7% (99/1427) of all patients with TC are refractory: 34% DTC, 19% MTC and 47% ATC. 74% were diagnosed refractory at diagnosis and 26% during the follow-up. We need prospectiv epidemiological studies to confirm these preliminary results.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1808

**Glucagonlike Peptide-1 Receptor Imaging as diagnostic tool in patients with Medullary Thyroid Carcinoma (MTC) - preliminary report.**

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Introduction and aim
MTC accounts for 5–10% of all thyroid cancers. Because of elevation postoperative calcitonin, short doubling time of calcitonin and no disease in available imaging techniques in some MTC patients, searching for new targets for radioisotope diagnostics is necessary.
Among other overexpression of GLP-1 receptors have been shown on parafollicular thyroid C-cells.
The aim of this study was to present the first experiences with the use of 99mTc-labeled GLP-1 analogue in MTC imaging.
Material and method
99mTc-GLP-1 receptor scintigraphy with [Lys40(Ahx-HYNIC-99mTc/EDDA) NH2]-exendin-4 was performed in 3 patients: man 70y, dissemination of MTC (2008), qualified to PRRT, local recurrence of MTC (2011); man 74y, metastatic lesions of MTC in liver and lymph nodes (2009), qualified to PRRT, since then stabilization of disease; man 22y, with genetically confirmed MEN2a syndrome, suspicion of local recurrence after abnormal pentagastric test (2009), USG revealed hypogenic leasion with following negative biopsy result. The lyophilized kit prepared by IAE POLATOM was used for preparing the tracer. WB scans were performed at 6 time points and SPECT at 3 points. The hybrid device SPECT/CT was used for performing examinations.
Results
In the first patient, 99mTc-GLP-1 scintigraphy confirmed the recurrence of the disease. In the second patient, a pathological tracer accumulation in the liver was found. For both patients the similar images to GLP-1 scintigraphy were obtained in SRS scintigraphy. In the third patient, the GLP-1 scintigraphy showed the tracer uptake in the same place as 99mTc and 131I scans. Due to lack of other lesion localizations, the patient was qualified to surgery. In all patients no side effects after the tracer injection were observed.
Conclusion
[Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 seems to be an promising new tracer for clinical practice in case of MTC patients, to assess recurrence and advancement of the disease especially when standard imaging techniques failed.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1809

**ARG982CYS is a new variant of the RET proto-oncogene: is it a polymorphism or a transforming mutation?**

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In this study we describe a new allelic variant of RET, identified in a family with Medullary Thyroid Cancer (MTC) and negative for the classical RET mutations, that causes an Arg982Cys substitution in exon 18.
The "in silico" analysis has shown that this variant has a reduced compatibility [score 15] with the normal biological activity of the native protein and could therefore be a transforming mutation.
The aim of this work was to verify whether this new variant was correlated to MTC development.
The study was conducted on 6 family members, 2 affected with MTC and 4 healthy subjects, and on 172 patients with sporadic MTC. We also studied 176 healthy controls. The new RET variant was analized by direct sequencing of PCR amplified DNA.
Our data showed the presence of the Arg982Cys variant in 3 subjects of the family, including 2 healthy subjects and 1 patient with MTC. In the group of sporadic MTC tumors the Arg982Cys variant was observed in 12/172 (6.9%) cases. The allelic frequency of Arg982Cys was 3.5% (12/344). Among the 176 control subjects, 10 (5.7%) had the Arg982Cys variant and the allelic frequency was 2.8% (10/352). Comparing the allelic frequency of the Arg982Cys variant in the two groups [MTC vs. healthy subjects] no statistically significant difference was observed.
In conclusion, this study describes a new variant of RET that does not seem to be predisposing to the development of MTC but may instead be considered a true polymorphism, never described up to now. The "in silico" analysis identifies an altered biological activity of the mutant protein that could still play a pathogenic role in other diseases. It should be noted that this variant has been described in some cases associated with Undine's syndrome, which was not observed in our subjects.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1810

**Utility of the perciutaneous ethanol injection (PEI) in the treatment of metastatic adenopathies of thyroid carcinoma**

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Introduction
Surgery is the usual therapeutic approach for treatment of recurrent lymph node metastases due to thyroid carcinoma. However, there is no evidence of improvement in the final prognosis and is not an option without risk, especially in patients such as those who already underwent several surgeries or suffered from comorbidities.
PEI is a useful alternative for treatment of selected thyroid diseases. Here, we present our experience in patients with recurrent lymph node metastases.
Patients and methods
Patients: 24 recurrent metastatic lymphadenophaties in 8 patients, previously diagnosed of thyroid carcinoma with high risk of surgical complications. Histology: 4 medullary and 4 differentiated thyroid carcinomas.
Methods: Ethanol (0.4–1 mL) was infused inside adenopathies under ultrasonographic control. Maximum diameter and vascularisation pattern of each adenopathy, serum thyroglobulin (TG) and calcitonin (CT) were measured previously and 3 months after the injection. Time of follow-up was 3 to 31 months. Persistence of vascularisation as well as positivity of biochemical markers were criteria for repeat PEI. We defined as success criteria: adenopathy disappearence or decreased in size with absence of vascularisation and decreament of CT or TG levels.
Results
Sixteen out of 24 adenopathies disappeared or decreased in size with absence of vascularisation (two embolizations were needed in four cases).
In the rest: Six out of 8 decreased in size and are waiting for a new PEI; the other two adenopathies were in the same patient, who developed distant metastases and died before receiving any other treatment.
TG values decreased in all patients (from 82 to 100% compared with its initial value), whereas serum CT showed no change in 2 patients and decreased in the other two.
Conclusion
PEI could be considered as a useful therapeutic tool for treatment of metastatic lymphadenophaties in selected cases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1811

**Genotype - phenotype correlations and timing of prophylactic thyroidectomy in patients with familial medullary thyroid carcinoma**

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Background
Recommendations on the timing of prophylactic thyroidectomy (PTTE) in patients with familial medullary carcinoma (FMTC) are based on classification of RET mutations into four risk levels according to genotype - phenotype correlations. In clinical practice it is/was not always possible to fulfill the recommended timing of PTTE.
Objectives
To study the clinical course of FMTC patients with various RET mutations who were operated with a time delay against recommended timing.
Patients and methods
Retrospective analysis of clinical course in 42 RET mutation carriers from 12 FMTC and MEN 2A families.
Results
###25 high risk mutation carriers (codone 634) undergone an operation at the age 13–48 years. In all but one aged 13 years MTC was histologically confirmed. 30% of them continued to suffer from persistent disease after delayed operation and 70% achieved remission. One patient operated after 40 years of age died from the progression of MTC after 6 years.
###12 patients with low risk mutations (codone 618 and 620) had surgery aged between 16–60 years. In all but one aged 52 years MTC was verified. In the whole group of patients remission was achieved only in 58%.
In 3 least high risk mutation carriers (codones 791) aged 36, 40 and 42 years respectively TTE was indicated after possitive calcium stimulation test. It was completelly prophylactic, only C-cell hyperplasia was revealed.
Conclusions
Our data confirm the necessity to fulfill the guidelines on the timing of PTTE at the age of 5 years for high risk mutation (codone 634) carriers, while in least high risk mutation carriers surgery may be postponed untill an abnormal stimulation test result is observed (delay even to young adulthood). Our results in a small group of "low" risk mutation carriers (codones 618 and 620) suggest the need to be much more radical. In the case of negative results of thyroid USG and calcitonin stimulation test PTTE can be postponed no later than to the age of 10 years.
Declaration of interest
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## P1812

**Morphology and metastatic potential of papillary thyroid carcinoma**

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Papillary thyroid carcinoma (PTC) is a malignant tumor of follicular cell origin that is characterized by a broad diversity of morphological features. There are 15 different histological types of PTC in the recent edition of WHO classification. Morphology of PTC is important to estimate a metastatic potential of tumor and to make an accurate prognosis of clinical behaviour.
Objective of the work is to study a relationship between frequency of regional metastasis and morphological variant of PTC, size of primary tumor and breadth of invasiveness.
Materials and methods. Histological sections, paraffin blocks and pathology reports of 325 PTCs in 322 patients (60 males and 262 females aged from 10 to 76 years) were studied. Histological typing was made in accordance with WHO classification (2004). Conventional type of PTC was subclassified into variants with and without focal tall cell component.
Results. Conventional type of PTC (86 tumors) is diagnosed in 85 patients, follicular variant - in 11, solid - in 26, encapsulated - in 7, oxyphilic type - in 4, Warthin-like - in 9, tall and columnar cell variant - in 7 cases. Conventional type of microPTC (122 tumors) is revealed in 120 patients, microcarcinoma with follicular architecture - in 25, with solid architecture - in 28 cases. Regional metastases are revealed with high frequency in PTCs of oxyphilic type (100%), tall and columnar cell (71%) and solid variants (62%); with moderate rate in PTCs of conventional (49%), follicular (45%), Warthin-like variants (44%) and microPTC of conventional type (32%); with low frequency in microPTC of solid-follicular type (11%). PTCs with regional metastasis have biger size of primary tumor and higher frequency of vascular invasion than PTCs without metastasis. MicroPTCs of conventional type with focal tall cell component are characterized by higher frequency of regional metastasis (50%) in comparison with microPTCs in patients of the same age, gender and tumor invasiveness without focal tall cell component (24%).
Conclusions. Frequency of regional metastasis is varied in different histotypes of PTC and is dependent on the size of primary tumor and vascular invasiveness. Papillary microcarcinomas of conventional type with focal tall cell component have higher metastatic potential in comparison with microcarcinomas without tall cell component in patients of the same age, gender, tumor size and invasiveness.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1813

**Surgical Management of Primary Thyroid Carcinoma Arising in Thyroglossal Duct Cyst**

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Background
Thyroid carcinoma in a thyroglossal duct cyst is very rare and surgical management is based on the individual experience.
Patients and methods
Twelve cases of primary TDC thyroid carcinoma operated in one Institution during last 25 years. Sistrunk's procedure was done in all cases followed by dissection of submental and prehyoid lymph nodes and bilateral biopsy of level 3. In the same act 11 of 12 patients underwent total thyroidectomy. In cases of synchronous thyroid gland carcinomas central neck dissection and frozen-section examination of level IV LN was done. In cases of LN involvement modified radical neck dissections (MRND) were performed.
Results
Definitive pathology revealed 11 papillary and one follicular TC in TDC. Synchronous thyroid gland carcinoma was found in 3 cases (27%). LN metastases were found in six patients (50%) MRND was done in 5 cases and central neck dissection in four cases. Radioiodine therapy was applied in five patients. All our patients are alive.
Conclusion
TDC carcinoma is essentially a thyroid carcinoma and must be treated as a primary thyroid cancer. Our results imply that TC in TDC were associated with synchronous thyroid gland carcinomas in one-third and LN metastases in half of cases Active searching for cervical metastases is highly recommended considering the high incidence of LNM in papillary thyroid carcinoma. In a lack of surgical consensus, this algorithm can be safely applied to obtain optimal radical surgery in those patients.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1814

**Differentiation of thyroid nodules using Acoustic Radiation Force Impulse Imaging**

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Objective
Acoustic Radiation Force Impulse (ARFI)-Imaging is a novel ultrasound-based elastography method enabling quantitative measurement of tissue stiffness. The aim of the present study was to evaluate ARFI for the differentiation of thyroid nodules and to compare it with the well known qualitative Realtime-Elastography (RTE)
Materials and Methods
ARFI-imaging involves the mechanical excitation of tissue using short-duration acoustic pulses to generate localized displacements in tissue. The displacements result in shear-wave propagation which is tracked using ultrasonic, correlation-based methods and recorded in m/s. Inclusion criteria were: nodules $\geqq 5$ mm, non-functioning or hypo-functioning on radionuclide scanning, and cytological/histological assessment. All patients received conventional ultrasound, real-time elastography and ARFI-imaging.
Results
###129 nodules in 113 patients were available for analysis. One-hundred-fourteen nodules were benign on cytology/histology, and 15 nodules were malignant (8xpapillary carcinoma, 4xfollicular carcinoma, 3xmedullary carcinoma). The median velocity of ARFI-imaging in the healthy nodule-free thyroid gland, as well as in benign and malignant thyroid nodules was 1.74m/s (range:0.89–3.33m/s), 1.91m/s (range:0.72–3.73m/s), and 3.63m/s (range:0.80–6.83m/s), respectively. The velocity in malignant nodules was significant higher than in benign nodules ($P=0.001$). The negative predictive value of ARFI for the diagnosis of malignant thyroid nodules was 94% using a cut-off of 3.0m/s. This was comparable to RTE with a NPV of 95%.
Conclusions
ARFI can be performed in the thyroid tissue with reliable results. This novel quantitative elastography-method can be performed with negative predictive value in the diagnostic work-up of thyroid nodules.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1815

**Calcitonin levels in washout of cytology needle on thyroid nodule without suspicion of medullary thyroid carcinoma**

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Introduction
We study the calcitonin levels measurement in the washout of FNAB needles after sampling each thyroid nodule (FNAB-CT) as an approach to calcitonin normal value in them and distinguish of MTC and to correlate with serum CT (sCT) and cytology FNAB.
Material & Methods
109 consecutive subjects (mean age $53.7 \pm 13.4$, range 18–85 yr, 79.8% females, 27.5% smokers, 18.3% autoimmune thyroid disease) initially no suspicious for MTC who underwent FNAB for thyroid nodule served as study poblation (mean size $20.1 \pm 9.3$ mm.) FNAB was performed in thyroid nodule and the same needle

and syringe used for FNAB was washed with 1 mL of % 0.9 sodium chloride solution and sent to the laboratory for calcitonin evaluation (FNAB-CT). Both serum and washout calcitonin were measured by chemiluminescence assay (IMMULITE 2000)
Results
In patients with a thyroid nodule under evaluation, sCT were $3.07 \pm 3.22$ ng/l (range 2–19.4 ng/l) and and FNAB-CT values $3.98 \pm 10.36$ ng/l (range 2–97 ng/l) , respectively. In 2 patients, FNAB-CT values were 10x higher than the highest values found, but cytology results were compatible with a benign thyroid lesion and papillary carcinoma; both were submitted to surgery found MTC (serum CT/FNAB-CT: 382/32.250 ng/ml, respectively) and microMTC/C-Cell hyperplasia (serum CT/FNAB-CT: 4.5/1.150 ng/ml, respectively) In the other cases FNAB-CT values not show significative correlation with any study parameters (age, sex, autoimmune thyroiditis, tobacco, BMI) except weakly with sCT ($r$: 0.17, $P < 0.04$).
Conclusion
Assaying calcitonin (CT) in the wash-out fluid from fine-needle aspiration biopsies (FNAB.CT) can be an additional and precocious approach to diagnosis of MTC
Values are expressed as mean $\pm$ DE and range. sCT (serum calcitonin) nCT (calcitonin in thyroid nodule FNAB-washout)
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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**Table 1** Levels of sCT & nCT in relation to ecographic and cytological diagnostics

| DIAGNOSTICS | sCT | nCT |
|---|---|---|
| Thyroid single nodule ($n=39$) | $2.49 \pm 1.33$ (2–8.1) | $6.36 \pm 16.3$ (2–97) |
| Multinodular Goitre ($n=42$) | $3.79 \pm 4.6$ (2–19.4) | $2.02 \pm 0.02$ ( 2–2.1) |
| Cystic lesion ($n=11$) | $4.48 \pm 5.26$ (2–19.4) | $\leq 2$ |
| Autoimmune Disease ($n=16$) | $2.6 \pm 1.15$ (2–6.5) | $3.59 \pm 1.15$ (2–13.2) |
| Follicular neoplasm ($n=4$) | $2.27 \pm 0.55$ (2–3.10) | $3.95 \pm 3.0$ (2–8.4) |
| Papillary Cancer ($n=4$) | $\leq 2$ | $\leq 2$ |
| Material hematic ($n=5$) | $2.6 \pm 1.34$ (2–5) | $2.31 \pm 0.77$ (2–3.9) |

## P1816

### Thyroid paraganglioma. Report of 3 cases and description of an immunohistochemical profile useful in the differential diagnosis with medullary thyroid carcinoma, based on complementary DNA array results

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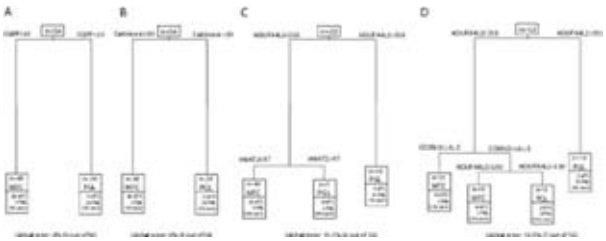
Thyroid paraganglioma (TP) is a rare disorder that sometimes poses problems in differential diagnosis with medullary thyroid carcinoma (MTC). So far, differential diagnosis is solved with the help of some markers that are frequently expressed in MTC (TTF1, calcitonin, and CEA). However, some of these markers are not absolutely specific of MTC and may be expressed in other tumors. Here we report 3 new cases of TP and describe our strategy to design a diagnostic immunohistochemical battery.
First, we performed a comparative analysis of the expression profile of head and neck paragangliomas and MTC, obtained after complementary DNA array analysis of series of fresh-frozen samples of paragangliomas and MTC, respectively. Seven biomarkers showing differential expression were selected (NDUFA4L2; COXIV2; VMAT2; CGRP/calcitonin; CEA; and TTF1) for immunohistochemical analysis. Two tissue microarrays were constructed from 2 different series of paraffin-embedded samples of paragangliomas and MTC.
We provide a classifying rule for differential diagnosis that combines negativity or low staining for CGRP (histologic score, $<10$) or calcitonin (histologic score, $<50$) together with positivity of any of NDUFA4L2; COXIV2; or VMAT2 to predict paragangliomas, showing a prediction error of 0%. Finally, the immunohistochemical battery was checked in paraffin-embedded blocks from 4 examples of TP (1 previously reported case and 3 new cases), showing also a prediction error of 0% (Default 1).
Our results suggest that the comparative expression profile, obtained by complementary DNA arrays, seems to be a good tool to design immunohistochemical batteries used in differential diagnosis.

**Table 1** Summary of the main immunohistochemical results

| Antibody | TMA MTC Hscore, Mean Range | TMA PGL Hscore Mean Range | Case 1 Hscore | Case 2 Hscore | Case 3 Hscore | Case 4 Hscore |
|---|---|---|---|---|---|---|
| Anti-NDUFA4L2 | 149 (100 –206) | 215 (112 –285) | 250 | 195 | 210 | 240 |
| Anti-COXIV2 | 126 (15 – 165) | 154 (101 260) | 220 | 210 | 250 | 210 |
| Anti-VMAT2 | 83 (20 – 100) | 98 (0 – 130) | 170 | 140 | 170 | 170 |
| Anti-CGRP | 242 (70 – 296) | 0.97 (0 – 7.5) | 0 | 0 | 0 | 0 |
| Anti-calcitonin | 253 (168 – 295) | 1.7 (0 – 40) | 0 | 0 | 0 | 0 |
| Anti-TTF1 | 72 (0 – 205) | 0 (0 – 0) | 0 | 115 | 0 | 0 |
| Anti-CEA | 150 (0 – 295) | 0 (0 – 0) | 0 | 0 | 0 | 0 |




Classification trees to predict tumor type, based on immunohistochemical features of the potential biomarkers. Prediction error is shown for each terminal node and globally for each tree.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1817

### Clinical trial of thyroid RFA for papillary microcarcinomas

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The management of papillary thyroid microcarcinomas (PTMC) has been controversial and only observation without surgery has been advocated for the low-risk PTMC. Radio-frequency ablation(RFA)has been applied to thyroid nodule, which shows safe and useful results for benign thyroid nodules. In our institution, not only benign nodules, but thyroid malignancies have been attempted to be therapeutic objectives by RFA. The aim of this study is to evaluate the efficacy of RFA for primary PTMC.
Objectives
Eighty-nine cases were treated by RFA from 2007 in our institution, which consisted of 55 hyperplastic nodules, 9 AFTNs, 5 local recurrences of papillary carcinomas, 11 metastatic LNs and 9 primary PMTCs. All of 9 patients with PTMC were initial cases without the history of previous thyroid surgery or irradiation, and refused the surgery because of cosmetic and private reasons.
Methods
RFA has carried out under general or local anesthesia and under careful US imaging guidance. The equipment of 17–18G needle electrodes were applied to thyroid RFA: STARmed RF electrode (STARmed, Korea) As an intra-operative assessment for the estimation of extent of ablated area, gray-scale US, color-Doppler imaging, Elastography and contrast enhanced US were applied to judge the satisfactory ablation.
Results
All cases showed no side effect or thermal damage, and discharged after one night observation. The efficacy of RFA has been verified with both imaging diagnosis and repeated FNA. Post therapeutic FNA revealed no cancer cells remained in 8 of 9 PTMC cases.
Conclusions
The efficacy of RFA has shown satisfactory results and a possibility to be one of alternative treatments for PMTC. Long-time follow-up and careful attention for thermal damage to surrounding tissues are required.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P1818

**Intraoperative calcitonin stimulation testing in an individualized surgical strategy of medullary thyroid cancer - primum non nocere**

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Context and Objectives
The prognosis of medullary thyroid carcinoma (MTC), derived from parafollicular C-cells, depends on the completeness of the first surgical treatment. The C-cells produce calcitonin, a peptid hormone that is used as biochemical and immunohistochemical tumor marker. The aim of study was to evaluate an individualized approach to patients with C-cell disease, i.e. MTC and C-cell hyperplasia (CCH), using the intraoperative calcitonin testing-assisted surgical strategy as a predictor of the final outcome after surgery.
Design
An unicentral cross-sectional study.
Patients and Methods
Since June 2009 to September 2011, sixteen patients with MTC/CCH were surgically treated primarily ($n=11$) or were reoperated for persistence of the disease ($n=5$). Depending on the result of an intraoperative calcitonin stimulation testing (iCST), patients underwent total thyroidectomy with or without lymph node dissection. All patients were tested repeatedly in postoperative period (range 2 to 27 months).
Results
The results of iCST correlated with postoperative findings - if intraoperative testing was negative, it stayed negative in postoperative follow-up period. If serum calcitonin showed increase in iCST, it persisted positive postoperatively.
Discussion
Surgical treatment is the only modality that possibly can lead to complete cure of the patient. An importance of initial operation, including total thyroidectomy and lymph node dissection of adjusting metastases including micrometastases is indubitable. The aim of the surgery is to be equally radical, avoiding an over-resection and considering complications.
Conclusions
The results encourage to use the individual approach to patients with MTC/CCH, e.g. to be less radical surgically in cases of negative iCST, and to be more radical in those patients with persistent increase of serum calcitonin. The absence of poststimulation calcitonin elevation in iCST seems to be a good prognosis indicator, but longer follow-up is needed.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1819

**Detection of novel genetic changes in the ras genes in papillary thyroid carcinoma**

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Introduction
Activating point mutations in the RAS genes (H-RAS, K-RAS, N-RAS) are reported in thyroid tumors. The aim of this study was to determine the frequency of RAS mutations in 98 patients with thyroid tumors.
Methods
DNA was extracted from 72 fresh frozen thyroid samples and 26 paraffin-embedded formalin-fixed samples. The cohort contained 83 PTCs (56 FVPTCs, 14 mixed follicular-classical types, 11 classical variants and two other rare variants), one FTA, 7 FTCs, 4 poorly differentiated carcinomas (PDC) and 3 anaplastic carcinomas (ATC). The presence of RAS mutations in exon 1 and exon 2 of the H-RAS, K-RAS, N-RAS genes was determined by direct sequencing and detected missence RAS alterations were evaluated in silico analysis using PolyPhen-2, Align-GVGD and SIFT software.
Results
Mutations in six PTC patients was found in codon 61 of the activating domain of K-RAS (2 patients) and N-RAS genes (4 patients). The polymorphism 81T-C in H-RAS gene was found in 41% PTC, in one FTA, 3 FTC, 3 PDC and one ATC. We detected other 7 silent, 6 missence and one nonsense genetic changes. The alterations were found only in follicular variant of PTC. The association with phenotype was not apparent.
Conclusion
RAS mutations in our cohort of thyroid cancer were screened. In addition to mutations in codon 61 (GTPase domain) in six patients, we revealed other genetic changes. However, their influence on the development of PTC needs to be confirmed.
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Declaration of interest
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## P1820

**Elastosonographical characteristics, cytological results and histopathological features of nodules in patients with hurthle cells**

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Objective
In this study, we aimed to evaluate ultrasonographical and elastosonographical characteristics, cytological results and histopathological features of nodules in patients with Hurthle cells in cytological examination and operated for various reasons.
Method:57 patients and 57 nodules detected to have Hurthle cells in thyroid fine needle aspiration biopsy (FNAB) were included in the study.
Results
Among 57 nodules with Hurthle cells in cytological examination, 49 (86%) were classified as Bethesda 1 and 8 (14%) were classified as Bethesda 3. Histopathologically, 45 (78.9%) nodules were benign and 12 (21.1%) were malignant. When nodules were grouped according to Bethesda results, nodule volume, ultrasonographic features in gray-scale and vascularization pattern were similar in two groups. There was no signicant difference in terms of elastosonographic scoring and mean strain index (SI) between groups. Elastosonography scores determined in transverse axis were found to be more predictive for malignancy compared to scores determined in longitudinal axis ($P=0.023$ and $P=0.867$, respectively). However, mean SI values in longitudinal axis were significantly higher than mean SI values in transverse axis ($P<0.05$). Hurthle cells were significantly higher in Bethesda 3 nodules compared to Bethesda 1 nodules ($P<0.01$). Cytological features were compared in histopathologically benign and malignant nodules. Nuclear groove, transgressing blood vessel (TBV) and absence of colloid were observed with a higher frequency in malignant nodules compared to benign nodules ($P<0.05$).
Conclusion
Nuclear groove, TBV and absence of colloid in cytomorphological evaluation seem to be the features that support malignancy in nodules including Hurthle cells cytologically. Higher SI values obtained during elastosonographical examination in malignant nodules compared to benign nodules suggest that SI might be used as a parameter to predict malignancy. Since malignancy rate in our patient group was 21.1%, we think total or near total thyroidectomy should be preferred in nodules with Hurthle cells in cytological examination.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1821

**Usefulness of selective sentinel lymph node biopsy in determining the surgical approach of papillary thyroid cancer**

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Introduction
Routine central lymph node dissection (CLND) in papillary thyroid carcinoma (PTC) is controversial. Selective biopsy of the sentinel lymph node (SLN) could

improve the surgical approach, as negative cases would benefit from avoiding a more aggressive technique and in positive samples a more accurate staging would be obtained.
Aim
To analyze the usefulness of SLN biopsy in the surgical treatment of PTC.
Patients and Methods
21 women and 4 men with diagnosed or suspected PTC were selected for the study. The day before surgery lymphoscintigraphy with intratumoral injection (99mTc-nanocolloid, 4 mCi, ultrasound-guided punction) was performed to obtain planar and SPECT-CT images 2–4 hours after injection. Localized SLN was biopsed with gammaprobe localization. They all underwent a total thyroidectomy and a CLND. Lateral LND was performed in the cases diagnosed as preoperative stage N1b and when a SLN was found in the lateral compartment.
Results
One case showed no drainage in lymphoscintigraphy. Nine cases (36%) had a negative biopsy for both SLN and CLND. Fourteen cases (56%) had a lateral drainage and in 4 of them (28.5%) a metastatic SLN involvement was detected. One negative SLN was reported to have micrometastases in the final biopsy.
The sensitivity of SLN biopsy was 95%.
Conclusions
SLN biopsy could be useful in staging and in surgical approach of PTC. CLND could be avoided when no SLN involvement was found and a lateral LND would be indicated in lateral SLN affected cases.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1822

**Prevalence of differentiated thyroid cancer is relevant in patiens with familial adenomatous polyposis: a case-control prospective study**

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Introduction
Familial adenomatous polyposis (FAP) is a dominantly inherited disease characterized by colorectal adenomas, extracolonic gastrointestinal manifestations and extraintestinal diseases. Among them, differentiated thyroid cancer (DTC) is considered very frequent with an approximately risk 160 times higher than in general population, and with a prevalence of the cribriform-morular variant. As this esteem comes from scattered data, we evaluated in a prospective controlled study the prevalence of DTC in patients with FAP.
Methods
We enrolled 54 outpatients with FAP and 196 healthy subjects involved in a program of screening of thyroid diseases. All subjects underwent hormonal assay for thyroid function, ultrasonography (US) of the thyroid, fine-needle aspiration biopsy (FNAB) of all detectable nodules. Subjects with cytology suspicious for malignancy were addressed to thyroidectomy for histological verification.
Results
From the 54 FAP patients 19 (35.1%) had thyroid nodules: 2 (3.7%; mean age 34.05 ± SD 13.77 yrs) had a confirmed diagnosis of papillary thyroid carcinoma. From the 196 subjects of the control group 97 (49.4%) had thyroid nodules and 2 had a diagnosis of DTC (1%; mean age 48.17 ± SD 0.12 yrs).
Conclusion
Thyroid nodules is a common disease in patients with FAP (35.1%), as it is in the general population and in the control group of this study (49.4%). The prevalence of DTC was higher in FAP than in the control group and speak in favor of screening patients with FAP for thyroid cancer. The mean age at the time of the diagnosis of thyroid cancer in patients with FAP was lower than in the general population. These data confirm a possible correlation between FAP and DTC, suggesting the importance of a careful follow-up of thyroid lesions in these patients, even at young age.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1823

**The role of serum levels of selenium, superoxide dismutase, catalase and thiobarbituric acid reactive substances in the association between Hashimoto's thyroiditis and papillary thyroid cancer**

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Introduction
Hashimoto's thyroiditis can be accompanied by thyroid cancer. It is reported that there is an association between selenium deficiency and thyroid cancer. In addition, it is known that chronic inflammation is another causal factor in the etiology of cancer.
In this study, we aimed to determine whether selenium or oxidative stress is a modifying factor for the association between Hashimoto's thyroiditis and cancer.
Methods
As indicators of lipid peroxidation and oxidative stress that occur during the course of these diseases, we determined the serum levels of thiobarbituric acid reactive substances (TBARS) as well as those of selenium and the activities of catalase and superoxide dismutase (SOD). We divided the subjects into 3 groups as follows: 20 subjects with papillary cancer plus Hashimoto's thyroiditis (1st group), 20 subjects with Hashimoto's thyroiditis alone (2nd group), and the subjects with papillary cancer without Hashimoto's thyroiditis (3rd group).
Results
Levels of selenium, SOD, catalase and TBARS were measured in serum. Selenium levels were 73.0 ± 3.83 μg/l in the 1st group, 65.0 ± 3.22 μg/l in the 2nd group and 68.1 ± 4.62 μg/l in the 3rd group. SOD levels were 0.27 ± 0.01 U/ml in the 1st group, 0.25 ± 0.02 U/ml in the 2nd group and 0.29 ± 0.01 U/ml in the 3rd group. Catalase levels were 47.34 ± 6.64 nmol/min/ml in the 1st group, 43.34 ± 5.13 nmol/min/ml in the 2nd group and 30.78 ± 4.73 nmol/min/ml in the 3rd group. TBARS levels were 8.2 ± 3.0 nmol/ml in the 1st group, 7.5 ± 0.86 nmol/ml in the 2nd group and 5.55 ± 1.01 nmol/ml in the 3rd group.
Conclusions
Antioxidants such as selenium, SOD, catalase and oxidative stress due to chronic inflammation were not found to be associated with the progression to papillary thyroid cancer in patients with Hashimoto's thyroiditis.
Serum selenium, SOD, catalase, TBARS levels of the patient groups.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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**Table 1** Serum selenium, SOD, catalase, TBARS levels of the patient groups.

| | Group 1 (HT + PC) | Group 2 (HT) | Group 3 (PC) | |
|---|---|---|---|---|
| Selenium (μg/l) | 73.0 ± 3.83 | 65.0 ± 3.22 | 68.1 ± 4.62 | $P > 0.05$ |
| SOD (U/ml) | 0.27 ± 0.01 | 0.25 ± 0.02 | 0.29 ± 0.01 | $P > 0.05$ |
| Catalase (nmol/min/ml) | 47.34 ± 6.64 | 43.34 ± 5.13 | 30.78 ± 4.73 | $P > 0.05$ |
| TBARS (nmol/ml) | 8.2 ± 3.0 | 7.5 ± 0.86 | 5.55 ± 1.01 | $P > 0.05$ |

## P1824

**Iodine-131 lung uptake in bronchiectasis: a potential pitfall in the follow up of differentiated thyroid carcinoma (DTC)**

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DTC carries a good prognosis when adequately treated by means of total thyroidectomy, iodine-131 (I-131) treatment and TSH-suppressive therapy, and carefully followed up (i.e. serum thyroglobulin, I-131 total). In some cases, however, false-positive scans can occur. A 42-yr-old woman with recurrent chest infections and bronchiectasis was submitted to total thyroidectomy because of a13 mm nodule in the right lobe that showed a papillary thyroid carcinoma at FNAB histological examination. The patient was then submitted to I-131 treatment with 3700 MBq after L-thyroxine withdrawal. The post therapeutic dose total body scan showed a residual iodine uptake in the thyroid bed attributable to thyroid remnant, compatible with the thyroglobulin serum level (tg = 6.49 ng/ml), and a low bilateral uptake in the lung fields. A I-131 total body scan (185 MBq) following recombinant (rh)-TSH administration four months after the therapeutic dose of radioiodine showed a marked and diffuse uptake in the lungs with tg levels below the detection levels. A CT of the chest confirmed the bronchiectasis appearance of the bronchial tree. No further therapeutic dose of

radioiodine was administered and the patient was followed up with periodic serum tg level determination during TSH suppressive treatment and neck echographic evaluation. Two years later, another I-131 scan (rh-TSH) showed the same picture of diffuse uptake of radioiodine in the lungs, with undetectable stimulated serum tg, while CT of the chest confirmed the presence of bronchiectasies with no other lesions referable to metastatic involvement of the lungs. This case claim attention about the possible false-positive results of I-131 scan in DTC follow up in patients affected by infective and inflammatory diseases of the lungs, emphasizing the importance of interpreting the I-131 uptake on the bases of clinical context, imaging and laboratory findings (serum tg). Recognition of false-positive cases permit to avoid further radioiodine therapeutic doses administrations that are not indicated and potentially associated by side-effects (sialadenitis, gastritis, other malignancies, and pulmonary disease with lung fibrosis). and that can cause an emotional trauma for the patient, often leading to legal controversies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1825

**Prognostic markers in Papillary Thyroid Cancer by immunohistochemistry**

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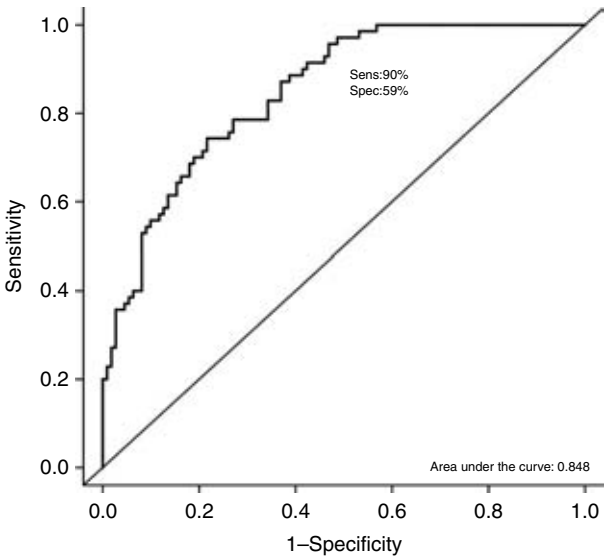
Papillary thyroid cancer (PTC) is the most common endocrine malignancy. Its management has not changed significantly in recent decades, and most patients receive the same treatment. Moreover, there are not alternative treatments for low response or aggressive neoplasias. The availability of reliable prognostic markers that would allow the PTC to be identified based on their aggressiveness at the time of diagnosis would derive in an individualized treatment. In a previous study, our group identified a transcript profile by DNA microarray that allows a prognostic classification with an accuracy level of 95% over its evolution. The current study aimed to establish an immunohistochemical profile to help determine the prognosis of evolution of the PTC

Materials and methods

We have performed Tissue Microarrays immunohistochemistry in a large series of 184 cases of PTC, clinically well characterized, with 17 antibodies (Ab) against proteins encoded by some of the genes identified in our previous study. For statistical analysis, we used the intra-individual mean obtained from four experimental replicates to measure Ab expression. We used the Mann-Whitney U to compare the values of the histoscore group as defined by the prognosis, and adjusted logistic regression model to identify those that contribute significantly to explain tumor poor prognosis




Results

Preliminary analysis of the samples showed differences between groups defined according to forecasts for 12 Ab. The multivariate logistic regression model to explain and distinguish poor from good prognosis showed that five Ab achieved an area under the curve of 0.85 [0.79, 0.90] and an optimal cut-off levels of sensitivity of 90% and specificity of 59%

Conclusions

The preliminary results provide promising results for differentiating good and poor prognosis of PTC. The combination of Ab identified may contribute to the prognostic stratification of PTC (with potential clinical applications)

Fx1

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1826

**Cytological thyroid: comparison (intra and interobserver) of results issued by pathologists with different skills of experience**

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The wide variability of analysis obtained from the fine needle aspiration cytology (FNAC) of thyroid nodules is attributed to experience differences in their interpretation. It is known that reproducibility is a critical parameter for the clinical usefulness of a diagnostic test. However, interobserver and intraobserver reproducibility of cytological examinations of the thyroid has been very little investigated. We studied 74 patients who underwent FNAC and a thyroidectomy. The examinations were reviewed by two pathologists, one experienced and one novice. Interpretations of the smears occurred on two separate occasions, with a time interval of six months between the first and second. The results revealed consistent intraobserver reproducibility after a lapse of six months, both for the experienced pathologist (K = 0.82 [0.71 to 0.94], Kw = 0.79, $P = 0.988$) and for the novice (K = 0.78 [0.64 to 0.91], Kw = 0.71, $P = 0.291$). There was no difference for the experienced and novice pathologist, respectively, in terms of rate for insufficient material, follicular neoplasm (FN), false-positive, false-negative and proportion of malignant tumors identified. The analysis of interobserver reproducibility showed a significant difference in both the first analysis (K = 0.55 [0.38 to 0.72], Kw = 0.50, $P = 0.011$) and the second (K = 0.54 [0.37 to 0.71], Kw = 0.44, $P = 0.021$). There was also a difference in the number of unsatisfactory results in both the first ($P = 0.001$) and second ($P = 0.021$) examination and FN in the second analysis ($P = 0.015$). However, there was no difference in the first and the second analysis, respectively, in terms of proportion of malignant tumors identified, false-positive and false-negative. The intraobserver reproducibility in the interpretation of FNAC is consistent and independent of experience to the examination, however, interobserver reliability is influenced. Examiner experience significantly influences the proportion of unsatisfactory results and cases compatible with FN. Surgical thyroidectomy should take into account the high rate of false-negative results, which occurs even when performed by an experienced pathologist, as well as the high rate of unsatisfactory results, mainly due to the high rate of malignancy observed in these samples.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Funding

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## P1827

**Follicular Neoplasms: Clinical, Sonographic and Cytological Determinants and its Histological Correlation**

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Introduction

Fine-needle biopsy(FNB) is an essential diagnostic tool in the evaluation of thyroid nodules, however, follicular neoplasm(FN) does not exclude malignancy. Some sonographic and cytological features have been described as being associated with increased risk of malignancy. The authors aimed to identify

demographic, sonographic and cytologic features associated with malignancy in patients with a cytological diagnosis of FN.
Methods
Descriptive study of all patients(pts) with a cytological result compatible with FN, who underwent thyroid surgery at our institution in 2010. Gender, age, nodule size, TSH, sonographic and cytological features were recorded. Statistical analysis was performed with SPSS, v.17.0, using chi-square and Mann-Whitney tests.
Results
78 pts, 58 F(74%), 20 M(26%), mean age 48.5±14.7 years(22–79). FN: 69 patients (88.5%), Hurthle cell neoplasm: 9 patients(11.5%). Average nodule diameter: 25.2 mm(8–81). Single nodule: 48 pts(61.5%), multinodular goiter: 30 pts(38.5%). Histology: 12 pts with malignant lesions(15.4%) - papillary carcinoma:9 pts, follicular carcinoma: 2 pts, medullary carcinoma: 1 pt; 64 pts with benign lesions(82.1%) - follicular adenoma(including Hurthle cell): 47 pts(60.2%), nodular hyperplasia: 17 pts(21.8%), well-differentiated tumor of uncertain malignant potential (WDTUMP): 2 pts(2.6%). Incidental histological findings: papillary microcarcinoma(MP): 12 pts(15.4%), WDTUMP: 1 pt(2.6%). The average age of patients with malignant lesions was lower than the group with a benign histology(37.7±12.5 vs. 50.0±14.3), $P=0.007$. No statistically significant differences were found between malignancy and sonographic characteristics(eg size, echogenicity, texture, peripheral halo, borders, micro-calcifications), cytological features and TSH.
Discussion
The retrospective nature of the study, the size and heterogeneity of the sample and the limited description of sonographic and cytological features were important limitations. The significantly lower age of pts with malignant disease may indicate the need for a more aggressive approach. Future studies will be necessary towards the confirmation of these results.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1828

**Ultrasoundelastography: a useful tool to differentiate benign and malignant thyroid nodules?**

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Introduction
Ultrasoundelastography (USE) evaluates the tissue elasticity according to the local tissue displacement after a small compression applied by an ultrasound transducer. This technique is based on the concept that a hard lesion is associated to a higher risk of malignancy. The USE has been recently applied for characterization of thyroid nodules. The aim of our preliminary study was to assess the validity of USE for screening benign and malignant thyroid nodules.
Methods
This preliminary study includes 57 thyroid nodules in 39 patients (32 females and 7 males, mean age: 52±11 years old) who were referred to the Endocrinological Unit at Atri hospital consecutively from March to December 2011. All nodules were examined using ultrasonography, USE and fine needle aspiration cytology. Nodules with cystic pattern or Thy 1 cytology were excluded. USE results were classified on the basis of the degree and the distribution of elasticity in 5 patterns: 1, 2, 3a, 3b and 4. All nodules were defined as benign or malignant on the basis of histological results.
Results and conclusions
The histological results revealed 50 benign nodules and 7 malignant lesions. The chi squared test showed that the association between the USE pattern and the histological definition tends to be statistically significant. In fact, USE pattern 4 was associated to a malignant lesion in 67% of the cases. In addition, the association with malignancy was found in 2% of the cases for the pattern 2, in 14% for pattern 3a and 8% for pattern 3b. However, a validation of these results might be obtained only by increasing our sample size.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1829

**Differentiated Thyroid Cancer in Diabetic Patients**

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Objective
Patients with differentiated thyroid carcinoma have an increased prevalence of insulin resistance and, diabetes increases the risk of thyroid cancer. In this study we aimed to evaluate tumor features in diabetic and non-diabetic differentiated thyroid cancer patients.
Methods
One hundred patients with differentiated thyroid cancer were enrolled in the study. Twenty one of the 100 patients were diagnosed as diabetic and 79 non-diabetic. All participants underwent a physical examination, detailed history and anthropometric measurements including BMI, waist to hip ratio and blood pressure. The characteristics of the thyroid cancer-histology, tumor size, tumor invasion, nodal and distant metastasis, radioiodine treatment and thyroglobulin levels were measured.
Results
Weight and waist-to-hip ratio were higher in diabetic patients compared to non-diabetic patients. Thyroglobulin levels were lower in diabetic subjects ($P<0.05$). We observed no significant difference between diabetic and non-diabetic patients in respect for age, gender, TSH levels, tumor invasion, nodal metastasis and persistant disease. The differences in risk by groups for the papillary and folicular types were not significant ($P=0.888$).
Conclusion
Although diabetes has been associated with increased risk of thyroid cancer, presence of diabetes was found not to be associated with more aggressive tumor features.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1830

**Diagnostic value of thyroglobulin and antithyroglobulin antibody in the early follow-up of well differentiated thyroid carcinoma**

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Objectives
Thyroglobulin (Tg) play an important role in managements of well-differentiated thyroid carcinoma (DTC). Undetectable TSH-stimulated serum Tg level in the absence of antithyroglobulin antibody (AbTg) indicates complete remission. Circulating AbTg may interfere with Tg assay and may cause false negative results. Moreover, undetectable Tg is observed in a part of patients after total thyroidectomy(TT), even if serum AbTg concentration is low.
The aim of our study was to evaluate a clinical significance of Tg and AbTg levels measured before and 6-12 month after thyroid ablation.
Materials and methods.
329 patients after TT because of DTC were treated with radioiodine(RIT). Diagnostic procedures - including whole-body scan (WBS) and measurements of Tg and AbTg levels were performed before and 6–12 month after 131I administration. Finally, we analyzed the subgroup of 104 patients, who had: 1) no evidence of distant metastases, 2) undetectable Tg (<1ng/ml) and/or elevated level of AbTg (>60U/ml) before RIT 3) complete data for study variables.
Results.
Among the 329 patients, 143 (43.5%) had elevated (↑) Tg and normal (n) AbTg level, 32 (9.7%) - Tg(↑) and AbTg(↑), 109 (33.1%) - Tg(n) and AbTg(n), 45 (13.7%) - Tg(n) and AbTg(↑)
Our group of 104 patients included
16 patients - Tg(↑) and AbTg(↑)
66 patients - Tg(n) and AbTg(n)
22 patients - Tg(n) and AbTg(↑)
6–12 months after 131I therapy, all these patients had undetectable Tg level. Among 38 patients with AbTg(↑) previously, 21 (55.3%) had AbTg (n)- subgroup A, 9 (23.7%) - AbTg decreased, but were still elevated- subgroup B, 5 (13.2%) - AbTg on the same level - subgroup C and 3 (7.9%) - AbTg increased - subgroup D. Diagnostic WBC was positive (persistent thyroid bed uptake) in 11/38 patients: 6 in A, 3 in B, 1 in C and 1 in subgroup D.
In the rest of 66 patients /(Tg(↑) and AbTg(↑) WBS was positive in 6.

Conclusions
In more than 50% of patients with DTC serum Tg concentration could not be used as an indicator of the effectiveness of thyroid ablation - because of coexistence of AbTg or/and undetectable level of Tg before treatment. WBS provides more important information and should be routinely used in early follow-up of patients after thyroid ablation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1831

**Prostate and bladder metastasis of Medullary Thyroid Carcinoma**

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Introduction
Medullary thyroid carcinoma MTC represents 5% of all thyroid cancers. It is familial in 30% of the cases. The most frequent metastatic localizations are lymph nodes, liver, lung and bone. We describe prostatic and vesical localizations of a MTC in a patient with multiple endocrine neoplasia (MEN 2B).
Case report
A 44 years old patient with MEN 2B operated in 1989 for MTC, bilateral pheochromocytoma and had a cutanuous neurofibroma. He presented with persistent dysuria and abdominopelvic pain with testicular irradiation. Computed tomography detected a heterogeneously enlarged prostate and another mass at left uretero-vesical junction. Histopathological study after a radical operation in 2009 confirms the existence of a neuroendocrine tumor (NET) with positive immunostaining to chromogranin A, synaptophysine and calcitonine. Re-analysis of the two lesions with the previous thyroid slides confirms the identity of a MTC with its metastatic lesions. Serum calcitonin declined postoperatively from 233 to 65 pg/ml.
Discussion and conclusion
Few metastatic localizations of NET in the prostate are described. Calcitonin positive prostatic tumor is reported in the literature. It might be an indicative of either a MTC or a NET, as calcitonin immunostaining is possible in a normal endocrine tissue of the prostate. Bladder localizations are never documented. Absence of a primary NET, bladder localization devoid of prostatic tissue and improvement of elevated calcitonin levels in our patient make us to consider these localizations as metastatic lesions of MTC rather than a NET of prostate.
Elevated neuroendocrine markers with unexplained chronic urinary tract symptoms and/or a prostatic tumor in young patients in the context of a NET should evoke a NE localization. Neuroendocrine immunohisochemical staining should be done to line out the management of these rare tumors, as the prognosis of a MTC, primary prostatic adenocarcinoma or a NET is not identical.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1832

**Role of FGR2 in thyroid tumor progression and their involement in the correlation between thyroid cancer and melanoma**

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Thyroid cancer is the most common endocrine malignancy. It is known that cancer progression involve dysregulation of growth factors (FGFs) and their receptors (FGFRs) have been identified. In thyroid tumors. In particular, FGFR1 and FGFR3 are expressed in most well differenziated tumors, FGFR4 is predominantly expressed in aggressive tumor types and cells lines, while FGFR2 is the only receptor consistently detected in normal human thyroid tissue and its expression is downmodulated in thyroid cancers and carcinoma cells lines.
The study is conduced on a series of 7963 patient undergoing t.t. in the Department of Surgical Science, Policlinico Umberto I, Sapienza University of Rome, from 1996 to 2011of these patients 1391 had a diagnosis of thyroid cancer.The aim of the study is the evaluation of a possible correlation between FGFR2 expression and progression/prognosis of thyroid cancer, analyzing FGFR2 downregulation in normal thyrocytes, in term of proliferation, differentiation, apoptosis and expression of tumoral markers.
Recent studies have identified an association between cutaneous malignant and thyroid carcinoma. Molecular studies show that TSHR is expressed by cutaneous melanocytic lesions, with higher expression in malignant and premalignant lesions, and that cultured melanoma cells, but not melanocytes, are induced to proliferate by TSH; this suggests that hypothyroidism may promote the development and progression of melanoma.
We will set up a retrospective analysis of FGFR2 expression in patients with thyroid carcinoma that have successively developed a melanoma or vice versa, in order to identify differences in FGFR2 expression in "double positive" patients versus patients affected by only thyroid cancer or melanoma.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector

## P1833

**Clinical and Pathological Features of Pediatric Thyroid Carcinoma**

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Introduction
Thyroid cancer in childhood and adolescence represents 1.5–3% of all carcinomas in this group of age. Our purpose is to define clinical and pathological features, therapeutical complications and follow-up characteristics and survival of a group of children and adolescents with thyroid carcinomas.
Design
We report a retrospective study of 26 children and adolescents with thyroid carcinoma who were investigated in our institution from January 2007 till July 2011. The study followed: historical data, clinical examination, hormonal profile, imaging, histological examination, side effects of therapeutic interventions, the locoregional/distant recurrence.
Results
There were 23 girls and 3 boys in our group. The average age at diagnosis was 14.9 years (15.1 years for girls and 13.33 years for boys). At the time of diagnosis 8 patients presented with unique nodule, 13 patients with nodule with laterocevical lymphadenopathy and 5 patients had multinodular goiter. The treatment applied was total thyroidectomy followed by radioiodine therapy. There was one case of persistent hipoparathyroidism and no other significant post-operative/radioiodine therapy were observed and no patients died from disease. Histological examination revealed papillary differentiated thyroid carcinoma: diffuse sclerosing papillary thyroid carcinoma and follicular variant were the subtypes observed. 30% of patients presented a tumor size between 3 cm and 4 cm. The involvement of thyroid capsule and surrounding tissue was observed in 40% of cases and multicentricity was described in 20% of cases. Postoperative surveillance was done by clinical examination, imaging and repeated dosing of thyroglobulin and antitireoglobuline antibodies. 4 patients exhibited local recurrence.
Conclusions
Compare with adult DTC, the pediatric DTC has an aggressive behavior at presentation, a higher risk of recurrences and a risk of induction of a secondary solid tumor after iodine therapy in later life, which require an improved protocol of treatment and follow-up.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1834

**Malignancy rate of thyroid nodules which defined as follicular lesion of undetermined significance in thyroid cytopathology**

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Objective
In the Bethesda classification (BC), which is used in cytopathologic evaluation of thyroid nodules, follicular lesion of undetermined significance (FLUS) does not involve sufficient cellular atypia for follicular neoplasia or malignancy, while it doesn't comprise the cytologic benign criteria. Therefore, there is no consensus for those nodules to follow-up and therapeutic approaches. In this study, we aimed to determine the ultrasonographic features and histopathologic results of the thyroid nodules which are defined FLUS in BC.
Methods
We evaluated 64 nodules of 62 patients, who have nodular or multinodular goiter and had diagnosed FLUS at least in one nodule with fine-needle aspiration (FNA). Operation indication was decided upon to the nodule size, suspected ultrasonographic feature (border irregularity, solid, hypoechoic nodule, presence of microcalcification), high elastosonographic score and strain index and family history of thyroid cancer.
Results
###87.5% of patients were female, 12.5% were male. Mean age was $46.5 \pm 12.6$ years. 51.6% of nodules were located in the right lobe, 45.3% in the left and 3.1% in the isthmus. According to the echogenite and component 43.8% of nodules were hypoechoic, and 57.8% were solid. Microcalcification, peripheral vascularization and border irregularity positivity was respectively 25.5%, 17.2% and 59.4% of nodules. After operation, 68.7% of nodules were benign ($n=44$), 31.3% ($n=20$) were malign. 85% of malign nodules were papiller thyroid carcinoma, 15% were follicular carcinoma.
Conclusion
In our study, we found malignancy rate 31.3% in FLUS compared to the 5–20% reported by different studies. Therefore, we think that, in the diagnostic and therapeutic approach of the FLUS nodules that challenge the clinician, this high ratio of malignancy must be kept in mind.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1835

**Appearance of secondary primary malignancies in patients with differentiated tumours of the thyroid gland**

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Introduction
thyroid carcinoma is an uncommon malignancy, but it's incidence appears to be increasing slowly (female 2–3.8/100.000 - male 1.2–2.6/100.000). This is the most common malignant endocrine tumour, represents about 1% of all malignancies. The majority of these patients females, mostly between the age of 30–60. The main differentiated histological types are papillary and follicular. The options of the treatment are surgical removal (mainly total), radioiodine therapy (ablation/repeated) and very rarely external beam radiotherapy, chemotherapy. The follow up of the patients are very important (postoperative levothyroxin therapy - TSH suppression and substitution, laboratory - thyroglobulin and thyroglobulin antibodies, ultrasonography).
Method and material
###341 patients are followed up with differentiated tumor of the thyroid gland at our Endocrinology Clinic continously. As they are regularly controlled, a lot of data are available to screen the patients.
Results
###8% of the patients had a secondary tumour (secondary primary malignancies), 25 female - 2 male case. The types of the tumours: breast, endometrium, cervix, skin, central nervous system, gastrointestinal tract. 60% of the patients the tumour of the thyroid gland was the first (50% breast cancer was the second tumour, averagely 16 years later than the thyroid), 22% of the patients the thyroid carcinoma was the second tumour (50% breast cancer was the first, followed averagely 7 years later by the thyroid), 18% of the patients unknown the order.
Conclusion
our data confirms that patients with thyroid gland tumours show a higher incidence of secondary malignancies. It emphasizes the role and importance of a follow up screening of the patients for a second malignancy. Furthermore we plan to investigate the oncogenetic background of these tumours.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1836

**Histological features of thyroid cancer in childhood and adolescence**

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Introduction Thyroid cancer (TC) is rare in childhood. The aim of the present study was to describe the tumoral characteristics of children and adolescents with TC and to investigate the frequency of microcarcinomas in that population.
Methods We studied the medical records of 101 children and adolescents ($\leq$ 21 years-old) who were diagnosed with TC between January 1977 and December 2011 in Theagenio Cancer Hospital. The following characteristics were recorded for each patient: year of diagnosis, patient's age and gender, histological type, tumor size, number of tumor foci, presence of: lymph node metastases, thyroid capsule invasion, vascular invasion and infiltration of the thyroid parenchyma or surrounding soft tissues. Microcarcinomas were defined as unifocal intrathyroidal papillary cancers $\leq$10 mm in diameter without lymph node involvement.
Results Tumor size was larger in patients with follicular, medullary and poorly differentiated thyroid cancer ($33.5 \pm 10.7$, $35.7 \pm 22.3$ and $32.7 \pm 21.9$ mm, respectively; $P<0.05$). Vascular invasion was more frequent in patients with follicular and poorly differentiated thyroid cancer (66.7% in both types; $P<0.005$). Patients older than 18 years showed infiltration of the thyroid parenchyma and the surrounding soft tissues less frequently than younger patients (42.4 vs. 64.3%, $P<0.05$ and 15.3 vs. 35.7% respectively, $P<0.05$). Other characteristics did not differ between patients older and younger than 18 years. There was no difference in any of the characteristics at diagnosis between males and females, or between the age groups $\leq$ 14, 15–18 and $>$ 18 years-old. Among the 89 patients with papillary TC and their variants, tumors $\leq$ 10 mm were present in 30 (33.7%) and microcarcinomas were present in 12 patients (13.5%).
Conclusions TC in children has more invasive characteristics than adolescents. 20.2% of patients had small ($\leq$ 10 mm) but invasive tumors. True microcarcinomas (13.5%) represent a minority of thyroid tumors.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1837

**A novel tandem germline RET mutations on the same allele in a patient with MEN 2B**

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Multiple endocrine neoplasia type2 (MEN 2) (OMIM 171400) is an autosomal dominant inherited cancer syndrome caused by activating mutations in the RET proto-oncogene. MEN 2 is classified into three subtypes: MEN 2A, MEN 2B and familial medullary thyroid carcinoma(FMTC). MEN 2B accounts for 5–10% of MEN2 cases. More than 95% of MEN 2B patients carry M918T mutation of RET, and 2–3% harbor A883F mutation. There has been three reports of cases with MEN 2B phenotype caused by double RET missense mutations of codons 805], 806and 904 in cis with V804M mutation. The V804M/Y806C mutations were the first case reported in a patient with MEN 2B like phenotype, functional tests of which suggest that the transforming activity of this double mutation was similar to that of the M918T defect.

Clinical case
A 32-year-old female presented with neck mass. She had had bumpy lips and multiple nodules on her lips, tongue, buccal mucosa and conjunctiva since childhood, but besides that she had been in good health. There was no family history of endocrine diseases. Fine-needle aspiration biopsy showed the characters of medullary cell carcinoma of the thyroid with lymphmetastasis. Histopathological examination of the buccal mucosa nodule showed multiple dermal nodules which was diagnosed as schwanoma. The serum calcitonin level was 6080pg/ml, and caricinoembryonic antigen levels was 435.2ng/ml. A total thyroidectomy was performed with central and right radical neck lymph node dissection.
Mutation analysis of the RET gene
The proband's genomic DNA was extracted from the blood samples and was screened for mutations in exons 10, 11, and 13–16 of the RET gene by the PCR-direct sequencing analysis. We detected two heterozygous missense mutations V804M and Q781R. Subcloning analysis of the gene revealed that the both mutations were present on the same allele. The genotype of her parents were examined under their informed consent. Q781R mutation was found in her father, and no mutation was found in her mother.
Discussion
We have identified a novel combination of RET missense mutation, V804M and Q781R. Subclone analysis had demonstrated that the de novo V804M mutation is on the paternal allele. Although several double mutation cases have been reported to cause MEN2B, the combination of the present case has not been reported.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1838

**Comparative examination of somatic oncogene mutation normal and pathologic thyroid tissues of Hungarian patients**

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It is established that somatic oncogene mutation (BRAF, NRAS, HRAS, KRAS) and gene translocations (RET/PTC, PAX8/PPAR-gamma) are associated with the development of thyroid cancer. Somatic single nucleotide polymorphisms were analyzed by LigthCycler melting method, while translocations were identified by real-time polymerase chain reaction technique. In 22 intraoperative thyroid tissue samples (11 pathologic and 11 normal). In tumorous sample 3 BRAF, 2 NRAS and one HRAS mutations were found, as well as one RET/PTC1 translocation. These results confirm international data. These oncogene mutations and translocations are linked to thyroid cancer. Cytological examination completed with genetic data may support the diagnosis of thyroid malignancies. In addition, genetic alterations may indicate malignant transformation and may become prognostic factors in future.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

## P1839

**Thyroid Metastasis of Clear Cell Renal Carcinoma**

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The clear cell renal carcinoma (CCRC) is the best known primary site of metastatic thyroid. The thyroid metastases can be the first sign of a kidney tumor, or appear long after the nephrectomy, in which case can may mistakenly diagnose a thyroid cancer early.
Goal
Examine ways of presentation, diagnosis and treatment of patients with thyroid metastases from clear cell renal carcinoma (TMCCRC).
Method
From January 1, 1985 to July 31, 2011 the diagnosis of TMCCRC was given to 9 patients at the Thyroid Center of Reggio Emilia. For the present study were enrolled eight because in one patient the clinical data were incompletes. In each patient we describe clinical manifestation, ultrasound description and anatomo-pathological aspects.
Results
Eight patients were enrolled: two women (25%) and six men (75%). The first diagnostic approach to TMCCRC was sporadic in three patients (37.5%), in two patients (25%) was by find increase in volume of the anterior region of the neck, in a patient (12.5%) for research of thyroid metastasis after nephrectomy for CCRC and in another two patient (25%) was the cytological reassessment after nephrectomy for a CCRC: both patients were previously cytological class T2. The TMCCRC were found in the right lobe in five patients (62.5%) and in the left lobe in three patients (28.57%).
Conclusions
The mode of presentation, the type of response, the clinical course and sonographic features of TMCCRC are heterogeneous. There is the possibility of confusing the TMCCRC with follicular neoplasm essentially in the absence of recognized previous CCRC. RECOMMENDATIONS. The finding of a nodule (s) of thyroid gland in patients with a history of CCRC must lead to the cytological analysis regardless of the ultrasound description. At the time of diagnosis of CCRC should evaluate the morphology of the thyroid.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1840

**Diagnostic power of conventional and doppler ultrasound in identifying malignancy in thyroid nodules**

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Background
Conventional ultrasound remains the most used imaging evaluation in case of a thyroid nodule.
Material, method
This prospective study included 69 patients (107 nodules). Conventional and Doppler ultrasound was performed with a HITACHI EUB 7500 HV machine with 6–13 MHz variable frequency linear probe, with Doppler software. All patients underwent surgery after complete evaluation.Postsurgical histopathological exam was performed. We analyses at each nodule the following parameters: echogenity, halo sign, margins, shape, homogeneity; presence of calcification, color flow Doppler pattern.
Results
We diagnosed 11 cancer nodules: 1 (0.93%) huge nodule with follicular cancer, 10 cases with papillary carcinoma (9.45%).
The most predictive parameters, appreciate by the AUC under ROC curve in descendant order were: inhomogeneity (0.7985), dominant intranodular vessel (0.789), absent halo sign (0.7581), intranodular vascularization (0.7206), nodule taller than wide (0.712).
All the diagnostic parameters are presented in Table 1.
Conclusion
In cases of thyroid nodules, inomogeneity, presence of intranpdular doppler signal, absence of halo and intranpdular calcifications are the most predictive ultrasound parameters in diagnosis of suspect thyroid nodules.
Diagnostic value of different ultrasound parameters in identifying malignancy

**Table 1** Diagnostic value of different ultrasound parameters in identifying malignancy

| parameter | aspect | BN ($n=96$ ) | CA ($n=11$) | sensitivity (%) | specificity (%) |
|---|---|---|---|---|---|
| shape | oval | 69 | 5 | 45.45 | 26.04 |
| | tall | 25 | 6 | 54.54 | 87.5 |
| margins | well defined | 78 | 8 | 72.73 | 20.83 |
| | irregular | 18 | 3 | 27.27 | 81.25 |
| halo sign | absent | 36 | 9 | 18.18 | 37.50 |
| | present | 60 | 2 | 81.82 | 62.50 |
| ecogenicity | izoecogenicity | 43 | 5 | 27.27 | 52.51 |
| ecogenicity | hypoecogenicity | 54 | 6 | 63.63 | 43.75 |
| homogenicity | inhomogenicity | 50 | 8 | 72.72 | 79.16 |
| composition | solid | 86 | 11 | 100 | 10.28 |
| calcifications | micro | 1 | 3 | 27.27 | 72.72 |
| | round | 3 | 2 | 18.18 | 95.28 |
| | egg shell | 0 | 1 | 0 | 98.96 |
| vascularisation | avascular | 46 | 1 | 9 | 67.77 |
| | perinodular | 50 | 5 | 45.45 | 52.08 |
| | dominant vessel | 10 | 6 | 54.55 | 89.58 |
| | intranodular nonspecific | 23 | 9 | 81.18 | 76.04 |

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1841

**Malignancy rate of thyroid nodules which defined as atypia of undetermined significance in the thyroid cytopathology**

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Aim
In this study, our aim is to evaluate the ultrasonographic features and histopathologic results of the thyroid nodules which are defined as AUS and contribute to the therapeutic approach of these nodules.
Methods
We evaluated 95 nodules of 92 patients, who have nodular or multinodular goiter and had diagnosed AUS at least in one nodule with fine-needle aspiration (FNA). Patients' thyroid function tests, ultrasonographic features of the nodules and histopathologic results were evaluated.
Results
###81.1% of patients were female, 18.9% were male. Mean age was 47.5 ± 12.1 years. In the ultrasonographic features presence of microcalcification, border irregularity, peripheral vascularization and absence of hypoechoic halo was respectively 28.4%, 47.4%, 20% and 60%. 43.2% nodules were hypoechoic and 47.4% were solid. According to the histopathology, 63.2% of nodules (n = 60) were benign, 36.8% (n = 35) were malign. In malign nodules papiller carcinoma, well-differentiated thyroid neoplasm, follicular carcinoma and Hurthle cell carcinoma were found respectively 88.6%, 5.7%, 2.9%, and 2.9%. In malign nodules mean tumor size was 1.2 ± 1.1 cm. No lymph node metastasis was found.
Conclusion
In AUS, malignancy rate is reported 25% of operated patients, but it is thought 5–10% of the total. In our study we found this ratio 36.8%. Our high ratio may be due to few and heterogeneous literature data outcomes. So, this high malignancy ratio in AUS nodules, have to be considered in the decision of operation.
Declaration of interest
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## P1842

**Rradioiodine-thyroid cancer link: re-evaluation of Chernobyl consequences**

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Chernobyl accident was followed by numerous publications overestimating its medical consequences, where spontaneous diseases have been without sufficient reasons classified as radiogenic. Among medical consequences of the accident, cause-effect relationship between ingestion of radioiodine and thyroid cancer in patients exposed at a young age is regarded to be proven. Significant incidence increase of pediatric thyroid cancer started 4 years after the accident, coinciding with the onset of large-scale screening programs. High percentage of advanced thyroid cancers among the cases found shortly after the accident can be explained by the screening effect with detection of neglected tumors and by the fact that patients from non-contaminated areas were registered as Chernobyl victims. Older neglected tumors, larger in size and less differentiated, prevailed early after the accident, being gradually replaced by smaller cancers detected by means of improving diagnostic methods. Accordingly, features of supposedly radiogenic post-Chernobyl cancers can be related to the disease duration and a later stage of tumor progression. Iodine deficiency in some of the contaminated areas contributed to a higher yield of thyroid nodules, found by the screening and offering an opportunity of overdiagnosis. In conclusion, mass screening in the areas, where pediatric thyroid cancer had been rarely diagnosed before, in conditions of outdated equipment of histopathological laboratories, shortage of modern literature, and high tumor expectancy after the accident, should predictably have resulted in the incidence overestimation.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1843

**Hyperfunctioning thyroid nodule and thyroid cancer**

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Thyroid nodules are common and majority is benign. Even so, clinicians and patient always concern about the risk for malignancy. In the past it was believed that hyperthyroidism excluded the possibility of a thyroid carcinoma. We describe here two patients presenting "hot" thyroid nodules with final diagnosis of papillary thyroid carcinoma. Informed consent was obtained. Case 1: A 39-years-old female, presenting with irritability, disquiet, and neck swelling. A nodule was palpable in left lobe. Cervical ultrasonographic studies showed a nonhomogeneous nodule in an enlarged left lobe. Serum examinations demonstrated hyperthyroidism. 99m Tc and 131I scintigraphy showed hot area corresponding to the nodule in the left lower lobe. A total thyroidectomy was performed and post operative histological examination revealed a papillary carcinoma within the nodule of the lower left lobe. Macroscopically the white nodule measured 1.4 cm and surrounding thyroid tissue had no evidence of infiltration. No metastatic foci were recognized in adjacent tissues. Case 2: A 46-years-old female developed symptoms of neck swelling and palpitations. A large painless hard nodule was palpable in the right lobe of thyroid. Ultrasonography revealed a large right lobe with solid nodule without lymphadenopaties. Thyroid function tests showed TSH undetectable of $<0.01$ μIU/mL, free thyroxine was 1.99 ng/dL (normal 0.7–1.8), and her triiodothyronine was 5.6 pg/mL (normal 2–4.4). A 99mTc scan demonstrated a "hot" area corresponding to the nodule with a suppressed uptake in the remaining thyroid tissue. RAIU value was 46% (normal = 15–35%). Right loboisthmectomy was performed and the histological examination revealed a papillary carcinoma with no signs of angioinvasivity. The surrounding thyroid tissue showed no signs of infiltration or spreading. A completion thyroidectomy revealed metastasis in 5/6 limph nodes. Thyroid carcinoma rarely occurs concomitantly with dominant hyperfunctional nodules. Papillary thyroid cancer within a "hot" nodule is reported to be much less than 1%. These cases illustrated that malignancy cannot be always excluded even in autonomously functioning thyroid nodules consequently all thyroid nodules require careful diagnosis and appropriate management.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1844

**Benefits and complications of total thyroidectomy as a surgical treatment for Graves'disease and for thyroid cancer**

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Introductions
Some surgeons are easy to avoid total thyroidectomy as a surgical treatment for Graves'disease and even for thyroid cancer, because of possible complications (i.e. laryngeal nerve injury, hypoparathyroidism, etc). We studied the benefits and complications of total thyroidectomy in 206 patients with Graves' disease or with thyroid cancer who were treated in our institution.
Methods
Surgical outcomes were analysed in the following two groups. 101 patients with Graves' disease (group A) and 105 patients with thyroid cancer (group B) underwent total thyroidectomy in the past three years. We evaluated post-operative hypocalcemia, laryngeal nerve injury, and pathological findings in the both group and transition of TSH receptor antibody for group A.

Results
Post-operative hypocalcemias were recognized in 45 patients (44.6%) in group A and 38 patients (36.2%) in group B. Post-operative hypocalcemia appeared early in the patients with hungry bone. All of them except one recovered after taking some alfacacidol and calcium lactate for a couple of weeks. One patient in group B turned out permanent hypoparathyroidism. No laryngeal nerve injury was recognized. TSH receptor antibody was successfully decreased in almost all patients of group A. Incidentalomas (papillary carcinomas) were recognized in 5 patients (5.0%) of group A. Contra-lateral metastases were recognized in 7 patients (6.7%) of group B.
Conclusions
5 patients with Incidentaloma and 7 patients with contra-lateral metastases were received benefit from the total thyroidectomy. Although 83 patients (40.3%) were suffered from post-operative hypocalcemia, 82 patients (98.8%) recovered within a short term. All patients successfully underwent the surgery and most of them felt happy being free from their diseases. Ultimately, to avoid relapse or recurrence of their diseases, total thyroidectomy is acceptable surgery even though they have to take thyroid hormone for the rest of their life.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1845

**Bilateral adrenal metastases due to follicular thyroid carcinoma: case report and litterature review.**

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Introduction
Apart from pulmonary and bone localizations, distant metastases from well differentiated thyroid carcinomas are very rare. Among them the adrenal ones are exceptional, and have generally a poor prognosis. The proof of the thyroid origin is not easy except if there is a histological confirmation by a CT guided biopsy of the adrenal tumor as in this observation
Observation
A woman aged 56 was referred to our unit for pulmonary metastases of a well differentiated thyroid carcinoma whose diagnosis was made by transthoracic puncture of one of the pulmonary nodules that reacted with anti thyroglobulin antibodies. In fact she was operated on twice (in 2005 and 2009) for thyroid disease diagnosed as an oncocytic metaplasia, and then she was lost in sight. During her hospitalization numerous bone and pulmonary metastases were found. Echosonography and CT scan showed two larges adrenals. A CT scan guided biopsy of the left adrenal confirmed the thyroid origin.
Conclusion
Adrenal metastases due to follicular thyroid cancer are exceptional. They may be uni or bilateral. Their prognosis is poor as they are diagnosed at the late stage of the disease as in our observation. A CT scan guided biopsy (when possible) is very helpful for the histological proof.
Declaration of interest
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## P1846

**Primary Thyroid Lymphoma Diagnosed During Pregnancy: Report of a Case**

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Introduction
The incidence of non-Hodgkin lymphoma (NHL) during pregnancy is quite low. There have been a few anecdotal reports of NHL that arises from the thyroid gland.
Case Report
A 28-year-old female patient presented with a neck swelling, pain and respiratory distress while she was in the 17th week of her first pregnancy. Subtotal thyroidectomy was performed and combined histological and immunohistochemical analyses led to the diagnosis of diffuse large B cell lymphoma with a high proliferation index (Ki-67: 80%) and positive CD20, CD10 and BCL6. Chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP), 6 cycles every 4 weeks) was planned and the patient underwent the first 4 cycles without vincristine. Twenty weeks after the initiation of chemotherapy she had cesarean delivery of a healthy, full-term male infant. Four additional courses of chemotherapy were administered, after which the patient was reevaluated. She had no complaints and a total body CT did not show relapse of the disease.
Conclusions
Because of the rarity of NHL and the variety of subtypes of NHL during pregnancy it is not possible to make accurate assessment of outcomes. Our case is a one of the few examples that thyroid lymphoma associated with pregnancy can be successfully managed with a combination of surgery and chemotherapy.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1847

**The efficacy of 50mCi versus 100mCi radioactive iodine for thyroid remnant ablation after total thyroidectomy among patients with low stage differentiated thyroid cancer**

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The recommended amount of administered radioiodine activity needed to successfully ablate thyroid remnants after total thyroidectomy is controversial. We compared the efficacy of 50mCi and 100mCi radioactive iodine for thyroid remnant ablation among patients with low stage and low to intermediate risk differentiated thyroid cancer who underwent total thyroidectomy through a retrospective cohort study in a tertiary multi-specialty referral center located in Quezon City, Philippines. A total of 74 patients who underwent total thyroidecomy for low stage differentiated thyroid cancer were included, with main exposure as the activity of radioactive iodine used for remnant ablation and main outcome measures were efficacy of remnant ablation, need for repeat RAI, adverse events and duration of stay at isolation. There were 16 patients (66.7%) in the 50mCi group and 38 patients (76%) in the 100mCi group who had successful remnant ablation (P value 0.526). Risk ratio for failure of remnant ablation among those who received 50mCi RAI is 1.389 (95% CI 0.656–2.942). There was no statistically significant difference in the need for repeat RAI between the two groups (RR 2.083, 95% CI 0.312–13.909, P value 0.440). The most common acute adverse events after administration of RAI reported were nausea (22% for the 100mCi group versus 4.2% for the 50mCi group) and neck pain (16% for the 100mCi group versus 4.2% for the 50mCi group). Adverse events were more likely to occur among those who received 100mCi versus 50mCi (36% versus 21%) although this did not achieve statistical significance (RR for total adverse events in the 50mCi cohort 0.579, 95% CI 0.244–1.372, P value 0.187). Nausea was more likely to occur among those who received 100mCi (RR 5.29, 95% CI 0.723–38.46). Duration of hospital stay was shorter among those who received 50mCi (median 2 days) versus those who received 100mCi (median 2.5 days), P value 0.090. Our results show that administration of 50mCi RAI may be as effective as 100mCi for ablation of residual thyroid tissue among patients treated with total thyroidectomy for low stage and low to intermediate risk of recurrence for differentiated thyroid carcinoma. Administration of 50mCi RAI is associated with fewer short term adverse events and a shorter stay in an isolation unit.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1848

**Radioiodine ablation can be avoided in intrathyroidal papillary thyroid cancers ≤2 cm (pT1N0M0)**

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The need for thyroid residue ablation in papillary thyroid cancer (PTC), classified with the TNM staging (AJCC, 2002) as pT1, with a maximum diameter comprised between 1 and 2 cm (pT1/1-2) and/or multifocal, is still debated. Aim of this study was to compare the outcome of pT1N0M0 patients submitted or not to postsurgical radioiodine ablation. Consecutive patients followed-up at this Institution from 1996 to 2010 were enrolled. All pT1/1-2N0M0 cases were radioiodine ablated before June 2002 and not ablated after that date. Patients with local or distant metastases at post-therapeutic whole body scan were excluded. The patients enrolled were 257 (214 F, mean age 47.3 yrs), the median follow-up was 88 months and the persistence/recurrence was evaluated according to the most recent European guidelines. Patients were submitted to total thyroidectomy, associated in 63 cases to central neck dissection. Tumors were classified as pT1/<1 (n=169, Group A) and pT1/1-2 (n=88, Group B). Multifocality was found in 35.5% of Group A and in 38.6% of Group B patients. Central neck dissection was performed in 18.3% (Group A) and 36.3% (Group B) of cases. Radioiodine ablation was performed in 26% of Group A (mean 131 dose: 74 mCi) and in 49% of Group B patients (mean 131 dose: 75 mCi). At the end of follow-up, persistent patients were 2/169 in Group A (both ablated) and 3/88 in Group B (all ablated). At a logistic regression analysis either age, gender, nodule diameter, central neck dissection, multifocality, or radioiodine ablation have been found not to influence the outcome. To our knowledge these are the first data showing that, in the pT1N0M0 category of PTCs, the outcome is not associated neither with the tumor size, nor with the therapeutic intervention, suggesting that radioiodine ablation can be avoided also in pT1/1-2 cases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1849

**Hürthle cell thyroid carcinoma metastatic to the choroid detected by radioiodine scintigraphy: a case report**

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In July 2010, a 69-year-old woman presented because of a CT proven 5th lumbar vertebral metastasis of thyroid carcinoma and in August she underwent a total thyroidectomy and central node dissection. Histopathology revealed a moderately differentiated Hürthle cell carcinoma which was according to TNM classification pT3N1M1 tumor. Thereafter, a right hemylaminectomy of L5 and metastasectomy was performed in order to decompress the L5 and S1 spinal nerves. The histopathology confirmed the diagnosis of metastatic Hürthle cell carcinoma. In October 2010, the patient had a radioiodine ablation of thyroid remnant using rhTSH, followed by an external beam irradiation of the lumbar spine with concomitant chemotherapy. Post-therapeutic whole body scintigraphy revealed lesions in bone and lungs and also an increased accumulation of radioiodine in the projection of the left orbit, which could be caused by a physiological accumulation of iodine in tear gland or a metastasis. In February 2011, the patient noticed an impaired vision in her left eye. During the next month a half of her left visual field was progressively appearing blurred. Echography of the left eye revealed a solid irregular choroidal lesion of approximately 6.5 mm thickness with some round hypoechogenic regions, causing exudative retinal detachment above the papilla. The echographic image was typical of an ocular metastasis. A MRI scan of the head confirmed a mass in the choroid of the left eye. Since radioiodine accumulated in this lesion, it was diagnosed as a thyroid carcinoma metastasis to the eye. Palliative external irradiation of the left orbit was performed, followed by administration of a therapeutic dose of radioiodine after rhTSH aplication. Only a trace of radioiodine accumulated in the projection of an eye metastasis. The control eye echography in July 2011 showed a partial regression of the metastatic choroidal tumor.

Magnetic resonance imaging coronal (A) and axial (B) scan showing left intraocular metastasis of Hürthle cell thyroid carcinoma.

Declaration of interest

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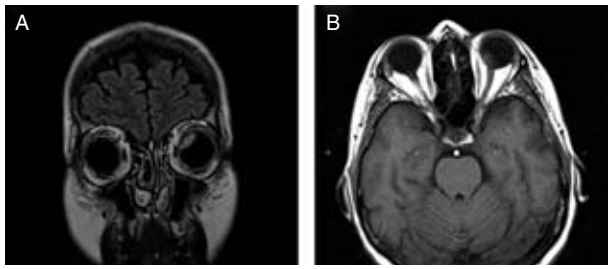


## P1850

**Features of incidental thyroid carcinoma in Gohierri county.**

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Introduction

In the last years, has produced an increment in diagnosis of thyroid cancer. Incidental thyroid carcinoma is defined as a tumour diagnosed after surgery for benign lesions.

Aim. The objective of this study was to assess the clinical presentation, tumour characteristics and follow-up.

Patients and methods

We analysed clinicopathologic data of incidentally thyroid carcinoma from 351 thyroid surgeries carry out in our hospital between 01-01-2005 and 31-06-2010, for uni or multinodular goiter.Surgical criteria was nodule size.

Results

Of 351 surgeries in 28 cases incidental thyroid cancer was detected of these 18 were papillary micro carcinomas (PMC), all females of 55.11±11.34 year-old (range 37–79)with a size of 5.44±2.66 mm (range 1–9 mm);10 papillary(2 follicular variant), all women of 9.55±13.8 year-old with a size of 28.4± 13.1 mm. FNA previous surgery, was carried out all patients and were negatives for malignity in all cases.Total thyroidectomy was carried out by multinodular goiter to all patients. Association with Hashimoto thyroiditis was detected in 7 patients, benign follicular lesions in 5, multicentric tumor 3 cases.Lymph-node metastasis were not disclosed in histological study.Patients with papillary tumours and multicentric PMC were treated with radioiodine.

Mean of follow up 2.35±1.56 years. No metastasis or relapses or death have been detected during follow-up.

Conclusion

Incidental thyroid carcinoma are more common in women and PMC is the main diagnosis after total thyroidectomy. Lymph-node metastasis were not found in our study. Treatment with radioiodine was performed in papillary and multifocal PMC. The prognosis in our study has been good.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1851

**The evaluation of fine needle aspiration biopsy results in thyroid nodules below and above one centimeter diameter**

S. Yazgan, F. Besir, O. Yazgan, R. Buyukkaya, H. Erdem, E. Onder, A. Buyukkaya, B. Erdogmus & Y. Aydin
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Objective

We aimed to investigate the ultrasound (US) features and malignancy rates of thyroid nodules, below and above 1 centimeter diameter, according to fine needle aspiration biopsy (FNAB) results.

Materials and Methods
Total 157 nodular goiter patients, 60 patients' nodules were below 1 cm and 97 patients' nodules were above 1 cm, analyzed retrospectively. The nodules with pure cystic, peripheral calcification and inadequate cytological result were excluded. As a malignancy criterion at US, including hypo echoic pattern, solid structure, micro calcification features of nodule and not having peripheral halo feature of nodule were used. In statistical analyze, $P<0.05$ was accepted as significant.
Results
The malignancy or suspicions cytology rate was found 5% in nodules with below 1 cm, 17.5% in nodules with above 1 cm (total malignancy or suspicions cytology rate: 12.7%) and statistically it was not observed significant difference ($P=0.548$) . The statistical significant differences was not observed between thyroid nodules below and above one centimeter in terms of the US features of nodules for malignancy or suspicions cytology results.
Conclusion
In this study, we found that the US features of the nodules were not significant for determine to perform FNAB or not. Also, the thyroid nodules between below and above 1 cm of FNAB results were not statistically significant difference. Therefore, in our opinions, FNAB should be performed all thyroid nodules regardless of size and US features for early diagnosis of thyroid malignancy.
Keywords: Thyroid, nodule, ultrasound, fine needle aspiration biopsy, size
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1852

**Sonographic characteristics of thyroid nodules and the corresponding cytological results from the fine-needle aspiration biopsies**
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Introduction
A major objective of the ultrasound of thyroid nodules is the stratification of the risk for possible malignancy and the selection of nodules for fine-needle aspiration biopsy (FNAB) and cytological examination.
Objective
The objective of this study was to assess the sonographic criteria used in the selection of thyroid nodules for FNAB and to compare them to the cytological reports.
Methods/Design
This was a retrospective study. 155 cases of FNAB of thyroid nodules for the last 3 years were suitable for analysis (147 women and 8 men). The thyroid sonography was performed on a Fukuda-Denshi 5500 device (Fukuda Corp., Japan) with a linear-array transducer 7.5 MHz. The ultrasound-guided FNABs were performed by the "free hand" technique; smears were fixed and processed according to Pappenheim.
Results
The following ultrasound features suspicious for possible malignancy were reviewed: hypoechogeneity to the surrounding parenchyma (which was the case in 51.6% of the biopsied nodules), spiculated margins (5.2%), blurred margins (48.4%), absence of halo (34.8%), presence of microcalcifications (5.8%), a taller than wide shape (7.7%), and a significant cervical lymphadenopathy (0.6%). 7.1% of all nodules had no suspicious features, 32.2% had only one, 45.8% - two, and 14.8% had three feautures. The cytology reports showed no risk of malignancy in the absence of suspicious features, a 10% risk - if one feature was present, a 16.9% - if two were present, and a 21.7% risk - if three ultrasound features were present.
Conclusion
The studied thyroid nodules had not been optimally selected by ultrasound prior to the FNAB.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1853

**Surgery of Papillary Thyroid Carcinoma in Children and Adolescents**
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Background
Papillary thyroid carcinoma in children and adolescents is rare but it shows extremely aggressive behavior. Gross lymph node metastases and distant metastases are common on first clinical presentation.
Patients and methods
Forty five children and adolescents were operated due to PTC. Mean age was 16.6 years (range 7–21). At the time of diagnosis 13% had lung metastases. Total thyroidectomy or completion of thyroidectomy was performed in all cases followed with central neck dissection and frozen section examination of lower jugulo-carotid compartments
Results
Median tumor size was 1.9 cm. PTC was found in 44 and FTC in one patient. Multifocal tumors were found in 37% and capsular invasion in 29% and vascular invasion in 24% of cases. LNM in either central or lateral neck compartments were found in 76% of patients. Capsular and vascular invasion were significantly more frequent in children less than 16 years of age. Median follow-up was 127 months. Overall survival rate was 100%.
Conclusion
PTC in children is characterized with high incidence of loco-regional aggressiveness, multifocality, lymph node metastases and distant metastases at the time of diagnosis. Extensive surgical approach should be performed in both primary and recurrent disease in young patients with PTC.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1854

**Diffuse Large B Cell Lymphoma of Thyroid Gland Presenting With Acute Tracheal Compression**
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Introduction
Non-Hodgkin lymphoma (NHL) involving the thyroid gland constitutues 2–3% of all NHL cases, 2–8% of all thyroid malignancies. The majority of lymphomas arising in the thyroid gland are MALT lymphomas and diffuse large B cell lymphomas commonly occuring in the setting of thyroiditis. Most cases are seen in adult or elderly females. The thyroid enlargement is often rapid and can lead to symptoms of tracheal or laryngeal compression. Herein, we present a case of diffuse large B cell lymphoma of the thyroid gland presenting with acute tracheal compression.
Case report
A 60 year-old woman had received a diagnosis of pneumonia with symptoms of cough, expectoration and dispnea. She had begun antibiotherapy. During treatment, a rapid progression of dispnea had occured. A huge, retrosternal goitre compressing trachea had been detected by computerized tomography. She had gave a history of a rapid increase in the size of swelling on her neck over 3-month period associated with difficulty in swallowing. She had been entubated and gone under urgent thyroidectomy and tracheostomy. After operation, she was extubated, followed by tracheostomy. Pathologic specimen established the diagnosis of diffuse large B cell lymphoma of the thyroid gland (Default 1). Bone marrow aspiration was negative for metastasis. Positron Emission Tomography performed for staging revealed involvement of locoregional lymph nodes; cervical, supraclavicular, and mediastinal lymph nodes (Figure 2).
She was given R-CHOP (Rituximab, Cyclophosphamide, Adriamycin, Vincristin, Prednisolon). A remarkable regression for the size of the lesions was observed after the first cure of therapy. Tracheostomy has been decanualeted and is being waited for healing.
Conclusion
Thyroid lymphoma is a rare disease. Fine needle aspiration and ultrasonography may be usefull for acutely swelling glands. Medical treatment and radiotherapy are first line treatments. Patients may present with acute compression symptoms as in our case. Surgery may be needed to overcome compression symptoms.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
Funding
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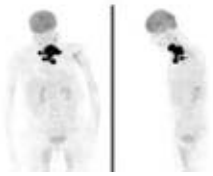
Thyroid folliculls infiltated by lymphoma cells/ Muscular invasion/ CD20 + lymphoma cells



PET-CT imaging

## P1855

**Papillary thyroid carcinoma with a sollitary central nervous system metastasis**

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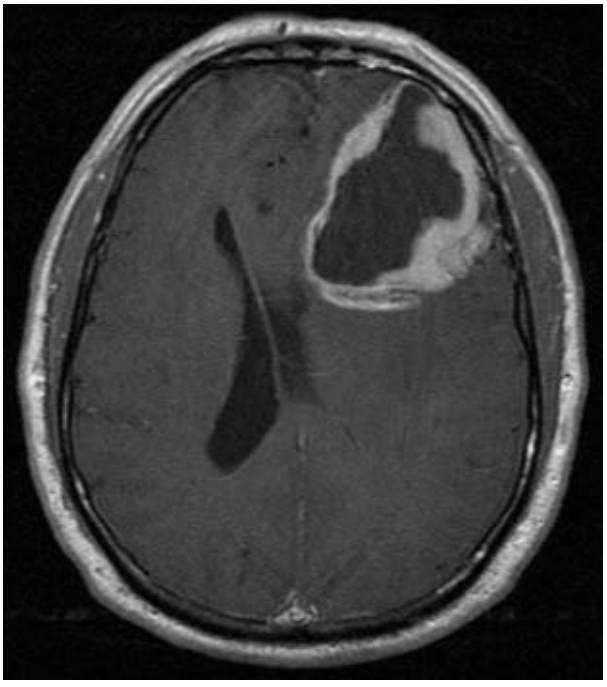
Introduction
Papilar thyroid carcinoma (PTC) is the most common thyroid neoplasia. It usually has a good prognose, being lymph node spread common. Distant metastasis are present in 9–10% of cases, worsening prognose. Cerebral metastasis are extremely rare, representing 1% of all metastasic PTC.



Case report
A 68-year-old man was sent to the Endocrinology Unit for follow-up of a PTC that had been operated (total thyroidectomy) 2 months before. After 100 mCi of I-131, total body scan(TBS) showed a pathological cervical lymph node. Due to this, a functional cervical lymph node dissection was performed. However, after stimulation with recombinant TSH, thyroglobuline was 59 ng/ml, so 100 mCi of I-131 were given again. The TBS showed a pathological uptake in the proximal third of the left femur, confirmed with a MRI, where a sollitary 15 mm bone lytic lesion was shown. No other images suggested metastasis, so excission of the bone lesion and hip replacement were done. The anatomic pathology report informed of a PTC metastasis with healthy tissue border. Thyroglobuline levels continued being high, so a PET with TSH-RH was done, reported as normal. However, due to high levels of thyroglobuline (138 ng/ml), additional150 mCi of I-131 were given. In the TBS done afterwards, no pathological uptakes were observed. The cervical ultrasound and CT-scan were normal. Six months later, the patient consulted urgently due to apathy and loss of language fluency that had started one month before. A CT-scan showed a 6.5 cm frontal left mass conditioning subfalcial and uncal herniation and hydrocephalus. The patient was operated, and excission of the mass took place. PTC hystology was confirmed.
Conclusions
Even if PTC usually has good prognose, practicians should bear in mind that distant metastasis are possible, and that an exhausting study including CNS should take place if the metastasic phocus is not localized in the most common territories.
Fx1
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.
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## P1856

**Papillary carcinoma in a hyperfunctioning thyroid nodule**

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Introduction
Thyroid hyperfunctioning nodule usually represents benign tumor. Less than 1% of all cases have been reported as malignant lesion, especially in the presence of hyperthyroidism.
Case report
An asymptomatic 46-year-old man with a history of diabetes mellitus type 2 came for regular diabetic check. Two palpable thyroid nodules were detected during a routine physical examination. Thyroid function tests showed hyperthyroidism: TSH $<0.005$ mIU/L (0.63–4.19), FT4 31.1 pmol/L (11.5–22.7), FT3 9.7 pmol/L (3.5–6.5). Antithyroglobulin and antiperoxidase antibodies were present in elevated titre. Thyroid scintigraphy (Tc-99m pertechnetate) revealed mildly decreased glandular activity, a hyperfunctioning nodule in the left lobe and a nonfunctioning nodule in the right lobe. Thyroid ultrasound showed hypoechoic nodule (volume 3.3 ml) containing microcalcifications in the left lobe and hypoechoic nodule (volume 1.5 ml) in the right lobe. US-guided fine-needle aspiration cytology (FNAC) of a nonfunctioning nodule showed clusters of thyrocytes, phagocytes, and diffuse colloid. FNAC of the hyperfunctioning nodule was also done due to suspicious ultrasound nodule features, enlarged ipsilateral lymph nodes highly suggestive for malignancy and it revealed features of papillary carcinoma. Treatment with thiamazole was started and patient was subjected to operation as soon as he became euthyroid. A total thyroidectomy and level III, IV and V selective neck dissection were performed. Histology confirmed follicular variant of papillary carcinoma with intraglandular tumor dissemination and regional lymph nodes metastases. Postoperative whole body scintigraphy with 131I showed large inhomogeneous RI activity in the projection of the anterior cervical region. The patient received 100 mCi of the 131I for thyroid remnant ablation and levothyroxine treatment was started.
Conclusions
Papillary thyroid carcinoma rarely exists in an autonomously hyperfunctioning nodule. Clinicians should be aware that hyperfunctioning nodule, even if hyperthyroidism is present, does not exclude the presence of a well differentiated thyroid carcinoma. Although this pathology is rare, it is important to be correctly diagnosed and appropriately treated.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

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## P1857

**Concomitant hyperthyroidism and thyroid carcinoma**

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Background
Recent reports have mentioned rising incidence of thyroid cancer (TC) detected before and after thyroidec-tomy (Tx) for hyperthyroidism but "hyperfunctioning" TC is however a rare entity.
Patients & Methods
Among a personal series of 464 patients which underwent Tx including 147 (33.6%) cases of thyroto-xicosis and 72 (13.6%) cases of TC, we founded 10 patients with hyperthyroidism and nodular or microscopic TC but only one case of TC with features of thyroid overactivity. History, clinical records, biohormonal picture, imaging studies, pathology findings and surgery notes with follow-up results were analysed.
Results & Discussions
The study enrolles 10 females and one male, age ranged from 16 to 62 years. Concurrent TC was present in 3 cases of Graves' disease, 5 cases of toxic multinodular goiter and 3 cases of toxic adenoma. None of these patients had recieved previous head or cervical irradiation. One woman underwent elsewere a subtotal Tx 18 years ago. In all but one cases the diagnosis was established by permanent section of the surgical specimen. There were 4 nodules with indeterminate results at frozen section but this procedure furnised a valuable indication in only one case with a follicular sample. Six papillary lesions was microcarcinomas. In the whole group we have 10 papillary and only one follicular cancer. Surgery was the mainstay therapy in all these lesions, the suspicion or presence of cancer imposing the extent of exeresis. Six near-total Tx, 4 hemiTx and one completion Tx were done. Appropriate additional mesures included radioiodine and hormonal therapy in 9 observations. Postoperative results were good with no morbidity and mortality. The long-term study showed 8 cases alive and disease-free in an average follow-up of 60 months.
Conclusion
Hyperthyroidism do not exclude the presence of TC, the latest being crucial for the choice of the surgical strategy and prognosis. The rising number of Tx in cases of hyperthyroidism will probably detect more observations with associated thyroid (micro)carcinoma.
Declaration of interest
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