

# Endocrine Abstracts

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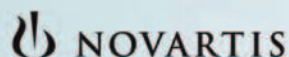
**STRENGTH, BUILT ON EVIDENCE.**

\*Range reflects results derived from pivotal trials and long-term follow-up data.

†Includes both ongoing and completed trials in acromegaly and functional GEP NETs.

††Combined experience with immediate release Sandostatin® and Sandostatin® LAR®.

**References:** 1. Data on file, Novartis Pharma AG. 2. Cozzi R, Attanasio R, Montini M, et al. Four-year treatment with octreotide-long-acting repeatable in 110 acromegalic patients: predictive value of short-term results? *J Clin Endocrinol Metab.* 2003;88:3090-3098. 3. Sandostatin® LAR® Basic Prescribing Information, Novartis Pharma AG. 4. Pubmed [database online], National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov/sites/gquery?term=sandostatin>. Accessed February 12, 2010.





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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.



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### Summer School on Endocrinology

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### 11th ESE Postgraduate Course in Clinical Endocrinology

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### ESE Clinical Update 2011

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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.

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Successful candidates will attend both The Endocrine Society and European Society of Endocrinology annual meetings. The successful candidates will take part in an interview process at both annual meetings, where they will have the opportunity to meet principal investigators of several laboratories with the purpose of choosing the most appropriate laboratory for postdoctoral training.

The closing date for 2011 applications has now passed but check the ESE website for the application requirements for 2012!



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

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IMPACT FACTOR: 3.539 (as at June 2009)

### **Journal of Endocrinology**

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## 13th European Congress of Endocrinology

30 April – 4 May 2011, Rotterdam, The Netherlands

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# Prize Lectures and Biographical Notes

## European Journal of Endocrinology Prize Winner

The *European Journal of Endocrinology* Prize is awarded to a candidate who has significantly contributed to the advancement of the knowledge in the field of endocrinology through publication. This year's recipient is Professor Manuel Tena-Sempere. The prize will be presented as part of the ECE 2011 opening ceremony where Prof. Tena-Sempere will deliver his lecture. Prof. Tena-Sempere will also write a review article based on this lecture to be published in the *European Journal of Endocrinology*. Further information can be found on <http://www.euro-endo.org/prizes/default.htm>

Manuel Tena-Sempere, Spain



Manuel Tena-Sempere (1969) MD, PhD is full Professor of Physiology at the Department of Cell Biology, Physiology and Immunology of the University of Córdoba, Spain. His expertise lies in Reproductive and Metabolic Endocrinology, with special attention to the neuroendocrine regulation of puberty onset and the reproductive axis (i.e. study of the molecular events responsible for the proper activation and function of the hypothalamic-pituitary unit and the gonads), and their modulation by energy balance and metabolic and nutritional cues. In this context, Dr Tena-Sempere and his group have significantly contributed to the characterization of the physiological roles of kisspeptins and GPR54 in the control of key aspects of reproductive function, including puberty onset, gonadotropin secretion, positive and negative feedback effects of sex steroids, ovulation and

metabolic gating of fertility. Overall, his research activities in this area have resulted to date in more than 55 articles and invited reviews in international peer-reviewed journals, and more than 40 invited lectures and international seminars over the last 6 years. In addition, Dr Tena-Sempere and co-workers have provided novel evidence for the reproductive effects and mechanism of action of different signals primarily involved in energy homeostasis, and several studies of his group have been recently published concerning the characterization of the physiological role of those signals (leptin, ghrelin, PYY3-36, GALP, 26RFa, neuromedins, nesfatin-1) in the control of several aspects of reproductive function, including the regulation of puberty onset as well as gonadotropin secretion and gonadal function. Overall, Dr Tena-Sempere has published 185 articles in international peer-reviewed journals over the last 18 years (PubMed database; February 2011), including a significant number (>95) of research papers and invited reviews in authoritative, highly-ranked journals in the last 6 years. The total number of citations of Tena-Sempere's work is presently >4495, with an *H*-index of 38.

In addition, Dr Tena-Sempere has participated as invited speaker in numerous international meetings and seminar series (>50 in the last 7 years), and has served/serves as member of the Editorial Boards of prestigious journals of the field, such as *Neuroendocrinology* (acting as Associate Editor), *Endocrinology*, *Molecular & Cellular Endocrinology*, *PLoS ONE*, *Journal of Neuroendocrinology*, *Peptides*, *Frontiers in Endocrinology*, *Frontiers in Pituitary Endocrinology* and *Journal of Physiology & Biochemistry*. Dr Tena-Sempere is scientific partner (PI) and member of the steering board of the Spanish Centre of Excellence for Research in Obesity and Nutrition, where he serves also as responsible for its formative program. In addition, Dr Tena-Sempere is PI of one of the groups of the recently created Maimonides Institute of Biomedical Research of Córdoba (IMIBIC), and panel member of the area of Biomedicine of the Spanish Agency for Evaluation of Research Activities (ANEP). The research group of Dr Tena-Sempere, that keeps active international collaborations with numerous leading groups in the field of Endocrinology, is currently composed of 18 members, including senior scientists, post- and pre-doctoral fellows and technicians, and is funded by numerous projects from different Spanish and European agencies, as well as private institutions.

The *European Journal of Endocrinology* Prize Lecture

**(Aiming at) Deciphering puberty**

Manuel Tena-Sempere, Department of Cell Biology, Physiology and Immunology, University of Córdoba, Córdoba, Spain

Puberty, as the key developmental period when reproductive capacity is attained and sexual and somatic maturation completed, is under the control of a complex series of regulatory mechanisms that are sensitive to endogenous factors and environmental cues. The mechanisms of normal puberty onset and its potential deviations have been deeply scrutinized in humans and model species. However, characterization of the neuro-hormonal basis of puberty remains incomplete. In addition, evidence (and concern) is mounting that the timing of puberty is changing in several countries, with a trend for earlier puberty onset, especially in girls, whose underlying causes are ill defined. In the last decade, our laboratory has aimed at deciphering some of the basic neuroendocrine and molecular mechanisms responsible for the control of puberty onset, using rodents as pre-clinical models. In this presentation, a synoptic overview of the experimental work conducted by our group towards this aim will be presented, with special attention to our studies on the roles of the Kiss1 system in the control of the gonadotropic axis in general and pubertal timing in particular, as well as on the mechanisms for the metabolic regulation of puberty. Likewise, recent studies of our group on the putative roles of additional neuropeptide pathways (e.g., neurokinin B and nesfatin-1) and molecular mechanisms (e.g., mTOR and AMPK signalling) in the central regulation of puberty will be briefly described. Our basic studies will be discussed in the context of recent seminal findings regarding key regulatory mechanisms of human puberty and their potential contribution to disorders of pubertal timing and reproductive function. Ideally, such debate will highlight the relevance of translational research for widening our understanding of the basis of human physiology and disease; fields where Reproductive Endocrinology has provided paradigmatic examples in recent years.

## Geoffrey Harris Prize winner

This prestigious prize is intended for established workers in the field of basic and clinical neuroendocrinology and is generally supported by Ipsen. This year's recipient is Professor Ezio Ghigo. The prize will be presented as part of the ECE 2011 opening ceremony where Professor Ghigo will deliver his lecture. Professor Ghigo will also deliver two other lectures at future ESE scientific meetings. Further information can be found at <http://www.euro-endo.org/prizes/default.htm>

Ezio Ghigo, Italy



Ezio Ghigo, MD, is Professor of Endocrinology in the School of Medicine at the University of Turin, Head of Endocrinology, Diabetology and Metabolism, and Chairman of the Department of Medicine in the SGB-Molinette University Hospital of Turin, Italy. He is currently also Dean of the School of Medicine of the University of Turin.

His research interests include the neural control of anterior pituitary function, ghrelin, glucose and lipid metabolism, the endocrinology of ageing, obesity and anorexia nervosa.

He has authored over 400 papers. He has been Editor-in-chief of the *Journal of Endocrinological Investigation* and is member of the editorial board of other international endocrine journals. He is a member of a many endocrine societies, was vice president of the European Society of Endocrinology and is now president elect of the Italian Society of Endocrinology.

The Geoffrey Harris Prize Lecture

**Ghrelin: a neuroendocrine and/or a metabolic story?**

E Ghigo, Turin, Italy

The acylated form of ghrelin (GRLN) was discovered as the natural ligand of the GHS-R1a. GRLN is acylated by a specific octanoyl-transferase (GOAT) and is predominantly produced by the stomach, although expressed by many other endocrine and non-endocrine, peripheral and central tissues. Also GHS-R1 is widely expressed by several central and peripheral tissues. Acylated GRLN displays strong GH-releasing activity but is not specific for GH exhibiting other neuroendocrine actions such as stimulation of PRL and ACTH and inhibition of LH. Acylated GRLN is now recognized as a potent orexigenic factor that also modulates energy expenditure as well as other important central functions. At the peripheral level, GRLN modulates gastrointestinal motility and secretion, exerts cardiovascular actions and, most of all, plays major physiological regulation of glucose and lipid metabolism. Acylated GRLN plays a diabetogenic role while non-acylated GRLN (like smaller fragments and its analogues) positively influences glucose and lipid metabolism, as indicated also by recent studies in GOAT KO mice. GRLN receptors different from GHS-R1a are likely to mediate the metabolic actions of non acylated GRLN. In all, acylated and non acylated GRLN, but also obestatin, another relevant product of the GRLN gene, play a major role in regulating peripheral metabolism and it is not by chance that their secretion is mostly under metabolic regulation.

# Plenary Lectures



## Disorders of sex development

### PL1

#### Disorders of sex development: recent progress

Tsutomu Ogata & Maki Fukami  
Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan.

Disorders of sex development (DSD) can occur as a single gene disorder, a multifactorial disorder, and an imprinting/epigenetic disorder. Here, I will report our recent progress on two causative genes for DSD.

**MAMLD1: Mastermind domain containing gene 1**

We have reported that MAMLD1 is a causative gene for hypospadias, a mild form of 46,XY DSD. This is primarily based on the identification of several pathologic mutations of MAMLD1 in patients with hypospadias. The murine homolog was specifically expressed in fetal Sertoli and Leydig cells around the critical period for sex development, and transient knockdown experiments showed significantly reduced testosterone production and Cyp17a1 expression in murine Leydig tumor cells. We also found that MAMLD1 was controlled by SF1 and transactivated the non-canonical Notch target gene Hes3 in nuclear bodies. Notably, Maml1 knockout mice had no abnormal genitalia but exhibited metabolic syndrome.

**POR: P450 Oxidoreductase**

POR deficiency (PORD) is a rare autosomal recessive disorder characterized by skeletal dysplasia referred to as Antley-Bixler syndrome, adrenal dysfunction, 46,XY and 46,XX DSD, and maternal virilization during pregnancy. We have studied 38 patients with POR, and classified them into two major groups: group A, homozygosity for R457H with low residual POR activity; and group B, compound heterozygosity for R457H and one apparently null mutation. Phenotypic comparison between group A and group B suggest that the residual POR activity reflected by the R457H dosage constitutes the underlying factor for clinical variability in some features but not in other features, probably due to the simplicity and complexity of POR dependent metabolic pathways relevant to each phenotype. Notably, PORD is almost invariably associated with 46,XX DSD, and this is primarily explained by placenta-derived testosterone exposure and backdoor pathway (fetal adrenal)-derived dihydrotestosterone exposure. Furthermore, we have identified the promoter region of POR gene by analyzing three patients with apparently heterozygous mutations.

**Conclusions**

Although DSD is a highly heterogeneous condition with many unknown underlying factors, the black box is gradually shrinking with progress in molecular endocrinology.

## Making sense with thyroid hormone

### PL2

#### Making sense with thyroid hormone

Douglas Forrest, Emily Cordas, Yulong Fu, Hong Liu, Ailing Lu, Michelle Ma, Lily Ng, Robin Peeters & David Sharlin  
Clinical Endocrinology Branch, NIDDK, NIH, Bethesda, Maryland 20892, USA.

Thyroid hormone has long been known as a key developmental factor in human and model species. Insufficient thyroid hormone during development can impair the growth and maturation of diverse tissue and organ systems. One of the most serious risks associated with congenital hypothyroidism is defective brain maturation and mental retardation. However, in recent years, targeted mutagenesis of the thyroid hormone receptor genes in model species has expanded our understanding of the neurodevelopmental role of thyroid hormone to include sensory systems. The development of all of the senses may be subject to some degree of control by thyroid hormone with the auditory and visual systems being particularly sensitive. Each of these systems is characterized by unique sensory cell types and specialized anatomy that allows the detection of stimuli as different as mechanical sound waves and photons of light. Our studies have indicated that the developmental timing and nature of a given cellular response to thyroid hormone reflect the cooperative action of several genes that mediate hormone action, including genes encoding different thyroid hormone receptor isoforms and other genes encoding hormone-activating and hormone-inactivating deiodinase enzymes. The evidence suggests that one of the most fundamental roles of thyroid hormone is to promote the development of sensory function.

## Targets for the management of diabetes in 2011

### PL3

#### Targets for the management of diabetes 2011

Jens Sandahl Christiansen  
Aarhus University Hospital, Brendstrupgårdsvej, Denmark.

The Management of both type 1 diabetes (T1DM) and type 2 diabetes (T2DM) involves careful monitoring and treatment of hypertension, hyperlipidaemia and hyperglycaemia. Aggressive treatment of high blood pressure and high cholesterol has been proven effective in reducing mortality in both T1DM and T2DM. Various guidelines advocate certain values for blood pressure and for cholesterol to be aimed for. These cut-off values have been pushed downwards constantly during the last 3 decades. 'The lower, the better' has been the mantra – although some have cautioned, that a lower limit exist from where a further reduction will not be beneficial, or even harmful (the J-curve phenomenon). Recent prospective studies indicate, that this phenomenon does exist as regards antihypertensive therapy – at least in certain patient groups.

The association between glycaemic control and the development of microangiopathy has been obvious for many years, and prospective studies have proven glycaemic intervention to be effective. The clinical problem here deals with the delicate balance between avoiding prolonged hyperglycaemia leading to late complications (Scylla) and hypoglycaemia threatening life and well being of the patients (Charybdis). Studies within the last 5 years have suggested the presence of the J-curve phenomenon as regard glycaemic control – leading to a revision of the therapeutic targets.

## Gonadotrophin Signalling in Health and Disease

### PL4

#### Gonadotrophin signalling in health and disease

J Huhtaniemi<sup>1,2</sup>  
<sup>1</sup>Imperial College London, London, UK; <sup>2</sup>University of Turku, Turku, Finland.

The main functions of the two pituitary gonadotrophic hormones, luteinising hormone (LH) and follicle stimulating hormone (FSH), and of the placental choriongonadotropin (hCG), are well characterized today. In clinical medicine, gonadotrophin measurements in peripheral serum form a valuable tool in the diagnosis of disorders in sexual development and fertility, and gonadotrophins are the mainstay in infertility treatments. A great deal of the new information about physiology and pathophysiology of gonadotrophin secretion and actions has emerged from mutations detected in the genes of gonadotrophins and their cognate receptors (R), as well as from genetically modified mouse models. The recent detection of gonadotrophin actions in extragonadal tissues both in humans and experimental animals is a topic of continuing controversy. The contributions of our laboratory to this new information include the detection of an inactivating mutation of FSHR and a functionally significant polymorphism in the LHBeta gene. We have also produced a knockout mouse for LHR, and transgenic mice overproducing hCG and expressing a constitutively activated mutant of FSHR. Our findings have clarified some of the controversies surrounding the extragonadal gonadotrophin actions. Our current research is concerned with gonadotrophin physiology and pathophysiology using a variety of genetically modified mouse models now at our disposal. Our key findings include the following: Neither the physiological high intratesticular testosterone concentration nor FSH action are necessary for the induction of spermatogenesis, extragonadal LHR expression is not necessary for the maintenance of female mouse fertility, and homodimerisation of LHR molecules is a physiologically important mode of their action. Whereas a constitutively activating mutation of FSHR induces a dramatic phenotype in the female, the consequences of such a mutation in the male are surprisingly mild. The recent developments in the field indicate that gonadotrophin function will remain in the focus of research in reproductive endocrinology.

## Endocrine hypertension

### PL5

#### Endocrine hypertension

W Young  
Mayo Clinic, Rochester, Minnesota, USA.

**Objective**

To review the first reported cases of successfully treated pheochromocytoma and primary aldosteronism and document the diagnostic and therapeutic advances that have occurred since the initial descriptions.

**Methods**

The original case descriptions and the subsequent pertinent literature were reviewed.

**Results**

The successful management of the initial cases of pheochromocytoma in 1926 and primary aldosteronism in 1954 was highlighted by keen clinical observation, clinical intuition, and the application of scientific principles. Since those prismatic case descriptions, the technological advances in laboratory-based diagnosis, radiology-based tumor localization, and surgical approaches to the adrenal glands have been truly remarkable.

**Conclusions**

The evolution in the diagnosis and treatment of pheochromocytoma will continue to progress as we identify more genetic causes, develop biochemical markers for 'pre-clinical' pheochromocytoma, identify better markers for malignant disease, and develop more effective treatment options for malignant pheochromocytoma. Over the next decade we hope to determine the pathophysiology for bilateral idiopathic hyperaldosteronism, develop less invasive and less technically demanding tests to distinguish between unilateral aldosterone-producing adenoma and bilateral idiopathic hyperaldosteronism, determine where low renin hypertension stops and primary aldosteronism starts, and determine the impact of genetic and environmental factors on aldosterone secretion in patients with and without primary aldosteronism.

## Neurosteroids as Regenerative Agents in Brain: Therapeutic Implications for Cognition and Neurodegenerative Disease

**PL6**

### Neurosteroids as regenerative agents in brain: therapeutic implications for cognition and neurodegenerative disease

Roberta Brinton

USC Clinical and Translational Science Institute, University of Southern California, Los Angeles, California, USA.

Allopregnanolone (AP $\alpha$ ), a neurosteroid, significantly increased proliferation of rodent and human neural progenitor cells *in vitro* via GABA receptor channel and L-type channel mechanisms that activated the cell cycle. We subsequently investigated the efficacy of AP $\alpha$  to promote neurogenesis in the hippocampal subgranular zone (SGZ), to reverse learning and memory deficits in the 3 month-old male triple transgenic mouse model of Alzheimer's (3xTgAD). AP $\alpha$  significantly increased BrdU<sup>+</sup> cells in SGZ in 3xTgAD mice and restored SGZ proliferation to normal magnitude. Further, AP $\alpha$  reversed the cognitive deficits to restore learning and memory performance to the level of normal non-Tg mice. In 3xTgAD mice, AP $\alpha$ -induced survival of neural progenitors was significantly correlated with AP $\alpha$ -induced memory performance. We then determined efficacy of AP $\alpha$  to restore neural progenitor cell survival and cognition subsequent to AD pathology in male 3xTgAD and nonTg mice. AP $\alpha$  significantly increased survival of BrdU<sup>+</sup> cells and cognition in 3xTgAD mice in the presence of intraneuronal A $\beta$  whereas AP $\alpha$  was ineffective subsequent to development of extraneuronal A $\beta$  plaques. Restoration of cognition was maximal by the first day and sustained throughout behavioral training. Learning and memory function in AP $\alpha$ -treated 3xTgAD mice was 100% greater than vehicle-treated and comparable to maximal normal nonTg performance. In aged 15-month-old nonTg mice, AP $\alpha$  significantly increased survival of BrdU<sup>+</sup> cells and cognition. Because long-term therapeutic clinical efficacy will be modified by progression of AD pathology, we determined the efficacy of AP $\alpha$  to promote neurogenesis while simultaneously reducing Alzheimer's disease (AD) pathology. Treatment was initiated either prior to or post intraneuronal A $\beta$  accumulation. Results indicated that AP $\alpha$  significantly increased survival of newly generated neurons and simultaneously reduced A $\beta$  pathology with greatest efficacy in the pre-pathology treatment group. AP $\alpha$  also significantly reduced microglia activation while increasing CNPase, an oligodendrocyte myelin marker. These results provide preclinical evidence that AP $\alpha$  promoted survival of newly generated cells and restored cognitive performance in the pre-plaque phase of AD pathology and in late-stage normal aging. *Supported by NIA to RDB.*

## Androgen use and misuse: A 2011 view

**PL7**

### Androgen use and misuse: a 2011 view

David Handelsman

ANZAC Research Institute, Sydney, Australia.

Testosterone is a well established, safe, effective and affordable hormone for use in androgen replacement therapy (ART) for androgen deficiency (AD) due to pathology of the hypothalamo-pituitary testicular axis. Newer testosterone products provide short-acting (transdermal) or long-acting (depot injectable) delivery with enhanced convenience to improve long-term compliance during life-long ART. Yet there is a striking disparity between major under-diagnosis of authentic AD (e.g. Klinefelter's syndrome) and the steeply increasing overuse of androgens in ageing men and women as well as abuse for bodybuilding and sports doping. Within the accepted framework of the pathological basis of disease, AD remains a clinical diagnosis with a pathological basis and confirmed by hormone assays. Biochemical confirmation of AD remains hampered by the unreliability (method-dependent bias, non-specificity) of direct testosterone immunoassays which are still universal in clinical pathology labs although almost completely supplanted by mass spectrometry in clinical research labs. The epidemic use of (meaning)-free testosterone, elevating an outdated heuristic concept ('free hormone hypothesis') into an unquestioned quasi-axiom, usually calculated by inaccurate formulae only adds confusion. Readily available, affordable testosterone assays used in a form of ad hoc population screening together with the pathologising of ageing by invention of 'andropause' as a disease, have created a 'eugonadal sick syndrome', analogous to what is now known as 'non-thyroidal illness syndrome', a reversible, non-specific pan-hypothalamic reaction to systemic illness. These factors have converged to foster a striking upsurge in testosterone prescribing, a marketing-driven distortion threatening to derail endocrinology practice from a needed focus on improving underdiagnosis of genuine AD into a lifestyle consultancy treating pseudo-AD of ageing. Whether testosterone can ameliorate any biological aspect of ageing (obesity, pre-diabetes, cardiovascular disease) remains to be established by adequately powered, placebo-controlled RCTs but the prospects of a WHI-style study focussed on male ageing *per se* seems a remote prospect of doubtful value.

## Genetics of bone disease

**PL8**

### Genetics of bone disease

Stuart Ralston

University of Edinburgh, Edinburgh, UK.

Over the past 10 years, tremendous advances have been made in understanding the role that genetic factors play in the regulation of bone metabolism and bone disease. Most bone diseases have a strong genetic component but for many of these the genetic architecture is incompletely understood. However it is clear that in some diseases, there is Mendelian inheritance of the phenotype due to alleles that have a large effect size, whereas in other diseases, the inheritance is polygenic with alleles of small effect size and additional contributions from environmental factors. Osteoporosis is the most common bone disease and is characterised by reduced bone mineral density (BMD) and increased fracture risk. The heritability of BMD is about 70% and fractures also have a heritable component although this decreases markedly with age as environmental factors assume a more important role. Multiple genes have been identified by genome wide association studies that regulate BMD and fracture risk but these have small effect sizes and (in total) so far explain only 5–6% of the genetic variance. Studies are in progress to determine if larger effect alleles also exist. Several rare bone diseases exist in which BMD levels are unusually high or low including osteopetrosis (due to mutations in genes that regulate osteoclast function) and sclerosing bone dysplasias (due to mutations in genes that regulate bone formation). Genetic analysis of families with these diseases has resulted in the identification of several key molecules that regulate bone turnover such as lipoprotein-receptor related protein 5 (LRP5), sclerostin (SOST) and Cathepsin K Paget's disease of bone (PDB) has a frequency of about 1–2% and has a strong genetic component. Current evidence suggests that PDB is caused by a combination of rare alleles of large effect that cause Mendelian inheritance of the disease and commoner alleles of medium effect that increase disease risk by 30–50%. Causal genes include *SQSTM1*, *TNFRSF11A*, *CSF1*, *OPTN*, and *TM7SF4* which are involved in regulating osteoclast differentiation and function. Advances in genetics have not only advanced our knowledge of the pathophysiology of bone disease but have also identified several molecules that are being targeted for the next generation of drug treatments for bone disease.

# Symposia

## Keep cool, lose weight: brown fat and energy metabolism

### S1.1

#### Brown adipocytes and energy homeostasis in mice

S Herzig<sup>1,2,3</sup>

<sup>1</sup>German Cancer Research Center, Heidelberg, Germany; <sup>2</sup>ZMBH University of Heidelberg, Heidelberg, Germany; <sup>3</sup>University Clinics Heidelberg, Heidelberg, Germany.

Adipose tissue can be subdivided into two distinct categories of fat cells: white adipocytes are specialized for the storage of chemical energy such as triglycerides, and under conditions of obesity white adipose tissue is characterized by tissue inflammation and energy overload.

In contrast, brown adipocytes dissipate energy in the form of heat (thermogenesis) by uncoupling of the mitochondrial electron transport chain from ATP formation through the specific expression of so-called uncoupling protein (UCP)-1. Lineage tracking studies in mice have demonstrated that white adipocytes are distinct from brown adipocytes during normal embryonic development. Indeed, progenitors of the brown adipocyte lineage have been shown to share a common precursor with the myogenic lineage.

In addition, a third distinct brown adipocyte-like cell type has been identified (brown-in-white, BRITE). In response to cold exposure, many WAT depots develop an increased number of BRITE cells, which seems to be mediated – at least in part – through sympathetic beta3-adrenoceptor agonist action. Despite typical BAT-like functional properties, such as UCP-1 expression, the BRITE adipocytes are developmentally distinct from the ‘classical’ brown cells and their functional contribution to systemic energy metabolism has only recently been documented.

Here, new developments and findings from animal models with respect to white versus brown versus BRITE adipocyte determination as well as its implication for the control of systemic energy homeostasis will be discussed.

### S1.2

#### Physiology of brown adipose tissue in humans

Pirjo Nuutila

Turku PET Centre, University of Turku, Turku, Finland.

Human brown adipose tissue (hBAT) has recently been re-discovered and found to be functionally and metabolically highly active when exposed to cold. Advanced imaging technology, namely the development of hybrid positron-emission tomography (PET)/computed tomography (CT) scanners has been the premise for the progress. The main areas of activated BAT have been found in supraclavicular regions and in the neck, and paravertebral regions. When we quantified glucose uptake in these supraclavicular regions during cold activation, it was found to be more than 10 times higher than in the scan performed in normal room temperature. Surgical biopsies from this location demonstrate significant mRNA and protein levels of uncoupling protein 1 (UCP1) and histology multilocular intracellular lipid droplets. In our study population of 27 normal-weighted adults <sup>18</sup>F-FDG uptake was activated in 60% of cases during experimental cold exposure. Obesity decreases this probability and female gender and younger age enhances it.

Animal studies have suggested, that BAT can be activated also by high fat diet. Our recent data shows that glucose metabolism of hBAT is activated by insulin. Activation by insulin but not activation by cold is perfusion-independent. Recent studies have shown that thyroid hormones play an important role in development and function of BAT. Our data suggests that glucose metabolism of hBAT is enhanced in hyperthyroid patients with Graves disease. An explanation for this could be increased activity of the sympathetic nervous system in hyperthyroid diseases.

Further studies are needed for the evaluation of the importance of hBAT in human physiology and for the possibilities for its manipulation. Brown adipose tissue also serves new pharmaceutical targets for drug development against the obesity pandemic.

### S1.3

#### Brown adipose tissue and thermogenesis in lean and obese subjects

Wouter Van Marken Lichtenbelt

Department of Human Biology, Maastricht University Medical Centre, Maastricht, The Netherlands.

The occurrence of obesity and the related metabolic syndrome increases dramatically worldwide. A positive energy balance causes obesity. Facultative

thermogenesis, which is the increase in energy expenditure in response to cold or diet, may be an effective way to affect the energy balance.

Several studies have confirmed that humans show significant (mild) cold induced thermogenesis, i.e. without shivering. Tissues shown to be involved in adults are skeletal muscle and brown adipose tissue (BAT). The most likely cellular mechanism in both tissues is mitochondrial uncoupling. Recently functional BAT has been rediscovered in adult humans.

At the functional level, adipocytes can be subdivided into white and brown. The most important function of white adipocytes is energy storage, while the main function of brown adipocytes is heat production. Brown adipocytes are located in BAT or white adipose tissue, the latter is called ‘BRITE’ (brown-in-white).

From metabolic studies it is known that the facultative thermogenesis is blunted in obese humans, although there is a large individual variation. Intriguingly, functional and active BAT is also inversely correlated with body mass index (BMI) and body fat percentage in humans. An important aspect in the current studies is to find out whether BAT can be activated and recruited in obese subjects. Animal and human studies indicate that recruitment and activation of brown and brite adipocytes can potentially be accomplished by pharmacological, nutritional or environmental intervention. Crucial in the activation of adaptive thermogenesis and brown adipocytes are the sympathetic nervous system and the thyroid hormone axis.

Finally, thermogenic BAT is a major site for lipid breakdown and glucose uptake, and thus the thermogenic capacity of even small amounts of brown adipocytes has emerged as an attractive target for anti-diabetes therapies.

Results will be given from studies on BAT activity in lean, obese and morbid obese subjects before and after weight reduction.

## Controversies in hyperaldosteronism

### S2.1

#### Controversies in hyperaldosteronism: whom and how to screen

P Mulatero, C Bertello, S Monticone & F Veglio  
University of Torino, Torino, Italy.

Primary hyperaldosteronism (PA) is the most frequent cause of secondary hypertension. PA detection is of particular importance, because it provides opportunity for targeted treatment (surgical for APA and medical for BAH), and because it has been demonstrated that PA patients are more prone to cardiovascular events and target organ damage than essential hypertensives. The Endocrine Society Guidelines stated the categories of hypertensive patients with relatively high prevalence of PA, that should undergo a screening test: - resistant hypertension; -hypertension grade 2 or 3; -hypertension with spontaneous or diuretic-induced hypokalaemia; -hypertension with adrenal incidentaloma; -hypertension and a family history of early-onset hypertension or cerebrovascular accident at a young age; -all hypertensive first-degree relatives of PA patients PA. However, recent studies indicate that other categories of patients may be at increased risk of PA, including hypertensive patients with metabolic syndrome (MS), type 2 diabetes mellitus (T2DM) and obstructive sleep apnoea (OSA). Aldosterone/renin ratio (ARR) is considered the best screening test. Several confounding factor, in particular interfering drugs, should be considered during the screening. Moreover, it should be noted that the test may perform differently if renin instead of plasma renin activity is measured together with aldosterone. It remains to be determined the potential benefit of screening all hypertensives for PA. Widespread screening would result in a large increase in costs for screening and confirmation of PA, but on the other hand, would offer to all PA patients the opportunity of a cure or targeted pharmacotherapy. In light of the high prevalence of low renin hypertension, it is important to emphasize that an increased ARR is not itself diagnostic of PA and a confirmatory test is always required to avoid a high number of hypertensive patients inappropriately undergoing costly and potentially harmful lateralisation procedures. Although Endocrine Society Guidelines clearly recommend performing a confirmatory test, the choice of confirmatory test remains a matter of debate and there is currently insufficient direct evidence to recommend one over the others. In our Unit we perform the intravenous saline load and we suggest to perform a confirmatory test that includes NaCl administration and to use captopril test only for those patients at risk for volume expansion.

**S2.2****Adrenal vein sampling**

Tomas Zelinka

First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic.

Adrenal venous sampling plays a central role in discriminating between unilateral (mostly aldosterone-producing adenoma) and bilateral adrenal disease (mostly bilateral hyperplasia) in primary aldosteronism. Although computed tomography or magnetic resonance are used to visualize adrenal glands, both methods are not sensitive enough to detect small tumors and they are not also specific for autonomous aldosterone production. With increasing age, prevalence of adrenal tumors or nodules is increasing reaching up to 7% in the age of 70. According to recent Recommendations for diagnosis and treatment of primary aldosteronism, all subjects who agree with adrenalectomy should undergo adrenal venous sampling to exclude incorrect subtype diagnosis.

To cannulate adrenal veins, in particular the right adrenal vein, skilled radiologist is essential due to difficult sampling. In the last time, visualization of right adrenal vein using the multi-detector computed tomography and rapid cortisol assays have been shown to increase the rate of successful cannulation of the right adrenal vein.

Although adrenal venous sampling is regarded method of choice, they exist according many authors uncertainties in using ACTH to stimulate the adrenal cortex in order to increase the success rate in cannulation of adrenal veins. However, stimulation of the non-adenomatous adrenal cortex with ACTH may be associated with the decrease of specificity. Another disadvantage of adrenal venous sampling is the absence of clear criteria for evaluating the success of adrenal vein cannulation (different cortisol<sub>(adrenal)</sub> to cortisol<sub>(periphery)</sub> ratios), and lateralization (different aldosterone<sub>(one side)</sub>/cortisol<sub>(one side)</sub> to aldosterone<sub>(other side)</sub>/cortisol<sub>(other side)</sub> ratios).

Some centers use as an alternative to adrenal venous sampling nuclear medicine imaging – scintigraphy using noriodocholesterol or positron emission tomography with <sup>11</sup>C-metomidate both after dexamethasone suppression.

**S2.3****Non-surgical therapy of primary aldosteronism**

M Stowasser

Endocrine Hypertension Research Centre, University of Queensland School of Medicine, Greenslopes and Princess Alexandra Hospitals, Brisbane, Queensland, Australia.

Unilateral laparoscopic adrenalectomy for unilateral primary aldosteronism (PA) results in cure of hypertension in 50–60% and improvement in all remaining patients. For those with bilateral PA or with unilateral PA but unsuitable for surgery, treatment with mineralocorticoid receptor (MR) blockers (spironolactone 12.5–50 mg/day or eplerenone 25–100 mg/day) or with sodium channel antagonists (amiloride 2.5–20 mg/day) is effective, but regular biochemical monitoring is required to avoid potentially dangerous hyperkalemia and azotemia. The extent to which renin becomes 'unsuppressed' can help indicate the degree of aldosterone blockade induced by any given treatment dose. Spironolactone is most effective but associated with side effects due to actions on sex steroid receptors, a problem addressed with introduction of the more specific but weaker eplerenone. One study reported the two drugs to be of similar efficacy in lowering BP in PA. Patients with glucocorticoid-remediable PA show excellent BP responses to low dose glucocorticoids (e.g. dexamethasone 0.25–0.5 mg/day), but can also be treated effectively by aldosterone blockade.

In addition to beneficial BP effects, both adrenalectomy and (possibly to a less rapid or pronounced degree) spironolactone result in improved cardiovascular function. Most importantly, the excess cardiovascular morbidity associated with PA (over 'essential hypertension') is reversed with surgical or spironolactone treatment. In addition, both surgery and (again to a possibly lesser degree) spironolactone and/or amiloride treatment are associated with improved quality of life. These data provide compelling support for detecting individuals with PA who may then benefit from the improved clinical outcomes afforded by specific therapy. Given that non-BP-dependent adverse effects of aldosterone excess appear salt-dependent, it is conceivable that amiloride, by promoting salt excretion, confers similar cardiovascular protection to that seen with spironolactone through mechanisms not requiring direct MR antagonism. Further study is required to address this question.

The emergence of aldosterone synthase inhibitors represents another promising new treatment option.

**Molecular mechanisms in neuroendocrine tumours****S3.1****Somatostatin and dopamine signalling in neuroendocrine tumours**

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The discovery of the new properties of somatostatin (SSTRs) and dopamine (DRs) receptors has led to a renewed interest in agents targeting these receptors, and has opened new perspectives for medical treatment of patients with neuroendocrine tumors (NETs). Indeed, patients unresponsive or resistant to the 'classical', currently available somatostatin analogs may benefit from these insights. Moreover, SSRs and DRs crosstalk at membrane level may trigger alternative intracellular pathways, or even enhance the signalling for the control of cell growth. However, the activity of SSTRs and DRs could be also driven by the specific cell types in which they are expressed, and the formation of receptor dimers on cell membrane may similarly vary, depending on the cells, rather than the receptors profile. Therefore, not only the receptor profiles but also the cell types, as well as the dimerization, are responsible for the final effect of a given ligand.

New somatostatin analogs and hybrid molecules, which display a broader and different spectrum of activities compared to conventional analogs, seem to be a promising therapeutic alternative for the control of hormone secretion and, hopefully, to reduce tumor burden. Few initial *in vitro* studies revealed that individual NETs exhibit substantially different SSTRs and DRs expression, which are differently coupled to the intracellular signalling pathways. Indeed, individual NETs seem to respond differently to specific somatostatin analogs, panagonists, and new SSTR/DR chimeric compounds, depending on the SSTR and DR profile coupling to signalling pathways. Although antiproliferative effects seem mainly driven by ERK1/2 activation, additional intracellular pathways, such as JNK signalling and other proteins involved in the control of cell cycle may be targeted. The crucial point emerging from these preliminary studies is that when, in the future, a therapeutic strategy is conceived, it will require a stronger and adequate delineation of the specific receptor profile, as well as a clarification of the linked intracellular pathways of the tumor, in order to optimize an effective treatment.

**S3.2****A new truncated variant of somatostatin receptor subtype five, sst5TMD4, is present in pituitary tumours and breast cancer, and impairs the normal response to somatostatin**

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Somatostatin receptors (sst1–sst5) comprise a family of G-protein-coupled, 7 transmembrane domain (TMD) receptors encoded by five separate, intronless genes widely distributed throughout the organism. Somatostatin (SST) and cortistatin (CORT), two highly-related cyclic neuropeptides, bind to sst1–sst5 with comparable subnanomolar affinity to exert a number of (patho)physiological actions, from inhibition of endocrine secretions (e.g. GH and insulin), to the control of metabolic homeostasis, neurotransmission, or gastrointestinal and immune function. In addition, SST can also act via sst to inhibit tumour cell growth. Hence, SST analogues are used to treat endocrine/tumour pathologies. This notwithstanding, there was evidence that not all SST/CORT actions could be explained by their interaction with the five known sst. In this scenario, our group has recently identified novel truncated variants of sst5 in human, pig, mouse, and rat, which lack one or more TMD and exhibit unique molecular and functional features. Thus, each truncated sst5 variant displays a distinct tissue distribution in every species examined, and exhibits disparate, selective responses to SST and CORT when expressed on heterologous cell lines. In general, truncated sst5 variants show a preferential intracellular distribution that contrasts with the typical plasma-membrane localization of full-length ssts. Moreover, in cells coexpressing truncated sst5 variants and full-length sst5 or sst2, we observe that truncated and full-length receptors colocalize and physically interact, such interaction likely providing the molecular basis for truncated sst5 variants to disrupt the normal response of sst2/sst5 to SST and CORT. In humans, sst5TMD4 expression was found to be very rare and scarce in normal tissues. However, this receptor was frequently and abundantly expressed in pituitary tumours, particularly in somatotropinomas, where its presence was inversely correlated with GH reduction by SST analogues both *in vivo* and *in vitro*. Furthermore, sst5TMD4 is present in a subset of breast tumours, where it is associated to bad prognosis markers, and increases malignancy features of MCF-7 breast cancer

cells by acting as a dominant negative for SST action. Study of these new truncated sst5 variants paves the way to explore novel aspects of sst pathophysiology and to expand their therapeutic potential. Funding: BFU2010-19300, BFU2008-01136/BFI, RYC-2007-00186, CTS-5051 BIO-139.

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### S3.3

#### Lessons from MENX

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MENX is a recently identified multiple endocrine neoplasia (MEN) syndrome in the rat. Affected animals develop multiple neuroendocrine tumors (NETs) with high penetrance and at young age, specifically: bilateral pheochromocytoma (100%), multifocal pituitary adenoma (100%), multifocal thyroid C-cell hyperplasia, parathyroid hyperplasia, extra-adrenal pheochromocytoma (paragangliomas) and pancreatic islet cell hyperplasia. MENX is caused by a germline frameshift mutation of the *Cdkn1b* gene, which leads to extremely reduced expression of the encoded mutant p27 protein *in vivo*. Capitalizing on this discovery, we and others identified germline mutations in *CDKN1B* in patients having multiple endocrine tumors (the MEN4 syndrome).

In order to exploit the MENX animal model in studies aimed at elucidating the molecular pathogenesis of NETs or in preclinical therapy-response studies, it is necessary to characterize the tumors associated with this syndrome. Thus, we assessed the histo-morphological and immunohistochemical features of the rat tumors, and demonstrated that the pituitary tumors in MENX rats are non-functioning adenomas of the gonadotroph lineage. Then, we performed transcriptome analysis to identify novel molecular mechanisms involved in the rat tumors, and correlated these findings with human NETs. Using this approach we identified novel candidate molecular markers for diagnosis and targeted therapy of pheochromocytoma. Gene expression analysis of the rat pituitary tumors is still ongoing and preliminary results will be presented. In conclusion, if properly characterized and exploited the MENX syndrome may represent a relevant translational platform for molecular and preclinical studies of NETs.

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### Subclinical hormone excess

#### S4.1

##### Subclinical adrenal hyperfunction

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The adrenal glands secrete a variety of hormones from the cortex (steroids) and the medulla (amines) that when in excess lead to characteristic clinical syndromes. Dysregulation of the secretory pattern of these hormones or hypersecretion not enough to cause a clinically obvious syndrome is termed subclinical hyperfunction and is mainly found in primary adrenal lesions in the form of adrenal incidentalomas (AI). These are adrenal mass lesions usually > 1 cm in diameter that are increasingly being discovered during testing, mostly via CT abdominal imaging, for conditions unrelated to adrenal diseases. Their overall incidence ranges between 3 and 10% increasing with age and it is clinically important to identify those that exert clinical or subclinical hormonal activity or have a malignant potential. Subclinical mainly hypercortisolism (Cushing's) (SC), is the most common hormonal hyperactivity encountered in a significant number of patients with AI; its prevalence ranges according to the diagnostic procedures used and may fluctuate with time. In some patients it may be associated with some of the detrimental effects of continuous autonomous cortisol secretion such as arterial hypertension, dyslipidemia, alterations of carbohydrate metabolism and an increased risk for osteoporotic fractures. It is still unproven whether treatment to reverse autonomous cortisol secretion is beneficial and further mainly prospective controlled studies are required to address this issue. Clinical significant autonomous aldosterone secretion is less common than SC using conventional screening tests, namely the aldosterone to rennin ratio. The introduction of more sophisticated tests based on complex pathophysiological mechanisms may reveal a significantly higher prevalence but further studies are needed to identify significant clinical correlates. Clinically unrecognized or silent pheochromocytomas may be associated with increased morbidity and mortality.

However, in the absence of prospective studies correlating hormonal activity with end organ damage, the long term consequences of these syndromes and their management remains largely pragmatic.

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### S4.2

#### Normocalcemic primary hyperparathyroidism

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The diagnosis of normocalcemic primary hyperparathyroidism (PHPT) can be made in subjects whose total and ionized serum calcium are completely normal but in whom the PTH level is persistently elevated. In order to make the diagnosis of normocalcemic PHPT, secondary causes for an elevated PTH level should be ruled out, such as vitamin D insufficiency, or renal insufficiency. Replacing the former patients with vitamin D to reach levels now considered to be normal (i.e. > 30 ng/ml) often returns the PTH to normal. Occasionally, however, these patients will become hypercalcemic with vitamin D replacement thus unmasking more typical hypercalcemic PHPT. If the PTH remains elevated and the serum calcium remains normal, following vitamin D repletion, and other causes of an elevated PTH have been excluded, then the diagnosis of normocalcemic PHPT can be considered. Since there are limited data on the natural history of normocalcemic PHPT, it is unclear how these subjects should be regarded vis a vis parathyroid surgery. A large population based study of over 5000 postmenopausal women who were screened and then retested 8 years later provided evidence for the development of hypercalcemia in some of these subjects. Two observational studies of normocalcemic PHPT have followed patients longitudinally. In one study 37 patients were followed for a mean of 3 years (range 1–9). Typical hypercalcemic PHPT emerged in 7 (19%) individuals. However, 40% developed evidence of disease progression with development of kidney stones, fractures, marked hypercalciuria or > 10% decline in BMD. Seven patients had successful parathyroidectomy, of whom three were hypercalcemic and the rest met other criteria for surgery. Clearly, normocalcemic PHPT is a newly recognized clinical phenotype of PHPT.

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### S4.3

#### Subclinical hyperthyroidism

Bernadette Biondi

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Subclinical hyperthyroidism (SHyper) is defined as serum FT<sub>4</sub> and FT<sub>3</sub> levels within their respective reference ranges in the presence of is low or undetectable serum TSH levels. The most common cause of SHyper is exogenous SHyper due to unintentional excessive replacement therapy in hypothyroid patients or to intentional TSH suppressive therapy for malignant thyroid disease. Endogenous SHyper is commonly associated with autonomous thyroid function as occurs in Graves' disease, multinodular goiter, and solitary autonomously functioning thyroid nodules. The prevalence of SHyper is dependent on age, sex, and to some extent, on the iodine intake of the population. Despite the high prevalence of SHyper and the potential progression to overt disease, the risk associated with this condition is debated.

Opinions are quite divergent regarding the deleterious effects of SHyper on cardiovascular mortality. Several meta-analyses have examined the cardiovascular risk associated with SHyper with the aim of establishing whether treatment should be considered. Their results support the concept that the cardiovascular risk of SHyper depends on the age of the patients and co-morbidity conditions. There is a higher cardiovascular risk in elderly persons with SHyper and potential harmful effects are present in patients with co-morbidity conditions. Most studies on endogenous SHyper and bone health have found a decrease in bone mineral density in postmenopausal women, especially in cortical bone-rich sites, but there is little evidence of a clinically significant effect on bone in premenopausal women. Before treatment is started, it should be established whether the subnormal serum TSH is related to endogenous SHyper and whether it is persistent. Although it has not been demonstrated that early treatment of symptomatic patients with SHyper improves clinical outcome, treatment of SHyper might improve quality of life, cardiovascular risk factors and bone mineral density and would prevent a possible progression to overt disease.

## Novel therapies in type 1 diabetes

### S5.1

#### Pancreas transplantation

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Vascularized whole pancreas transplantation is currently the only therapeutic approach consistently able to restore euglycemia in patients with type 1 diabetes. More than two-thirds of all pancreas transplants are performed simultaneously with the kidney (SPK: simultaneous pancreas kidney) in patients with end-stage diabetic nephropathy. The results of SPK transplantation are excellent with rates of insulin-independence >80% at 1 year and >70% at 5 years. Venous thrombosis is the major cause of early pancreatic graft loss. Successful SPK transplantation significantly prolongs patient and kidney graft survival. Additionally, there is growing evidence that successful SPK transplantation can stabilize or even reverse some of the chronic secondary complications of diabetes. Pancreas-after-kidney (PAK) transplantation is an interesting option for candidates for SPK transplantation who have a living kidney donor. Pancreas transplantation alone (PTA) is increasingly performed and is offered to type 1 diabetic patients with glycemic lability (hypoglycaemia unawareness). PAK and PTA together comprise <30% of all pancreas transplants, and their success rate is improving, but slightly lower than for SPK, probably because rejection is more difficult to diagnose.

Current research efforts in the field are mainly focusing on the following issues:

1. Defining strategies to reduce the ischemia-reperfusion injury thought to be responsible for the majority of early 'technical' pancreatic graft losses.
2. Expanding the pancreas donor pool, notably through the use of living donors.
3. Exploring the potential of early PTA in preventing the development of end-stage nephropathy.
4. Identifying subsets of patients with type 2 diabetes eligible for pancreas transplantation with similar chances of success.
5. Exploring the role of minimally invasive surgery and surgical new technologies in pancreas procurement and transplantation.

### S5.2

#### Islet transplantation and regeneration

Eelco de Koning

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In patients with longstanding type 1 diabetes  $\beta$ -cell regeneration or  $\beta$ -cell replacement are the only options that result in normoglycemia without the risk of hypoglycemia. Although persistent C-peptide is present in ~10% of patients with type 1 diabetes, currently regeneration of eutopic pancreatic islets remains elusive due to the minimal regenerative capacity of the human pancreas and the presence of persistent autoreactivity. Islet transplantation is a form of  $\beta$ -cell replacement therapy that is a treatment option for a specific group of patients with type 1 diabetes that suffers from glycemic lability, recurrent hypoglycemic episodes and hypoglycemia unawareness. The minimally invasive nature of percutaneous intraportal infusion of isolated islets from human donor pancreas makes the procedure attractive compared to pancreas transplantation. There is only a small risk of portal vein thrombosis or hepatic bleeding. However, usually islet infusions from 2 to 3 donors are necessary to obtain insulin independence in islet transplant recipients. This is mostly related to the loss of islets during transplantation and islet engraftment. Although insulin independence may not be achieved after one islet infusion, partial islet graft function already leads to a substantial improvement in hypoglycemia-related problems. Over time islet graft function declines probably due to a combination of factors involving a suboptimal liver microenvironment for long-term islet function, impaired vascularisation, alloreactivity, recurrent autoreactivity and toxic effects of immunosuppressive agents. So a substantial number of transplant recipients that once were insulin-independent after islet transplantation have to resume insulin therapy but continue to benefit from partial graft function. This has resulted in most islet transplantation centers now regarding an absence of severe hypoglycemic episodes and return of hypoglycemia awareness as indicators of successful islet transplantation.

### S5.3

#### Stem cells as a treatment option in diabetes

Julie Kerr-Conte

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Cell therapy is an emerging, realistic treatment for diabetes. First we will quickly review alternative stem cell approaches including autologous Hematopoietic stem cells (CEB Couri JAMA 2009) and allogeneic Mesenchymal stem cells (Prochymal, Osiris Therapeutics, Inc.: Clinicaltrials.gov NCT00690066) as an attempt to cure recent onset type 1 diabetes, before focusing on  $\beta$  cell replacement therapies including an update on composite islet grafts (islets plus stem cells), or islets derived *in vitro* and *in vivo* from human embryonic stem cells (Viacyte Kroon *Nat Med* 2008), and induced pluripotent stem cells human (iPS). The proof of principle that type 1 diabetic patients' cells can give rise to iPS lines which subsequently differentiate into insulin secreting human islets has been demonstrated (Maehr R/Melton D *PNAS* 2009). The advantages and disadvantages of each pluripotent source with regards to regulatory and ethical issues, teratoma formation, genomic stability will be presented. Immunoisolation strategies of pluripotent cells will attempt to allow both transplantation with minimum to no immunosuppression, and confinement in a closed chamber to reduce teratoma formation. Theracyte chambers (Baxter USA) previously used in rodent islet transplantation (Sharma *Diabetologia* 2006) successfully allow human fetal and embryonic derived islet survival and function but not human islets in rodent models (Itkin-Ansari P *Transplantation* 2009). Finally, we will discuss the strategies and progress that companies are making towards pushing pluripotent stem cells ahead to clinical application.

## Long term effects of bisphosphonates

### S6.1

#### The risk of fractures and osteonecrosis

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Bisphosphonates are effective in the prevention of osteoporotic fractures and have been the mainstay of osteoporosis therapy for almost two decades. Being anti-resorptives, bisphosphonates bring about a gain in bone mass that is due to diminished bone remodelling accompanied by a higher mean age of the bone tissue.

The presentation aims to review the evidence linking bisphosphonates to skeletal complications and to discuss the magnitude of the risk of these potential complications relative to the benefit in terms of osteoporotic fractures averted by treatment.

First, bisphosphonates can cause osteonecrosis of the jaw (ONJ). This is particularly true when given intravenously with a short dosing interval as is the case in the oncology setting. Though this complication is relatively rare in patients treated with oral bisphosphonates, clinicians, dentists and patients must be aware of this potential complication to bisphosphonates. The clinical diagnosis is based on the observation – by a health professional – of exposed bone in the oral cavity that fails to heal within 8 weeks. Though severe cases requiring surgery have been reported, most cases resolve on conservative treatment such as antiseptic mouth washes and in some cases antibiotics. In oncology patients treated with IV bisphosphonates at short dosing intervals it may be desirable to avoid bisphosphonates for the first 3 weeks following dental extractions, provided this does not delay treatment of patients with uncontrolled skeletal metastatic disease.

Second, in the last five years, several case series have described an unusual femur fracture (atypical femur fracture) occurring in patients who had been treated with oral bisphosphonates. These rare fractures are often spontaneous and differ from classical shaft fractures of the femur in having a transverse or short oblique presentation. Such fractures can be bilateral and are often but not always accompanied by a cortical thickening or stress reaction. More than half of the patients complain of thigh pain in the weeks prior to the fracture. Case control studies have established bisphosphonate use as a strong risk factor, though it remains unclear if bisphosphonates are the cause. Pending additional research, physicians should exercise caution and avoid long term bisphosphonate treatment in patients who are not at high risk of osteoporotic fractures.

## S6.2

### Long term management of osteoporosis

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Over the last 25 years we have been expanding our choice of treatments for osteoporosis. The chronic nature of the disease makes long term treatment necessary. It has long been known that long term management of osteoporosis suffers the same compliance problems as chronic treatment regimens for other disease like hypertension, hyperkolesterolemia, etc.

The most widely used treatment for osteoporosis is still oral bisphosphonates, which have come under increased scrutiny due to side effects like osteonecrosis of the jaw and atypical femoral fractures being linked to long term exposure. The available data suggest, however, that the risk of these adverse events in an osteoporosis population are extremely low. These events together with the unfounded fear of oversuppression of bone turnover during long term treatment with bisphosphonates have elicited increased interest in drug holidays. The available data suggest that such holidays are possible, because bone turnover remains low many years, but in high risk individuals the risk of vertebral fractures increases. No significant increase in non vertebral fractures have been seen however.

Anabolic treatment with PTH for 2 years is necessary to exploit the full potential of the drug in terms of non-vertebral fracture reduction. Discontinuation of anabolic treatment should always be followed by an antiresorptive regimen, otherwise the bone gain is lost over a period of 2 years. Here the new long acting, parenteral regimens like Zoledronic acid and Denosumab seem especially attractive.

## S6.3

### Bone turnover markers in the management of osteoporosis

Abstract unavailable.

## Novel approaches to *in vitro* and *in vivo* imaging

### S7.1

#### How to visualize G-protein-coupled receptor signaling by FRET

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G-protein-coupled receptors (GPCRs) constitute the major family of cell surface receptors. They comprise receptors for light, taste and smell as well as ions, small transmitters, peptides and large protein hormones. Given their involvement in fundamental biological processes and their accessibility, GPCRs serve as targets for many classes of drugs, including beta-blockers, antihistamines and opiates.

Whereas many biochemical steps involved in GPCR signaling have been described in good details, their spatio-temporal dynamics in living cells is still largely unknown. This is because biochemical techniques have limited temporal and, generally, no spatial resolution.

The recent introduction of a series genetically encoded fluorescent sensors allows for the first time to monitor key steps of GPCR signaling, such as ligand binding, receptor activation, G-protein coupling and second messenger generation, directly in living cells<sup>1</sup>. These sensors are largely based on the physical phenomenon of fluorescence resonance energy transfer (FRET), which can be measured by different microscopy techniques. Since the temporal and spatial resolution are those of fluorescence microscopy, i.e. up to a few milliseconds in time and a few hundred nanometers in space, these techniques can capture fast signaling events and, at the same time, define their subcellular location. These methods have been instrumental for new and important advances in the field, including the recent finding that GPCRs can continue to signal to cAMP after agonist-dependent internalization into endosomes<sup>2-4</sup>.

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## S7.2

### Multiphoton microscopy to visualize cell migration and invasion

Abstract unavailable.

## S7.3

### Molecular and clinical imaging of adrenal steroidogenesis by iodometomidate

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Molecular imaging technologies are emerging tools providing both researchers and clinicians with technologies for non invasive *in vivo* studies. Steroidogenic enzymes are abundantly and specifically expressed in steroidogenic tissue and represent an interesting target for tissue specific characterization. Iodometomidate is a highly potent inhibitor of cortisol and aldosterone synthesis due to specific and high affinity binding to both CYP11B1- and CYP11B2-enzymes. Our aim was to specifically depict adrenocortical tissue in animal models and for clinical characterization of adrenal disease. Therefore, radiosynthesis of iodometomidate and several analogues has been established and compounds were evaluated *in vitro* and *in vivo*. In both animal and clinical studies iodometomidate revealed to be a suitable SPECT-tracer for detection of adrenocortical tissue and differential diagnosis of adrenal masses. Due to the high specificity of tracer uptake enabling high local activities, iodometomidate furthermore demonstrated potential for radiotherapy of adrenocortical carcinoma.

## The biological clock and metabolism

### S8.1

#### Clock genes in human adipose tissue

Marta Garaulet

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Circadian rhythms are such an innate part of our lives that we rarely pause to speculate why they even exist. Some studies have suggested that the disruption of the circadian system may be causal for obesity and manifestations of metabolic syndrome (MetS). Shift-work, sleep-deprivation and bright-light-exposure at night are related to increased adiposity (obesity) and prevalence of MetS. It has been provided evidence of clock genes expression in human adipose tissue and demonstrated its association with different components of the MetS. Moreover, current studies are illustrating the particular role of different clock genes variants and their predicted haplotypes in MetS.

In the present lecture, the molecular mechanisms implicated in the interaction between chonodisruption and metabolic-related illnesses such as obesity and MetS will be treated with different approaches: i) molecular approach: to achieve a better knowledge of the adipose tissue (AT) internal clock and to identify differences among subcutaneous and visceral fat in AT cultures obtained from obese subjects (genomics techniques); ii) different epidemiological approaches in large populations will be mention considering different single nucleotide polymorphisms (SNPs) of clock genes that are related to obesity and weight loss and whether which are modulated by external factors, such as sleep and diet (nutrigenetic studies with DNA isolation and clock genotyping); iii) clinical approach: behavioral (sleep quality and duration, eating patterns and chronobiological characteristics) and hormonal factors which could explain the previously reported association between the clock genes SNPs and weight loss will be also note.



**S8.2****Circadian clock regulation of glucocorticoid synthesis**

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Under non-stressed conditions adrenal glucocorticoid (GC) secretion shows strong circadian rhythms with blood levels peaking around wake-up time (i.e. in the morning in humans and at the beginning of the night in nocturnal rodents). These diurnal variations are primarily regulated by rhythmic activation of the hypothalamic–pituitary–adrenal (HPA) axis and the secretion of ACTH from the pituitary. While circadian HPA axis activity is ultimately controlled by the circadian pacemaker residing in the suprachiasmatic nuclei (SCN) of the hypothalamus, we found that clock gene activity at the level of the adrenal cortex regulates the sensitivity of the steroidogenic machinery to ACTH stimulation and, hence, the GC responsiveness during the course of the day. Using a combination of molecular, genetic and surgical approaches we describe the mechanisms of SCN-to-adrenal clock interaction in the regulation of GC rhythms in the mouse. Using a rapid light/dark cycle shifting paradigm we discovered a role for the adrenal clock and adrenal GC rhythms in the re-synchronization of rest-activity cycles during jet lag and devised a novel pharmacological strategy to accelerate jet lag adaptation based on ‘pre-flight’ manipulation of circadian GC secretion.

**S8.3****Feeding the clock**

J Johnston

Abstract unavailable.

**New endocrine aspects of diabetes mellitus****S9.1****The incretin system: from discovery to drug development**

T Vilsboll

Abstract unavailable.

**S9.2****GLP-1 and the cardiovascular system**

Michaela Diamant

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The gut hormone glucagon-like peptide (GLP)-1 lowers blood glucose by stimulating meal-related insulin secretion and lowering hyperglucagonaemia in a glucose-dependent manner. Currently, injectable GLP-1 receptor agonists (GLP-1RA) and oral inhibitors of the dipeptidyl peptidase (DPP)-4 enzyme, that degrades native GLP-1, are successfully employed in the treatment of type 2 diabetes (T2DM). Clinically relevant extrapancreatic effects of GLP-1 (RA) involve slowing down gastric emptying, inducing satiety, reducing food intake and decreasing bodyweight. Additional benefits are improvement of islet-cell function. More recently, direct effects on the cardiovascular (CV) system have been described. Indeed, GLP-1 receptors are present on vascular cells and cardiomyocytes. In rodents, GLP-1, but also GLP-1RA and DPP-4 inhibition, were shown to reduce myocardial infarct size in ischaemia-reperfusion models and pretreatment with GLP-1 improved cardiac function after ischaemia/reperfusion injury. In small-sized human studies, GLP-1 improved cardiac function in post-myocardial infarction patients with left ventricular dysfunction and in those with heart failure. Also, DPP-4 inhibition resulted in an improved myocardial response to dobutamine stress in patients with coronary artery disease. In addition, beneficial effects of GLP-1 (RA) were observed on endothelial function. However, to date it is unclear whether these effects are directly mediated by activation of the GLP-1 receptor or by another mechanisms involving the metabolite GLP-1 (9–36). Similarly, it is unknown whether the GLP-1 effects are direct or whether the improvement of systemic metabolism is the key contributor

to the observed beneficial effects. GLP-1 (RA) improved obesity-related CV risk factors, thus theoretically reducing CV risk. However, the recently initiated large-sized long-term outcome studies with the various incretin-based compounds should ultimately determine whether these promising agents indeed reduce the excessive risk of CV disease in people with T2DM.

**S9.3****GLP-1 biology: beyond the pancreas**

Darleen Sandoval

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Glucagon-like peptide 1 (GLP-1) is a gut hormone that broadly promotes glucose homeostasis through the regulation of islet hormone secretion. Interestingly, GLP-1 has been reported to be both cause and cure of type 2 diabetes mellitus (T2DM). Decreased GLP-1 is thought to cause the progression from impaired glucose tolerance to frank T2DM. Conversely, the GLP-1 system has been successfully targeted as a treatment option for T2DM patients and increases in GLP-1 have been implicated in the rapid resolution of T2DM after bariatric surgery. We have confirmed that animal models of two bariatric surgeries, roux en Y gastric bypass (RYGB) and vertical sleeve gastrectomy (VSG), drastically increase meal-induced GLP-1 and also increase insulin levels, and improve hepatic insulin sensitivity. The benefits of VSG and RYGB on glucose homeostasis may be mediated in part through increased circulating GLP-1. However, there are few, if any, hepatic GLP-1 receptors, which argues for alternative sites of action, at least for regulation of hepatic glucose production. GLP-1 is also synthesized in the brain, where it is thought to primarily regulate food intake. Our work suggests that the CNS GLP-1 system also provides important input to regulate glucose homeostasis. Importantly, we have found that central administration of a GLP-1R antagonist caused relative hyperglycemia during a glucose tolerance test suggesting that activation of CNS GLP-1R contributes to normal post-prandial glucose homeostasis. We have also found direct infusion of GLP-1 into the arcuate, but not the paraventricular nucleus of the hypothalamus reduced hepatic glucose production. Thus, both CNS and peripheral GLP-1 regulate normal glucose homeostasis. We hypothesize that after a meal the coordinated effects of the CNS GLP-1 system are to inhibit glucose production as well as to increase satiety. These CNS effects of GLP-1 may explain the benefits of VSG and RYGB on hepatic insulin sensitivity.

**New developments in the therapy for NETs****S10.1****New developments in the therapy for NETs: risk stratification**

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Neuroendocrine tumors (NETs) represent a rare and diverse group of tumors with predominant location in the gastro-entero-pancreatic and bronchopulmonary system. At initial diagnosis 50% of the patients present with metastatic disease whereas more than 80% develop metastases in the course of the disease with liver metastases as the most frequent ones. In consideration of the variable and sometimes indolent tumor growth behaviour, risk stratification is an important need for therapeutic decision making. Patient prognosis is currently best reflected by different classification systems according to WHO 2000/2010 and ENETS TNM staging system<sup>1</sup>. These are based upon prognostic determinants such as histological differentiation (well-differentiated NET/neuroendocrine carcinoma and poorly differentiated neuroendocrine carcinoma) and biological/pathomorphological signs of malignancy, and more recently on the TNM staging and grading system. Grading of the tumor based on the proliferation index as measured by Ki67 immunostaining (G1 <2%, G2 3–20%, G3 >20%) next to staging seems to be the strongest predictor of survival<sup>2</sup>. Location of the primary tumor in the pancreas (compared to GI), high liver tumor burden/extended disease and carcinoid heart disease as well as highly elevated circulating chromogranin A, are worse prognostic parameters. In contrast, surgery of liver metastases and removal of the primary tumor are reported to be beneficial for patient outcome. By integrating these and additional significant prognostic factors, a nomogram can be developed to better assess an individual patient's disease-specific survival<sup>3</sup>. However, it warrants prospective evaluation. Molecular imaging by 18F FDG PET may be helpful in identifying more dedifferentiated tumours and its prognostic value has to be validated in the future<sup>4</sup>. More recently molecular tissue markers (e.g. FGF13, TSC-2, and PTEN expression) were identified to predict survival in pancreatic NET<sup>5</sup>. In summary WHO/TNM classification provide currently the best tool to stratify patients, however specific and reliable molecular

markers have to be identified to better select patients for specific treatments and determine surveillance strategies.

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## S10.2

### Peptide receptor targeted radiotherapy

D Kwekkeboom

Abstract unavailable.

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## S10.3

### New developments in the therapy for NETs: new drugs including mTOR inhibitors

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Mammalian target of rapamycin (mTOR), a main serine/threonine protein kinase in the phosphoinositide 3-kinase (PI3K)/Akt/p70S6K signalling pathway, is an important intracellular mediator involved in multiple cellular functions including proliferation, differentiation, apoptosis, tumorigenesis, angiogenesis, and also longevity.

Recent studies indicate that mTOR is central in the integration of a multitude of signalling pathways activated by growth factors and nutritional status, receiving stimulatory signals from Ras and PI3K. PI3K localizes Akt to the cell membrane where it can be phosphorylated and activated by PDK1. Activated Akt phosphorylates tuberin (TSC2), resulting in TSC1/2 complex instability and inhibition of the tumor suppressor function of the TSC2. Noteworthy, different components of the Ras/MAPK/ERK and PI3K signalling pathways are mutated in most human cancers, inducing abnormal regulation of mTOR signalling, and therefore possibly increasing its susceptibility to mTOR inhibitors. There is emerging data indicating that genetic and metabolic changes accompanying malignant transformation might cause hypersensitivity to mTOR inhibition. Rapamycin, a known immune suppressor and a macrocyclic lactone, and its derivatives temsirolimus (CCI-779), everolimus (RAD001) and deforolimus (AP23573), known as rapalogs, specifically inhibit the function of mTOR. Based on overexpression of the components of PI3K/Akt/mTOR signaling pathway in many different types of cancer, the use of mTOR inhibitors may result in effective anti-tumor activity in various endocrine tumor types. Combining an mTOR inhibitor with other anti-cancer drugs can sensitize endocrine tumor cells to these agents, producing additional activity and preventing drug resistance. The results of preclinical and phase I-II-III clinical studies show that mTOR inhibitors have promising anti-cancer activity, as single agents or in combination with other anti-tumor drugs, in neuroendocrine tumors.

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## Genetics of male infertility

### S11.1

#### Update on genetics in male infertility

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Despite spermatogenesis is among the most finely regulated processes in our body, few genetic tests are currently routinely used in infertile males. These include analysis of karyotype, Yq microdeletions, CFTR and androgen receptor gene mutations, which collectively account for 15–20% of male infertility. Very little is known about the pathogenic mechanism leading to spermatogenesis disruption in many of these patients, especially those carrying Yq microdeletions. Recent field of research of our group focused on the identification of molecular pathways leading to spermatogenic damage in men with AZFc microdeletions and idiopathic infertility. Testicular gene expression profiling carried out by microarray assay revealed that all the AZFc deleted samples clustered together and showed a downregulation of several genes (331) related to spermatogenesis.

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Interestingly, some idiopathic patients clustered together to AZFc-deleted patients, suggesting that several forms of infertility can be triggered by a common pathogenic mechanism that is likely related to alterations in testicular mRNA storage. Our data suggest that a lack of testicular DAZ gene expression may be the trigger of such mechanism and DAZ gene dysfunctions could therefore account for a larger number of previously thought 'idiopathic' infertility cases. A second line of research includes the contribution of genetic polymorphisms to male infertility and, more importantly, as markers for a pharmacogenomic approach to the treatment of infertile males. In this light, we demonstrated the diagnostic and therapeutical validity of polymorphisms in the FSH receptor and FSH beta genes. These results, taken together with the most recent research published on the genetics of male infertility are shedding light on novel molecular events involved in 'old' causes of male infertility and are suggesting new pathogenic mechanisms of spermatogenesis disruption. It is believable that new genetic tests could be in a near future introduced in the clinical practice as diagnostic, susceptibility or pharmacogenetic tests.

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## S11.2

### Clinical aspects of Klinefelter syndrome in childhood, adolescence and adulthood

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Klinefelter syndrome (KS) which affects 1–2 per 1000 males, is not only challenging for the patients and their families. Professionals, who manage KS patients need a much better scientific foundation for decision making in patient care. Surprisingly little research on how to manage boys, adolescents and men with KS has been carried out in comparison to management of other clinical problems like, e.g. Turner syndrome. One of the problems has been that management, which must be a live long process in KS, most often is split between paediatricians, who take care of the boys with KS, and andrologists and endocrinologists who manage fertility problems and androgen insufficiency later in life. However, to obtain knowledge on the full spectrum of the syndrome, and the needs for intervention we should have life-long perspective from birth to old age in mind when we make our treatment strategies.

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## S11.3

### Genomics of azoospermia

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Azoospermia is a common condition of about 10% of all infertile men and this most severe clinical phenotype of male infertility can – in an otherwise healthy man – regularly be considered to have a genetic basis. An association of chromosomal aberrations and Y-chromosomal AZF-deletions with spermatogenic failure is well-established. However, since the latter were described over 10 years ago, basically no genetic causes have been found although many re-sequencing studies of candidate genes were performed. However, with the advent of new whole-genome analytic methods, there is hope to further elucidate the complex genetic origin of azoospermia. To-date, only one recent study analysed a relatively low number of 40 men with non-obstructive azoospermia (histology not mentioned) using whole genome single nucleotide polymorphism (SNP) arrays. Some SNPs were found significantly associated with the phenotype, but essentially none could be unequivocally replicated in a larger follow-up study. Two studies using array comparative genomic hybridisation (CGH) to identify chromosomal gains and losses (copy number variants, CNVs) not detectable by routine chromosome analysis are currently underway: a study of 9 patients with meiotic arrest and 20 controls yielded six 'interesting regions' not further described. The second study analysed larger numbers of azoospermic ( $N=54$ ) men with customised array-CGH specific for the X-chromosome. However, only one CNV was found significantly more frequently in patients after several CNVs had also been analysed in 150 controls. In our own experience of array-CGH in well-characterised and highly-selected patient groups with azoospermia due to mixed atrophy, meiotic arrest or Sertoli-cell-only syndrome, we could identify a number of putatively causal recurring, patient-specific and private, sex-chromosomal CNVs/genes. The novel method of genome analysis by next-generation sequencing may hold the potential to identify additional causative genes for azoospermia.

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**Novel insights into phosphate metabolism****S12.1****The maintenance of phosphate homeostasis by FGF23**

Abstract unavailable.

**S12.2****Genetic disorders of phosphate metabolism**

Abstract unavailable.

**S12.3****FGF23: marker or predictor of progression in chronic kidney disease**

Abstract unavailable.

**Endocrine response to critical illness****S13.1****The HPA axis in critical illness**

D Annane

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The role of the hypothalamic–pituitary adrenal axis in host response to an infection is crucial. The initial inflammatory response to sepsis activates the endogenous release of cortisol which in turn will modulate the synthesis and release of both pro- and anti-inflammatory mediators to restrict inflammation to the infected tissues. However, a number of factors including vascular or ischemic damage, inflammation and apoptosis within the hypothalamic–pituitary adrenal axis, and drugs induced altered cortisol metabolism, may cause adrenal insufficiency. One major problem ICU physicians are faced with is the diagnosis at the bedside of sepsis induced adrenal insufficiency. A recent international task force recommended that sepsis induced adrenal insufficiency is best recognized by basal cortisol is of  $<10$   $\mu\text{g}/\text{dl}$  or delta cortisol of  $<9$   $\mu\text{g}/\text{dl}$ . The diagnostic value of salivary free cortisol in this setting remains to be investigated. While sepsis adrenal insufficiency is undoubtedly associated with a poor prognosis, the indication and practical modalities of corticosteroids therapy remained controversial. Based on the two largest randomised, placebo-controlled trials, this author suggested that septic shock patients with hypotension poorly responsive to fluid replacement and vasopressors should receive a seven day treatment with the combination of hydrocortisone at a dose of 200 mg/day and fludrocortisone at the dose of 50  $\mu\text{g}/\text{day}$ .

**S13.2****Thyroid hormone metabolism in inflammation and sepsis**

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During illness changes in thyroid hormone metabolism occur, collectively known as the non-thyroidal illness syndrome (NTIS). NTIS is characterized by low serum thyroid hormone levels, while TSH and TRH expression do not increase, indicating a disturbance of the normal thyroid hormone feedback regulation.

Although the common view was that NTIS results in overall downregulation of metabolism in order to save energy, recent work has shown that genes involved in thyroid hormone (TH) metabolism show remarkably variable, illness-induced changes in key metabolic organs such as liver, muscle and adipose tissue, ranging from inhibition to activation. That illness-induced changes in peripheral organs appear to be very different during acute or chronic inflammation adds an additional level of complexity. Organ- and timing-specific changes in the TH deiodinating enzymes (deiodinase type 1, 2 and 3) highlight deiodinases as proactive players in the response to illness. Furthermore, the granulocyte is a novel and potentially important cell type involved in NTIS during bacterial infection. Finally, complex changes in thyroid hormone metabolism occur in muscle during critical illness that may be relevant for the pathogenesis of respiratory failure. Although acute NTIS may represent an adaptive response to support the immune response, NTIS may turn disadvantageous when critical illness enters a chronic phase necessitating prolonged mechanical ventilation, dialysis and inotropic support.

In sum, NTIS appears to be a timing-related and organ-specific response to illness, occurring independently from the decrease in serum thyroid hormone levels and potentially relevant for disease course.

**S13.3****Novel insights in endocrine and metabolic changes during critical illness**

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Host responses to critical illness, such as excessive inflammation and hyperglycemia, trigger detrimental chain reactions that damage cellular proteins and organelles. Such responses to illness contribute to the risk of non-resolving multiple organ dysfunction and adverse outcome. Autophagy is a bulk degradation pathway able to remove toxic protein aggregates and damaged organelles. Morphologically, both liver and muscle of critically ill patients reveal an autophagy-deficiency phenotype. Proteins involved in initiation and elongation steps of autophagy are induced several-fold by critical illness, but mature autophagic vacuole formation is impaired and proteins normally degraded by autophagy accumulate dramatically. Also mitophagy is down-regulated. Artificial nutrition, most specifically the amount of infused amino-acids, appears to be an important factor contributing to suppressed mature autophagy in the critically ill. Although insulin-titrated maintenance of normal blood glucose preserves hepatocytic mitochondrial integrity by prevention of direct damage, the infused insulin may have a downside as it may further impair the autophagic machinery. Insufficient autophagy in prolonged critical illness may cause inadequate removal of damaged proteins and mitochondria. Such incomplete clearance of cellular damage, inflicted by illness and aggravated by hyperglycemia, could explain lack of recovery from organ failure in prolonged critically ill patients. These novel insights open perspectives for therapies that activate autophagy during critical illness.

**Female contraception update****S14.1****Compliance and the risk of pregnancy**

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Unintended pregnancy is common. Most European governments are concerned by abortion rates which, if not rising, seem stubbornly difficult to reduce. Moreover by no means all unintended pregnancies are aborted: in the UK, almost one third of pregnancies which end in childbirth are unplanned or mistimed. While no method of contraception is 100% effective, few induced abortions are the result of true method failures; most are due to unprotected sex or incorrect/ inconsistent use of contraception.

Most currently available methods of contraception rely on correct and consistent use for their effectiveness. The oral contraceptive pill has a theoretical failure rate during perfect use of only 1 in 1000 but in reality and during typical use the failure rate is actually 8 in 100. So-called 'pill failures' account for a substantial percentage of abortions in countries where oral contraceptives are widely used. Forgetting to take a pill (or pills) is extremely common and continuation rates are usually  $<50\%$  at the end of 1 year of use.

The so-called long acting reversible contraceptives (intrauterine, implantable and injectable methods) are much less (or not at all) dependent on compliance for their effectiveness and typical use failure rates are not different from those estimated for perfect use. Moreover for contraceptive implants and intrauterine methods discontinuation rates are much lower because the method cannot simply be stopped but has to be removed by a health provider.

Interventions to assist women with compliance with contraception have proved disappointing. Reminding women to return to the clinic for their next injection of Depo Provera made no difference to compliance or to continuation rates at the end of a year of use. A randomised trial of daily telephone text messages reminding women to take their pill was similarly ineffective.

Since compliance with regular medication is often poor, even when the result of non-adherence is death; since most European women have to use contraception for more than 30 years of their lives; and since each act of sexual intercourse carries with it a risk of pregnancy of only 30% it is important that new contraceptive methods take into particular account the ease or difficulty of compliance.

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## S14.2

### New Pills

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The balance between the benefits and the risks of contraceptive steroids is generally positive in particular when comparing to the risks of pregnancy and especially in women with risk factors. Since recently the most common estrogen contained in the OCs remained ethinyl-estradiol although the doses used per tablet decreased dramatically in the most recent combinations. In parallel with the change in the progestin content from androgenic to non or anti-androgenic molecules, the estrogen have also been shifted recently to more natural compounds such as estradiol (E<sub>2</sub>) and estradiol valerate (E<sub>2</sub>V) with the objective of improving the safety of OCs.

Ethinyl-estradiol (EE) exerts a stronger effect than natural estradiol (E<sub>2</sub>) on hepatic metabolism including estrogen-dependent markers such as liver proteins. Current combined oral contraceptives (COC) affect a variety of hemostatic variables and estrogen-sensitive liver proteins, and these effects can be modulated by the associated progestin. In contrast, metabolism studies using the same surrogate markers as end-points indicate that the impact of estradiol-based novel COCs is similar or even lower than that of the second-generation COCs combining EE and an androgenic progestin such as Levonorgestrel.

Several new progestins have been designed to minimize side-effects related to androgenic, estrogenic or glucocorticoid receptor interactions. Dienogest (DNG), and drospirenone (DRSP) and the 19-norpregnanes including Nestorone (NES), norgestrol acetate (NOMAc) and trimegestone (TMG) have been combined with estrogen either EE or E<sub>2</sub> or estradiol valerate (E<sub>2</sub>V).

The lower metabolic impact of novel estradiol-based oral contraceptives may result in an improved safety profile, but large surveillance studies are warranted to confirm this plausible hypothesis. So far, the contraindications and warnings for use of current COCs also apply to the estradiol-based COCs.

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## S14.3

Abstract unavailable.

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## Emerging therapies in type 2 diabetes

### S15.1

Emerging therapies in type 2 diabetes: bariatric surgery

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The biggest achievement of bariatric surgery was to demonstrate that obesity co-morbidity improve or even reverse with long-term weight control. This

evidence was achieved with the Swedish Obesity Study for mortality and diabetes incidence.

Diabetes resolution rates range from 55 to 96% depending on the surgical procedure and length of follow-up. Diabetes improvement or resolution almost reaching the 100%. The type of surgery seems to play a role in diabetes and other co-morbidity improvement, the procedures associated with a greater percentage of excess weight loss presenting better results. This could suggest that diabetes improvement is directly related to excess weight loss. Although systematic review and meta-analysis suggest this is true, changes in gut hormones have been claimed for procedures involving changes in gut anatomy. Several hormones, namely GLP-1, GIP, PYY and ghrelin, have been carefully studied, results changing with type of surgery and protocols used. The role of changes of GLP-1 and GIP in diabetes improvement deserves great interest. The roles of caloric restriction, weight loss and alterations in the small intestinal anatomy, the importance of foregut exclusion and of rapid ileal exposure to nutrients, the changes in gut hormones, changes of the gut brain axis and the role of the vagus nerve as well as changes in gastric emptying and gut motility need to be better elucidated. Experimental procedures such as ileal interposition and foregut exclusion trying to better elucidate the importance of changes in gut hormones in diabetes improvement.

Controlled studies comparing diet versus surgery and different types of surgery are scarce and are urgently needed.

The length of follow up is of utmost importance to characterize diabetes improvement. Even with diabetes recurrence will there be a legacy effect as happened with other type of interventions as in UKPDS, DPS and DPP?

Several reports related poor responders to longer duration of diabetes preoperatively, revealing the importance of early surgical intervention for diabetes resolution. Bariatric surgery in type 2 diabetic patients poorly controlled with a BMI  $\geq 30$  is matter of debate.

Last but not least short term and long-term safety is a very important issue.

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## S15.2

### 11 $\beta$ -Hydroxysteroid dehydrogenase inhibitors for treatment of metabolic syndrome

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Harvey Cushing's work informed us of the deleterious consequences of circulating cortisol excess – hypertension, osteoporosis and obesity that contributes to diabetes and premature mortality. Conversely, Hench, Kendall and Reichstein were Nobel Laureates in Physiology 1950 for the discovery of cortisone and demonstrating efficacy in patients with Rheumatoid Arthritis – in effect the birth of the anti-inflammatory actions of glucocorticoids.

The tissue-specific generation of cortisol, independent of circulating levels, is catalysed by 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD1) that converts cortisone (E) to cortisol (F). High levels are expressed in liver and omental adipose tissue where the enzyme amplifies glucocorticoid-mediated hepatic glucose output and adipocyte differentiation. The former effect occurs through induction of hepatic gluconeogenesis (GNG); recombinant mice with global deletion of 11 $\beta$ -HSD1 or liver-specific deletion of the GR demonstrate reduced GNG. Conversely over expression of 11 $\beta$ -HSD1 in both liver and fat reproduce features of the metabolic syndrome.

Selective 11 $\beta$ -HSD1 inhibitors lower blood glucose, improve insulin sensitivity and cause weight loss in animal models. Biomarkers have been validated to confirm target inhibition in primate and human studies. Recent clinical trials show reduction in HbA1c and blood pressure in obese patients with diabetes mellitus who have failed on metformin therapy. Potentially the therapy offers a 'magic bullet' for patients with Metabolic syndrome with reduced blood glucose accompanying improved insulin sensitivity, lower lipids and blood pressure and reversal of hepatic steatosis secondary to reduced autocrine generation of cortisol in liver, adipose tissue, pancreas and muscle. Liabilities include activation of the HPA axis secondary to increased cortisol clearance with hyperandrogenism, though the extent and significance of this is debated. Perhaps of more concern is the potential impact upon the inflammatory process since 11 $\beta$ -HSD1 expression is significantly induced at the site of inflammation.

Modulation of glucocorticoid hormone action via selective 11 $\beta$ -HSD1 inhibitors represents a novel therapeutic advance to treat the global epidemic of metabolic syndrome.

**S15.3****Future therapies – what is in the pipeline?**

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Incretin-based therapies, such as the injectable GLP-1 receptor agonists and orally administered DPP-4 inhibitors, have recently been introduced into clinical practice. At present, the GLP-1 receptor agonists need to be administered once or twice daily. Several once weekly GLP-1 receptor agonists are in phase 3 development. This review examines the efficacy, safety and perspective for the future of the once weekly GLP-1 receptor agonists: exenatide once weekly, tasoglutide, albiglutide, LY2189265 and CJC-1134-PC and compare them to the currently available agonists, exenatide BID and liraglutide QD. A greater reduction in HbA1c and fasting plasma glucose was found with the once weekly GLP-1 receptor agonists compared with exenatide BID, while the effect on postprandial hyperglycemia was modest with the once weekly GLP-1 receptor agonist. The reduction in HbA1c was in most studies greater compared to oral antidiabetic drugs and insulin glargine. The reduction in weight did not differ between the short and long acting agonists. The gastrointestinal side effects were less with the once weekly agonists compared with exenatide BID, except for tasoglutide. Antibodies seem to be most frequent with exenatide once weekly, while hypersensitivity has been described in few patients treated with tasoglutide. Injection site reactions differ among the long acting GLP-1 receptor agonists and are observed more frequently than with exenatide BID and liraglutide. In humans no signal has been found indicating an association between the once weekly agonists and C-cell cancer. The cardiovascular safety, durability of glucose control and effect on weight will emerge from several ongoing major long-term trials. The once weekly GLP-1 receptor analogues are promising candidates for the treatment of type 2 diabetes although their efficacy may not be superior to once daily analogue liraglutide.

**TSH Receptor and thyroid disorders****S16.1****TSH-receptor antibodies**

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TSHR autoantibodies with stimulating (agonist) activity cause the hyperthyroidism of Graves' disease, while the rarer blocking (antagonist) type TSHR autoantibodies are responsible for hypothyroidism in some patients. We have isolated four human monoclonal autoantibodies (MAbs) which bind the TSHR with high affinity. Two antibodies, M22 and K1-18 have potent thyroid stimulating activity, one MAb K1-70 has strong antagonist (blocking) activity and the fourth MAb 5C9 has strong antagonist and inverse agonist activity. Stable complexes of the TSH receptor (amino acids 1–260; TSHR260) bound to the blocking type human MAb K1-70 and to the stimulating MAb M22 were prepared and analysed by X-ray diffraction. The structure of the TSHR260 in complex with K1-70 Fab (solved at 1.9 Å resolution) is virtually identical to the structure of the TSHR260 in complex with the thyroid stimulating MAb M22 previously solved at 2.55 Å resolution. The M22 (agonist) positions itself on the TSHR in a similar orientation as TSH (agonist) with the M22 heavy chain mimicking the interactions of the TSH  $\alpha$  chain and the M22 light chain mimicking the interactions of the TSH  $\beta$  chain. In contrast, the K1-70 MAb binds more N-terminally on the concave surface of the TSHR interacting extensively between amino acids D36 and D203, whilst the binding site of M22 extends further towards the C terminus and covers TSHR amino acids D36 to N256. These differences may reflect the distinct functional activities of M22 and K1-70. However, the binding sites of M22, TSH and K1-70 overlap extensively with 12 TSHR amino acids interacting with all 3 ligands. The availability of high resolution crystal structures of the TSHR in complex with a blocking monoclonal autoantibody (K1-70) and stimulating monoclonal autoantibody (M22) provides a foundation for developing new strategies to understand and control TSH receptor activation and the autoimmune response to the TSHR.

**S16.2****Activating and inactivating TSH receptor mutations**

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More than 21 families with inactivating TSHR mutations have been published. Their phenotype varies from euthyroid hyperthyrotropinemia (compound heterozygous) to severe congenital hypothyroidism (homozygous) with thyroid hypoplasia.

Current methods to precisely classify mutants with only a slight increase of the basal activity as constitutively active are limited. The results concerning the level of the basal activity can be influenced by the vector and/or the cell system. Reexamination of previously described constitutively active TSHR germline mutations did not show constitutive activity for R310C and N670S as tested in COS-7 cells. Moreover, mutations L677V and T620I identified in hot thyroid carcinomas and previously characterized in CHO and in 3T3-Vill cell lines, respectively, F666L identified in a patient with hot thyroid nodules, I691F in a family with nonautoimmune hyperthyroidism displayed no constitutive activity for  $G_{\alpha s}$  signaling. Therefore, beside the extensive clinical characterization, future characterizations of TSHR mutations regarding their constitutive activity status should also include linear regression analysis (LRA) of constitutive activity at least for borderline cases.

For 17 constitutively activating TSHR mutations found in 24 families with familial non autoimmune hyperthyroidism (FNAH) no direct relation of the *in vitro* activity of the TSHR mutation (basal cAMP value) and the age at onset of hyperthyroidism and the severity of clinical course was found and even for the same TSHR mutation different clinical courses in different families were described. For twelve case reports of sporadic non-autoimmune hyperthyroidism (SNAH) no clear evidence for a consistent relation of the TSHR mutation's *in vitro* activity determined by LRA with the clinical course of patients with SNAH was found. This suggests that the clinical course is more likely influenced by further genetic/epigenetic and environmental factors than by the *in-vitro* activity of the TSHR mutation itself.

However, a systematic analysis of published constitutively activating TSHR mutations and their clinical and *in vitro* activities provides evidence for a higher *in vitro* activity of shared sporadic and somatic TSHR mutations as compared to familial TSHR mutations. The few cases of sporadic non-autoimmune hyperthyroidism for which very active clinical progression were reported most likely contributed significantly to the higher LRA activity of the shared sporadic and somatic TSHR mutations.

**S16.3****Small molecule TSH receptor agonists and antagonists**

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TSH activates the TSH receptor (TSHR) thereby stimulating the function of thyroid follicular cells (thyrocytes) leading to biosynthesis and secretion of thyroid hormones. Because TSHR is involved in several thyroid pathologies there is a strong rationale for the design of small molecule 'drug-like' ligands (SMLs). rhTSH (Thyrogen<sup>®</sup>) has been used in the follow-up of patients with thyroid cancer to increase the sensitivity for detection of recurrence or metastasis. rhTSH, which is a heterodimeric 30 kDa glycoprotein, is difficult to produce and must be administered by injection. A small molecule TSHR agonist could produce the same beneficial effects as rhTSH but with greater ease of oral administration. We developed a SML that is a full agonist at TSHR with an EC<sub>50</sub> of 40 nM and interacts with the receptor's serpentine domain. In primary cultures of human thyrocytes, the agonist increases mRNA levels for thyroglobulin, thyroperoxidase, sodium-iodide symporter and deiodinase type 2. More importantly for its clinical potential, this agonist elevated serum thyroxine and stimulated thyroidal radioiodide uptake in mice after its absorption from the gastrointestinal tract following oral administration.

Graves' disease (GD) is caused by persistent, unregulated stimulation of thyrocytes by thyroid-stimulating antibodies (TSABs) that activate TSHR. We identified the first small molecule TSHR antagonists that inhibited TSH- and TSAB-stimulated signaling. Noteworthy, in primary cultures of human thyrocytes one of the antagonists inhibited cAMP production stimulated by all thirty GD sera tested by an average of 39%.

Our results provide proof-of-principle for effectiveness of small molecule agonists and antagonists for TSHR. Chemical optimization of these SMLs will be performed in an effort to produce more potent molecules for future clinical development.

## Pituitary update

### S17.1

#### Radiosurgery and radiotherapy of pituitary adenomas

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Transsphenoidal neurosurgery is the gold standard treatment for pituitary adenomas; however, it can be contra-indicated or ineffective. In these cases, medical treatment based on somatostatin analogs or GH receptor antagonist, can be used. Another approach is to use radiation therapy.

Stereotactic radiosurgery (SR) is a procedure aiming at controlling hormone hypersecretion and tumor size of pituitary adenomas. This talk will mainly deal with the long-term efficacy and adverse effects of Gamma knife, a modality of stereotactic radiosurgery, in secreting and non-secreting pituitary adenomas. Recent long-term data confirm the anti-secretory efficacy of the procedure (about 50% remission in hypersecreting tumors) but also a previously unknown low risk of recurrence (about 2–10% cases), in contrast with conventional radiotherapy. The main drawback is the time to remission estimated to range from 12 to 60 months. The anti-tumoral efficacy is observed in about 90% cases. Hypopituitarism is the main side-effect, observed in 20–40% cases. Comparison with conventional radiotherapy reveals a lower rate of remission counterbalanced by a more rapid efficacy, and a lower rate of hypopituitarism for radiosurgery. Recent short-term results about stereotactic fractionated radiotherapy suggest a risk of hypopituitarism similar to the one observed with radiosurgery. SR is thus probably still useful in the therapeutic algorithms of pituitary adenomas, despite the fact that antiseecretory drugs, particularly for acromegaly, are becoming more and more effective and well tolerated.

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### S17.2

#### Update on pituitary surgery 2011

Juergen Honegger

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The transsphenoidal surgical technique has dominated ever since Jules Hardy introduced microsurgery with the use of an operation microscope in the 1960s. Since the 1990s, endoscopic techniques have alternatively been applied in transsphenoidal surgery. Both microscopy and endoscopy should be accepted as established techniques which can often be used in a complementary fashion. It must be understood that microsurgery and endoscopy differ only in that they use different optical systems. The surgical access selected and the neurosurgeon's experience are much more decisive for the surgical outcome.

Extended transsphenoidal approaches have found their way into pituitary surgery during the past decade. This enables excision of pituitary tumours which extend well beyond the borders of the sella turcica. Using the 'transtuberculum sellae approach', for example, suprasellar tumours which used to be approached only via a craniotomy can be excised via a transnasal procedure.

Pituitary surgery is accepted for primary treatment in non-functioning adenomas, in acromegaly, and in Cushing's disease. In recent years, pituitary surgery is experiencing a renaissance in the treatment of prolactinomas. Surgical remission rates >90% with minimal surgical risk have been demonstrated for microadenomas. For this reason, surgical treatment is regaining acceptance as an alternative to often life-long dopamine-agonist therapy of prolactinomas.

Neuronavigation is widespread these days as a modern technology for intraoperative orientation. In pituitary surgery, it is preferentially used in difficult recurrence operations, in carotid artery narrowing or encasement by tumour, and in children with non-pneumatized sphenoid bone. Intraoperative magnetic resonance imaging (MRI) enables a valid control of the resection result and, if necessary, further resection during the same surgical intervention. Intraoperative MRI is, however, available in only a few large centers.

Over 90% of pituitary tumours can be excised these days via classical or extended transnasal approaches. The use of modern intraoperative technologies increases the safety and radicality of tumour removal.

### S17.3

#### Pituitary late effects of radiotherapy for brain tumours

M Bolanowski

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Radiotherapy of brain tumours given to children and adults is connected with various hormonal dysfunctions later in the life. There are anterior pituitary hormones deficiencies, hyperprolactinemia and precocious puberty. Their severity and frequency depends on the dose applied, age of the radiotherapy and time after radiotherapy. In children, the most important is early recognition of them, with growth hormone deficiency as most common. The impact of cranial irradiation on gonadotrophin secretion may result in precocious puberty and hypogonadism. Other cause of hypogonadism could be a deleterious effect of chemotherapy due to influence both on the pituitary and gonadal hormones secretion. TSH and ACTH deficiencies are less common. The occurrence of radiation-induced anterior pituitary hormonal deficiencies is high, they are progressive and irreversible. So, regular hormonal testing is crucial for early diagnosing of hypopituitarism and replacing of deficient hormones.

## Management of endocrine disease in pregnancy

### S18.1

#### Type-1 diabetes and pregnancy

P Damm

Abstract unavailable.

### S18.2

#### Type 2 diabetes and pregnancy

Eleni Anastasiou

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Obesity and DM2 increasingly affect young adults worldwide, to the point of warranting the description of 'epidemic'. This means that greater numbers of women of reproductive age are at risk. Frequently DM2 remains undiagnosed until pregnancy. The rate of congenital malformations seems to be the same with that of women with DM1, but the rate of perinatal mortality appears to be increased, according to a recent meta-analysis. These findings occur despite the fact that DM2 pregnant women have lower duration of disease, rate of complications and levels of HbA1c throughout pregnancy compared to DM1 women. The increased risk may be attributed to obesity, ethnicity, social deprivation and lack of preconception care. During pregnancy the management includes: i) self-monitoring of blood glucose in order to achieve the recommended glucose levels; ii) medical nutrition therapy, which has to be individualized taking into account the BMI; iii) exercise; iv) insulin. Most organizations do not recommend oral glucose lowering agents during pregnancy, because apart from the unknown long-term risks for progeny, it is difficult to achieve excellent glucose control in order to avoid fetal macrosomia. During labor maternal tight glucose control has to be maintained in order to prevent neonatal hypoglycemia. After delivery insulin requirements drop dramatically. Breastfeeding has to be encouraged. If required, metformin and possibly glibenclimide may be used. Finally, it is important to advise these women for the need to plan any future pregnancy.

### S18.3

#### Thyroid autoimmunity in pregnancy

Kris Gustave Poppe

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To adapt the working of the thyroid to the changes occurring during pregnancy, an adequate availability of iodine and the integrity of the thyroid gland are needed. With regard to thyroid function assessment during pregnancy, it is important to be aware of the differences in accuracy of the different FT<sub>4</sub> assays. Thyroid autoimmunity (TAI) is the most common cause of hypothyroidism before and during pregnancy and many women with TAI and a normal thyroid function in

early gestation tend to become hypothyroid during gestation. In euthyroid women, with and without TAI, similar pregnancy rates have been reported, but many studies showed that the presence of TAI is associated with a significantly increased risk in the overall miscarriage rate, independently of the presence of other autoantibodies. The association between TAI and miscarriage does not imply a causal relationship, and underlying causes might be (a combination of) immune disorders, inappropriate TH levels for the pregnancy period, and an increased maternal age in women with TAI. Concerning intervention trials, Negro *et al.* showed that L-T<sub>4</sub> in TAI + euthyroid pregnant women, reduced the miscarriage rate to that in women without TAI, a finding that was recently confirmed in an assisted reproductive setting by another group. The risk of spontaneous abortion during early gestation is also known to be increased in women with overt and subclinical hypothyroidism independently of the presence of TAI and in one study a positive linear relationship between fetal/child loss and serum TSH was shown. L-thyroxine is the drug of choice for the treatment of hypothyroidism and in women already receiving it, it should be adjusted before or during pregnancy to reach a serum TSH level  $\leq 2.5$  mIU/l during the first trimester. In women in whom hypothyroidism is diagnosed during pregnancy, L-T<sub>4</sub> should immediately be initiated according to their body weight. The increase in the L-T<sub>4</sub> dose becomes manifest after 4–8 weeks gestation and the magnitude of L-T<sub>4</sub> changes depends on the etiology of the hypothyroidism. For all hypothyroid L-T<sub>4</sub>-treated women, serum TSH levels should be monitored every 6–8 weeks unless an increase in dosage is needed. After parturition, most hypothyroid women need to decrease the L-T<sub>4</sub> dose received during pregnancy, and when TAI was associated, monitoring of thyroid function for at least 6 months after delivery is advised.

## Management of endocrine disease in adolescence

### S19.1

#### Management of congenital adrenal hyperplasia (CAH) in adolescence

H L Claahsen-van der Grinten

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Aim of the current glucocorticoid treatment strategies in patients with classic types of CAH due to 21 hydroxylase deficiency is to replace the lack of cortisol (substitution) and, secondly, to suppress excess adrenal androgen production by restoring the negative feedback on ACTH release (suppression).

In adolescence, besides adequate substitution, achieving normal puberty and menstrual cycle, preventing hyperandrogenism (hirsutism) and prevention of the development of testicular adrenal rest tumors in male patients are considered relevant medical treatment goals. All these aims require individualized treatment strategies. Mostly supraphysiological dosages of glucocorticoids are necessary to suppress the ACTH release. Glucocorticoid overtreatment could lead to iatrogenic Cushing's syndrome and increased cardiovascular risk. In adolescence, there is a decreased conversion of cortisol to and an increase in renal clearance of cortisol contributing to a relative lower cortisol concentration. Therefore, it is often necessary to increase dosages of hydrocortisone. However, current studies show that at this age glucocorticoids may have a deleterious effect on pubertal growth and therefore daily hydrocortisone dosage should not exceed 17 mg/m<sup>2</sup>. Therefore, it is a difficult task to find the right balance between under- and overtreatment especially in adolescence thereby avoiding important long-term complications.

In adolescence issues of sexual activity and contraception have to be discussed and genetic counseling has to be given to the patients. General information about CAH (including the need for wearing medical identification) should be given together with a crisis prevention training performed by an experienced nurse. In female patients with virilising CAH consultation of an experienced gynecologist should be arranged before or during puberty to decide whether additional surgery is needed.

A good preferably gradual transition to the adult endocrinologist has to be planned carefully at the end of adolescence.

### S19.2

#### The challenges of managing childhood cancer survivors during adolescence

H K Gleeson

Leicester Royal Infirmary, Leicester, UK.

In the UK 1 in 500 children will develop cancer in childhood. Survival from childhood cancer has more than doubled since the 1960s. Survivorship is

increasingly on the agenda and the National Cancer Survivorship Initiative has been launched in the UK.

During adolescence the endocrinologist is faced with four challenges in managing childhood cancer survivors:-

1. Managing the immediate endocrine consequences of cancer therapy, in particular, those relating to timing of puberty and growth.
2. Recognising that these patients are young people first and childhood cancer survivors second, and the importance of addressing the wider issues of adolescence.
3. Working as part of a multidisciplinary team and considering the wider implications of cancer therapy on reproductive potential, bone and cardiovascular health, risk of second malignancies, morbidity and mortality.
4. Identifying a suitable adult endocrinologist with the expertise to provide ongoing endocrine care for childhood cancer survivors in adulthood.

### S19.3

#### Management of type 1 diabetes in adolescence

Z Sumnik

2nd Medical School, University Hospital Motol, Charles University, Prague, Czech Republic.

The management of type 1 diabetes (T1D) in puberty and adolescence is characterized by special challenges related to the dramatic hormonal, emotional and social changes in the teenage years. It is a period of transition from physical immaturity to maturity and from parental dependency to independence. The daily application of insulin, necessity of frequent self-monitoring of blood glucose and awareness of diabetic diet represent additional burdens for teenagers with diabetes. It is thus not surprising that blood glucose control is the worst in adolescents compared to younger children and adults with T1D. In addition, adolescents are exposed to physiological puberty-related changes significantly influencing the course of diabetes and its management. Excessive height and weight gain and changes in the GH/IGF1 axis lead to an increase in insulin resistance and enhancements in hepatic glucose production, which are typical for this period of life. The management of T1D during adolescence is complex. The increasing insulin requirement is considerable and insulin doses may need to be increased to more than 1.0 units/kg per day which is the highest relative insulin requirement in life. The need of this amount of insulin can only be successfully achieved with either multiple daily injection (MDI) regimen or with insulin pump (CSII). The use of rapid insulin analogues may provide some advantage by offering a more physiological control of the post-prandial glycemias. Nevertheless, the long-term efficacy of insulin pumps and continuous glucose monitoring systems are limited in non-selected adolescent population. Therefore, a careful selection of suitable patients for this type of therapy is mandatory. Increasing the motivation for compliance to treatment is one of the most important parts of T1D management in adolescents. Multidisciplinary approach including educators, psychologists and dieticians is the basic pre-requisition for successful outcomes.

## Thyroid function: it is in your genes

### S20.1

#### Genetics versus environment: what determines thyroid function?

P S Hansen<sup>1,2</sup>

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The circulating levels of serum TSH and thyroid hormones represent biochemical phenotypes reflecting thyroid homeostasis. Studying the regulation of biochemical measures related to the thyroid in healthy individuals could prove essential in understanding the pathways that eventually lead to thyroid disease. It is the combined effect of genetic and environmental factors that give rise to these endophenotypes. It has been established that the measures reflecting thyroid homeostasis have a substantial heritable component. Thyroid hormone levels are partly genetically correlated – although most of the genetic variance for these measures is trait specific. Genetic as well as environmental risk factors (such as iodine intake and smoking) with significant influence on the thyroid related phenotypes have been identified. Several specific polymorphisms within obvious candidate genes are associated with serum TSH and thyroid hormone levels, respectively; however these specific polymorphisms account for <2% of the total

phenotypic variance. Using simple interaction models it has not been possible to detect any modifying effect of iodine intake (measured as iodine excretion) or cigarette smoking on the effect of a specific polymorphism in the TSHR gene.

#### Conclusion

Genetic as well as environmental determinants with significant influence on various thyroid related phenotypes in healthy twins has been identified, but neither of these factors have a strong impact on the various thyroid related phenotypes. Many unmeasured genetic variants as well as environmental exposures with influence on thyroid homeostasis remain to be uncovered.

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## S20.2

### Polymorphic variation in thyroid pathway genes

Robin Peeters

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Adequate thyroid hormone (TH) levels are essential for normal growth and differentiation, for the regulation of energy metabolism, and for the physiological function of virtually all human tissues. Epidemiological evidence shows that minor variation in TH serum levels, even within the normal range, can have important effects on different TH related clinical endpoints, such as bone mineral density, atrial fibrillation, metabolic syndrome and cardiovascular mortality.

In healthy subjects, serum TSH and TH levels show substantial inter-individual variability leading to wide laboratory reference ranges, whereas the intra-individual variability is within a much more narrow range. This suggests that every person has its own individual thyroid function 'set-point'. Approximately 45–65% of this inter-individual variation in serum TSH and TH levels is determined by genetic factors, but the causative genes are not yet well established. Recent genome wide association studies have demonstrated associations of polymorphisms located in Phosphodiesterase 8B and the *CAPZB* locus with serum TSH levels, and well-known TH pathway genes such as the deiodinases, TSH receptor and TH transporters have been associated with TH serum levels in candidate gene analyses.

However, the variation that can be explained by these gene variants so far is only modest. This could be due to the presence of not-analyzed rare variants, or gene-environment and gene-gene interactions. Novel analytic approaches and the availability of whole-genome sequencing of large numbers of individuals will provide important new information on this topic in the upcoming years.

This presentation will focus on the interpretation of different genetic studies in thyroid hormone research and discusses its clinical relevance.

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## S20.3

### Genetics of autoimmune thyroid disease

Stephen Gough

Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK.

Autoimmune thyroid disease (AITD) is the commonest of the autoimmune disorders. Strong familial clustering supports a hereditary component to the development of disease. However, the pattern of inheritance suggests that many genes with relatively small effect size are contributing to the genetic architecture of both Graves' disease and autoimmune hypothyroidism. Whilst early candidate gene studies helped to identify some of the major effects conferring risk to AITD, including the HLA status of an individual and other genes involved in regulating the immune system, such as *CTLA4* and *PTPN2*, many loci remained unknown. In the last 5 years or so, new technologies and large DNA collections have facilitated the use of genome-wide association studies in many common diseases in which over 500 000 genetic markers have been examined in many thousands of people with disease. This approach has focused on the study of the most common variants in the human genome, namely single nucleotide polymorphism (SNPs), and delivered many new disease-risk genes, including for Graves' disease a more detailed understanding of the TSHR locus. The most recent studies have also looked at other DNA variants such as common number variants (CNVs) and large scale detailed resequencing of the genome. Through these efforts we have made significant advances in our understanding of the genes that confer risk to many common diseases including AITD. Many loci however, remain undetected. Moreover, one of major challenges ahead will be trying to understand the functional consequences of the DNA variants conferring risk to disease.

## Vitamin D, pregnancy and development

### S21.1

#### Evolutionary perspective in vitamin D and its receptor VDR

Zeev Hochberg

Rambam Medical Centre, Haifa, Israel.

Vitamin D is photosynthesized in all forms of life from the phytoplankton 750 mya. Evolutionary pressures due to variation in climate play an important role in shaping phenotypic variation and influence variation in phenotypes. Migration out-of-Africa and change of living latitude placed human vit D as part of an evolutionary complex that adapted hominids to changing u.v. radiation. The 'vit D hypothesis' to explain skin pigmentation is based on the observation that the skin color follows a clinal distribution: the darkest populations inhabit the equatorial and tropical belt; the most pale-skinned the regions above 50°N; and those of intermediate pigmentation the middle latitudes. Exposure to u.v. light has to balance the need for UVB for vit D photosynthesis and damage by u.v. for folic acid generation. This balance is maintained by melanism, which determines skin color, reflecting a compromise solution to the conflicting physiological need for vit D and the detrimental effect of u.v. on folic acid generation. A multi-stage genome-wide association study of natural hair color identified several loci highly associated with hair color. But the strongest determinant of skin color remains *MC1R*. I propose that the *VDR* gene is epistatic with skin color-determining genes for the phenotype of fitness; both *MC1R* and the *VDR* are strongly implicated in immune system regulation, with obvious fitness consequences. *VDR* may be the original nuclear receptor gene, functioning initially as pregnane X receptors and constitutive androstane receptors, to induce P450 enzymes for xenobiotic detoxification. The broad abundance of the *VDR* may be also related to the multitude of recent reports claiming a role for vit D in cell differentiation and proliferation, immune function, muscle strength, blood pressure control and more.

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## S21.2

### Vitamin D deficiency and supplementation in pregnancy

P Lips

VU University Medical Center, Amsterdam, The Netherlands.

Vitamin D deficiency is more common in pregnant women than in non-pregnant women of similar age. Clinical vitamin D deficiency, associated with rickets and osteomalacia, may occur when serum 25-hydroxyvitamin D (25(OH)D) is lower than 25 nmol/l. The required serum 25(OH)D is currently set at 50 nmol/l, as lower levels are associated with lower bone mineral density, increased fracture risk and other outcomes such as lower physical performance than a vitamin D adequate state, i.e. serum 25(OH)D above 50 nmol/l. Many studies show low serum levels of 25-hydroxyvitamin D (25(OH)D) in pregnant women and in their offspring. Serum 25(OH)D is about 20% lower in the neonate (cord blood) than in the mother. During normal pregnancy, the concentration of the active metabolite 1,25-dihydroxyvitamin D (1,25(OH)2D) increases in the first trimester, due to the increase of the vitamin D binding protein. The free concentration of 1,25(OH)2D increases in the third trimester when the daily transfer of calcium through the placenta increases to more than 300 mg/day. Vitamin D deficiency in the mother is associated with a higher risk of pre-eclampsia and gestational diabetes. Severe vitamin D deficiency in the mother can lead to neonatal hypocalcemia and tetany, low birth weight and rickets. Vitamin D deficiency in the mother is also associated with asthma in the child, with lower bone mineral density in the first year and at 9 years of age. The most important risk group for vitamin D deficiency in pregnancy are non-western immigrants. A survey in The Hague in the Netherlands showed very low mean serum 25(OH)D levels in Turkish and Moroccan women (15.2 ± 12.1 and 20.1 ± 13.5 nmol/l respectively), and 25(OH)D was not detectable in 22% of the Turkish women.

The most recent guideline of the Institute of Medicine (2011) concluded that the required serum 25(OH)D level meeting the needs of 97.5% of the population is 50 nmol/l. The recommended dietary allowance for pregnant women is 600 IU/day. However, many uncertainties remain regarding the consequences of vitamin D deficiency in pregnancy. There is a lack of double blind randomized trials in pregnant women with sufficiently long follow-up to determine the effects of vitamin D supplementation in mother and child.



**S21.3****Maternal vitamin D intake during pregnancy and the risk of type 1 diabetes in the offspring**

Suvi Virtanen

Tampere University Hospital, Tampere, Finland.

The pre-diabetic autoimmune process leading to clinical type 1 diabetes may start already during early infancy. As the incidence of type 1 diabetes is increasing most rapidly among children younger than 5 years in many high and middle income countries (Patterson *et al.* 2009), there is increasing interest in the impact of early exposures such as diet even before birth.

Vitamin D has several effects on the immune system, which could be of potential relevance in the pathogenesis of type 1 diabetes. Maternal vitamin D intake and status during pregnancy affects the vitamin D status of the fetus and the newborn baby. Vitamin D supplementation during infancy may protect from type 1 diabetes as suggested by case-control findings from an European wide study (The EURODIAB Substudy 2 Study Group 1999) as well as by cohort findings from Finland (Hyppönen *et al.* 2001). In a Norwegian case-control study, the use of cod liver oil (rich in vitamin D) but not vitamin D supplementation as such during the first year of life was associated with lower risk of type 1 diabetes (Stene *et al.* 2003).

Findings whether maternal vitamin D intake protects the child from pre-clinical type 1 diabetes are discordant. In an US cohort, maternal intake of vitamin D from food, but not from supplements during pregnancy was associated with a decreased risk of early islet autoimmunity appearance in the offspring (Fronczak *et al.* 2003). In a Swedish cohort study a weak inverse association was observed between maternal vitamin D supplementation and appearance of diabetes-associated autoantibodies at the age of 1 year but not at 2.5 years (Brekke & Ludvigsson 2007). In a Finnish cohort study in which a more advanced pre-type 1 diabetes endpoint was used and where the number of endpoints outnumbered the previous studies, no association was shown between maternal intake of vitamin D either from food or supplements (Marjamäki *et al.* 2010). Neither was vitamin D intake related to the development of clinical type 1 diabetes in that study.

**Non traditional effects of pituitary hormones****S22.1****Non-thyroidal effects of TSH**

G R Williams

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The glycoprotein hormone, TSH, is synthesized and secreted by thyrotrophs in the anterior pituitary gland. It acts at the TSH receptor (TSHR), a 7-transmembrane G-protein coupled cell membrane receptor expressed in thyroid follicular cells. The TSHR, thus, plays a key role in the regulation of thyroid status and growth of the thyroid gland. In recent years TSHR expression has also been identified in a wide variety of extra-thyroidal tissues including: anterior pituitary; hypothalamus; ovary; testis; skin; kidney; immune system; bone marrow and peripheral blood cells; white and brown adipose tissue; orbital preadipocyte fibroblasts and bone. Considerable data are emerging that describe possible functional roles of TSHR at these various sites, but their physiological importance in many cases remains a subject of controversy and much interest. In particular, expression of TSHR in bone and the possible role of TSH in the skeleton have attracted considerable controversy. Thus, TSH has been proposed to act as a negative regulator of bone remodeling, acting directly via effects on the TSHR expressed in bone-forming osteoblasts and bone-resorbing osteoclasts. This hypothesis has challenged the conventional view that that skeletal responses to abnormal thyroid status result from altered thyroid hormone action in bone. Thus, in thyrotoxicosis, high bone turnover and osteoporosis are considered to result from thyroid hormone excess. By contrast, in the TSH model, these consequences are proposed to result from TSH deficiency. Unfortunately, because of the reciprocal physiological relationship between thyroid hormones and TSH it has been difficult to distinguish the actions of thyroid hormones from those proposed to result from TSH deficiency. This presentation will address these issues in particular as well as considering the wider actions of TSH in other extra-thyroidal tissues.

**S22.2****Vasopressin and social odour processing in the olfactory system**

M Ludwig

University of Edinburgh, Edinburgh, UK.

Central vasopressin facilitates social recognition and modulates numerous complex social behaviours in mammals, including parental behaviour, aggression, affiliation and pair-bonding. In rodents, social interactions are primarily mediated by the exchange of olfactory information, and there is evidence that vasopressin signalling is important in brain areas where olfactory information is processed. We recently discovered that the rat olfactory bulb (OB)<sup>1</sup> and the anterior olfactory nucleus (AON)<sup>2</sup> contain large populations of interneurons which express and release vasopressin. In the OB, single cell recordings from mitral cells *in vivo* showed that vasopressin modulates the processing of information by olfactory bulb neurones. Blocking the actions of vasopressin in the OB (using antagonists or small interference RNA against the vasopressin V1a receptor, or local selective destruction of vasopressin cells with diphtheria toxin in transgenic rats) impairs the social recognition abilities of rats. The treatments impaired habituation/dishabituation to juvenile cues, but not to volatile odours or object recognition, and did not affect locomotor activity or anxiety-related behaviours. Adult rats exposed to a conspecific juvenile showed increased Egr-1 expression in vasopressin neurones in multiple subdivisions of AON as compared to animals exposed to no odour or a non-social odour. These data suggest that vasopressin neurones in the AON may also play an important role in the coding of social odour information. The findings indicate that the vasopressin process olfactory signals relevant to social discrimination and vasopressin release may be involved in filtering out familiar signals.

**S22.3****Prolactin, stem cells and prostate cancer**

Vincent Goffin

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Androgen-independent recurrence is the major limit of androgen ablation therapy for prostate cancer. Identification of alternative pathways promoting prostate tumor growth is thus needed. Signal transducer and activator of transcription 5 (Stat5) has been recently shown to promote human prostate cancer cell survival/proliferation and to be associated with early prostate cancer recurrence. Stat5 is the main signaling pathway triggered by prolactin (PRL), a polypeptide hormone mainly secreted by the pituitary. PRL is also expressed by prostate epithelial cells, and increased PRL expression has been documented in high-grade prostate cancers. Prostate-specific PRL transgenic mice were earlier reported to develop benign prostate hyperplasia (BPH). We have used these mice to address the mechanisms by which local PRL induces prostate tumorigenesis. We observed that i) Stat5 is the major signaling cascade triggered by local PRL in the mouse dorsal prostate, ii) this model actually recapitulates prostate tumorigenesis from pre-cancer lesions in young animals, to invasive carcinoma in older mice, iii) tumorigenesis involves dramatic accumulation and abnormal spreading of p63-positive basal cells, and of stem cell antigen-1 positive cells identified as a stem/progenitor-like subpopulation. Since basal epithelial stem cells have been proposed to serve as tumor-initiating cells, we challenged the relevance of local PRL as a novel therapeutic target. Using a double transgenic approach, we show that Del1-9-G129R-hPRL, a competitive PRL receptor antagonist developed by our group, prevents early stages of prostate tumorigenesis by reducing or inhibiting Stat5 activation, cell proliferation, abnormal basal cell pattern and frequency/grade of intraepithelial neoplasia.

Although PRL is currently viewed by clinical endocrinologists as a pituitary hormone whose unique associated pathology is hyperprolactinemia leading to reproductive disorders, our study identifies autocrine PRL/Stat5 as a novel pathway initiating prostate tumorigenesis by altering basal/stem-like cell subpopulations. These findings strongly support the importance of further developing strategies to target locally over-expressed PRL in human prostate cancer, which cannot be achieved using current anti-PRL approaches involving dopamine agonists.

**Optimising thyroid hormone replacement****S23.1****Is there a place for combined T<sub>4</sub> and T<sub>3</sub> replacement therapy?**

A Weetman

University of Sheffield, Sheffield, UK.

This review will address the following questions i) what is the evidence that levothyroxine replacement alone is insufficient to deal with the treatment of

hypothyroidism; ii) what is the evidence from randomised control studies that combinations of tri-iodothyronine and levothyroxine are superior to levothyroxine alone in replacement treatment for hypothyroidism; iii) what other factors may be involved in the optimal replacement of thyroid hormone deficiency and iv) what conclusions may we draw and what future directions are likely in this area?

There is accumulating evidence that around 10% of patients feel dissatisfied with levothyroxine replacement although it is not clear whether these results in part stem from selection or treatment biases. There is no evidence so far that animal studies showing inadequate tissue levels of T<sub>3</sub> on T<sub>4</sub>-only replacement are replicated in man. A meta-analysis of 11 trials of T<sub>3</sub> and T<sub>4</sub> combination treatment up to 2006 concluded that this was not superior to T<sub>4</sub> alone although three subsequent trials have found some weak evidence for benefit.

Amongst the factors that must be taken into account in judging these trials are the heterogeneity of the causes of hypothyroidism, different durations of disease and treatment, variation in T<sub>4</sub>:T<sub>3</sub> dose ratio (by weight) of 20:1–1:1 and the use of differing outcome measures. Liothyronine is so far available only as a 20 µg tablet: unavoidable supraphysiological peaks of T<sub>3</sub> are inevitable. Moreover T<sub>3</sub> displays a circadian rhythm and this is not mimicked with current preparations. Variability in deiodinase enzyme and thyroid hormone transporter distribution, including genetic polymorphism, might mean that some tissues are underexposed to sufficient T<sub>3</sub> without the endogenous T<sub>3</sub> secreted by thyroid gland. There is also preliminary evidence that a SNP in *DIO2* may be associated with a greater improvement on combination T<sub>3</sub> and T<sub>4</sub> treatment.

Although combination T<sub>3</sub> and T<sub>4</sub> treatment cannot be recommended for routine use, there is still a need for physiological replacement trials, stratified by genotype and with meticulous, dynamic TSH monitoring.

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### S23.2

#### Thyroid dysfunction in the elderly

Kristien Boelaert

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The thyroid gland undergoes several anatomical changes with age and both overt and subclinical thyroid dysfunction is more prevalent in elderly patients. Recent reports have convincingly shown a physiological rise in serum TSH concentrations in the elderly and studies on autoimmunity have indicated an age-related prevalence of antithyroid antibodies. Interpreting thyroid function tests in elderly patients may be complicated by an increased prevalence of co-existing chronic diseases and the use of medication, which may affect laboratory tests.

We have recently demonstrated significantly reduced prevalences of symptoms and signs of overt hyperthyroidism in older patients with hyperthyroidism, independent of the underlying disease aetiology and the biochemical disease severity, indicating that diagnosing hyperthyroidism in the elderly represents a challenge. Since both subclinical and overt hyperthyroidism are associated with a number of negative outcomes including increased cardiovascular risk, reduction in bone density and decline in cognitive function, it is important to diagnose and treat hyperthyroidism in a timely fashion. A number of age-related factors may also influence the choice of treatment modality to be employed. Despite a physiological age-related increase in serum TSH, hypothyroidism is more prevalent when comparing older adults with younger individuals. Whilst there is clear evidence that significantly raised serum TSH concentrations in association with below normal concentrations of free T<sub>3</sub> and T<sub>4</sub> are associated with significant cardiovascular morbidity and mortality, the treatment of subclinical thyroid dysfunction in the elderly remains controversial. Paradoxically, recent evidence has demonstrated mildly raised serum TSH concentrations in subjects with extreme longevity and the question whether to treat mild hypothyroidism in the elderly remains unanswered.

In view of our ageing population it is important to consider challenges posed by diagnosing and managing thyroid dysfunction in the elderly and these will be addressed in this symposium.

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### S23.3

#### Failure of thyroxine replacement to suppress TSH: what to do now?

Jacques Orgiazzi

Service d'Endocrinologie, Centre Hospitalier Lyon, Lyon, France.

Persistence of elevated TSH levels despite large doses of L-thyroxine is not uncommon, although thyroxine substitution is usually easy and convenient. The first cause to be considered is thyroxine malabsorption, whether organic, due to

concomitant gastric or intestinal disease, or bariatric surgery, or, more frequently, related to dietary or drug interference. Proton-pump inhibitors, antacids and a long list of drugs may decrease thyroxine absorption. Drugs and thyroid disruptors may also affect thyroid hormone metabolism at many levels. Factitious disease, the so-called pseudo-malabsorption, is related to poor patient compliance, a difficult diagnosis which can be confirmed by a L-thyroxine absorption test. Whether patient education, as well as the method of education, is beneficial in this condition remains to be clarified. The recent advent of generic preparations of L-thyroxine is to be taken into consideration since the absorption or the quality of some preparations might be questionable. Finally, still another concept is of importance. Indeed, thyroid function parameters in healthy subjects may show considerable inter-individual variability indicating that each individual has a unique set point of thyroid function. In addition to clinically parameters such as body mass index, genetic factors, some directly involved in thyroid hormone metabolism, are important determinant of the pituitary–thyroid axis homeostasis.

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## Management of pheochromocytoma

### S24.1

#### Biochemical screening for pheochromocytoma using plasma free metanephrines: utility beyond diagnosis

G Eisenhofer

Technische Universität Dresden, Dresden, Germany.

Measurements of plasma concentrations of the *O*-methylated metabolites of catecholamines including metanephrine, normetanephrine and methoxytyramine have advantages over other biochemical tests used to diagnose pheochromocytoma for several reasons: i) the metabolites are produced within chromaffin cells continuously from catecholamines leaking from storage vesicles; ii) the single largest source of these metabolites is from adrenal medullary chromaffin cells, but this production normally represents a minor pathway of catecholamine metabolism; iii) in pheochromocytoma tumour cells the *O*-methylation pathway dominates; iv) intra-tumoural production of the metabolites occurs independently of variations in catecholamine release; and v) other catecholamine metabolites, including sulfate-conjugated metanephrines measured in urine, are produced largely downstream of chromaffin tumour cells. The consequences of these influences, as shown using data from 365 patients with pheochromocytoma, go well beyond utility of the metabolites for diagnosis. Increases in the metabolites can be used to predict tumour size, important for assessing disease burden and response to therapy in patients with malignant disease. Measurements of plasma methoxytyramine also provide a novel biomarker for malignancy and together with *succinate dehydrogenase type B subunit* mutation status, tumour size and location provide useful information to assess malignant risk. Furthermore, patterns of increases of metanephrine, normetanephrine and methoxytyramine show distinct differences according to hereditary syndrome and thus provide an easily utilised tool to guide cost-effective genotyping of underlying disease-causing mutations. Differences in ages of tumour presentation according to different biochemical phenotypes and tumour locations also suggest origins from different chromaffin progenitor cells with variable susceptibility to disease causing mutations.

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### S24.2

#### Imaging of pheochromocytoma and paragangliomas

H J L M Timmers<sup>1,2</sup>

<sup>1</sup>Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>2</sup>National Institutes of Health, Bethesda, Maryland, USA.

The standard technique for the functional imaging of pheochromocytoma and paraganglioma (PPGL) is [123I]-metaiodobenzylguanidine single photon emission computed tomography (MIBG SPECT). However, metastases are more accurately detected by 2-[18F]-Fluoro-2-deoxy-D-glucose positron emission tomography (FDG PET). In a large prospective study of 216 patients evaluated at the NIH for benign or malignant PPGL, we investigated the sensitivity and specificity of FDG PET as compared to MIBG SPECT for localization of (metastases of) PPGL. We also explored whether clues for a genetic diagnosis underlying PPGL can be gained from functional characterization by FDG PET. Cut-off values for FDG PET SUVs to distinguish PPGL from normal adrenal glands were established.

**S24.3****Surgical therapy**  
B Niederle

Abstract unavailable.

**GNAS locus: imprinting, animal models and human diseases****S25.1****Genomic imprinting of the *GNAS* locus**

G Kelsey

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Genomic imprinting is an epigenetic mechanism by which a subset of our genes displays unequal expression of the maternal and paternal alleles; in many cases, one allele is fully silenced. It occurs because these genes are marked differently (by DNA methylation and/or histone modifications) in male and female gametes. These gamete-specific marks are maintained at fertilisation and perpetuated throughout development and adult life, such that the alleles of imprinted genes retain a permanent memory of the parent in which they originated.

The *GNAS* locus on chromosome 20q13.11 (and chromosome 2 in mouse) is the archetypal complex imprinted locus. It comprises a set of overlapping transcripts determined by alternative promoters with different patterns of imprinted expression. It also provides the classic example of tissue-specific imprinting, in which the canonical *GNAS* transcript coding for *Gsz* is expressed predominantly from the maternal allele in some tissues. Functionally, this incomplete imprinting is clinically significant, as it dictates the nature of the disease caused by inactivating mutations in *Gsz*, with end organ hormone resistance specifically on maternal transmission (pseudohypoparathyroidism type 1a, PHP1a). In addition to *Gsz*, the locus encodes the proteins *XLzs* and *NESP55* from the overlapping transcripts. *XLzs* is expressed exclusively from the paternal allele and functions similarly to *Gsz* at the biochemical level. However, gene knock-out studies in mice show that *Gsz* and *XLzs* have opposite physiological effects, for example, in sympathetic nervous activity and metabolism, suggesting that *XLzs* acts as a counter-regulator of pathways dependent on *Gsz*. Despite the importance of *XLzs* demonstrated in the mouse, the impact of *XLzs* function in human is not understood.

An alternative form of pseudohypoparathyroidism, PHP1b, is an imprinting disorder characterised by DNA methylation defects in the differentially methylated regions (DMRs) that determine tissue-specific monoallelic expression of *GNAS*. Aberrant DNA methylation in PHP1b is caused by deletions in *cis*, and these mutations are helping to provide insights into the mechanisms of imprinting establishment at *GNAS* and generally.

**S25.2****Epigenetic defects at the *GNAS* locus and human diseases**

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The two main subtypes of pseudohypoparathyroidism (PHP), PHP-1a and -1b, are caused by mutations in *GNAS* exons 1–13 and methylation defects in the imprinted *GNAS* cluster, respectively. PHP-1a patients show Albright hereditary osteodystrophy (AHO), together with end-organ resistance to the action of different hormones that activate the Gs-coupled pathways, primarily PTH, TSH, gonadotropins, and GHRH. In PHP-1b patients AHO is classically absent, *Gsz* activity in erythrocytes and fibroblast is normal and hormone resistance is limited to PTH and TSH, the latter being essentially always subclinical. This disorder is caused by the disruption of long-range imprinting control elements in *GNAS* locus. In particular, the most consistent defect is the loss of imprinting at the exon A/B differentially methylated region (DMR), with consequent decreased *Gsz* transcription in tissues where this protein is derived from the maternal allele only. The familial form of the disease (AD-PHP-1b) is typically associated with an isolated loss of imprinting at the exon A/B DMR due to microdeletions disrupting the upstream STX16 gene. In addition, deletions removing the entire *NESP55* DMR, located within *GNAS*, have been identified in some AD-PHP-1b kindreds in whom affected individuals show loss of all the maternal *GNAS* imprints. Conversely, most sporadic PHP-1b cases have *GNAS* imprinting abnormalities that involve multiple DMRs, but the genetic lesion underlying these defects

remains to be discovered. Recently, methylation defects have been detected in a subset of patients with PHP-1a and variable degrees of AHO, indicating a molecular overlap between the two forms. Imprinting defects do not seem to be associated with the severity of AHO neither with specific AHO signs.

In conclusion, the latest findings of frequent *GNAS* imprinting defects in patients with major clinical and biochemical differences in respect with classical PHP-1b, further confirm the existence of an overlap between the molecular and clinical features of PHP-1a and PHP-1b, and highlight the necessity of a new clinical classification of these diseases, taking into account the recent knowledge on the molecular basis underlying these defects.

**S25.3*****Xlas*, *in vitro* functions and possible physiological and pathogenic roles**  
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The *GNAS* locus is a complex imprinted locus which encodes *Gsa* and four additional alternative transcripts including *Xlas*. *Gsa* is the ubiquitous  $\alpha$ -subunit of the G-protein involved in major biological pathways including hormonal signaling and cell differentiation. *Xlas* is the only transcript sharing with *Gsa* the protein domain encoded by *GNAS* exons 2 to 13 of, which plays a key role in coupling activated receptors to adenylate cyclase stimulation. However, *Xlas* differs from *Gsa* by its tissue-specific paternal exclusive expression. Epi-genomic defects at the *GNAS* locus cause a series of diseases called pseudohypoparathyroidisms (PHPs) whereas post-zygotic mutations in the domain of the *Gsa* protein coding for the GTPase intrinsic activity cause endocrine activation and McCune–Albright syndrome (MAS). In both contexts, the symptoms were until recently attributed solely to the modified function or expression of *Gsa*. Recent findings clearly indicate that changes in *Xlas* expression or function may contribute to these phenotypes. *In vitro* in transfected cells that endogenously lack both *Gsa* and *Xlas* (*Gnas*<sup>-/-</sup> cells), human *Xlas* shares with *Gsa* the ability to couple Gsa-coupled receptors to adenylate cyclase upon agonist stimulation (including PTHR1 activation by PTH), thereby generating an increase in intracellular cAMP. Loss of function mutations of *GNAS* (exons 2–13) impair receptor-mediated cAMP signaling for both *Gsa* and *Xlas*; interestingly in these studies, *Xlas* appears more potent than *Gsa* in mediating basal and stimulated cAMP generation. Animal models demonstrated the importance of *Xlas* in many processes including post-natal adaptation to feeding, energy metabolism and adipocyte biology. In humans, indirect observations suggest that *Xlas* contributes to pre and post-natal growth and hormonal signaling in early life and that *Xlas* alteration in PHPs or MAS influences the patient phenotypes.

Although there is no reported human 'model' of *Xlas* inactivation, the above data point to a specific role for *Xlas* in major biological functions. However, it is still debated if *Xlas* potentializes, replaces and/or antagonizes *Gsa* function.

**The role of plasma binding proteins in tissue hormone delivery****S26.1****Sex hormone-binding globulin: beyond plasma transport**G Hammond<sup>1,2</sup><sup>1</sup>Child and Family Research Institute, Vancouver, British Columbia, Canada; <sup>2</sup>University of British Columbia, Vancouver, British Columbia, Canada.

Sex hormone-binding globulin (SHBG) transports sex steroids (androgens and estrogens) in the blood and regulates their access to tissues. In humans and other vertebrates, the liver produces the SHBG that circulates in the blood, and in most species the gene encoding SHBG is also expressed in the testis. The testicular cell types in which SHBG is produced vary between species; in most mammals, expression of the *Shbg* gene in Sertoli cells gives rise to a secreted protein that binds androgens in the seminiferous tubules and male reproductive tract. By contrast, the human *SHBG* gene is expressed in developing sperm cells rather than Sertoli cells. In spermatids and spermatozoa, human SHBG accumulates as an amino-terminally truncated isoform in the acrosome, the concentrations of which decrease with aging and are correlated with sperm motility. Studies in transgenic mice have indicated that human SHBG is also produced as distinct glycoforms in

several other tissues, and accumulates inside sex steroid-sensitive cells of the kidney and small intestine. The physiological role(s) of these intracellular forms of SHBG remain to be established, but recent studies indicate that intracellular SHBG may influence the access of sex-steroids to the respective receptors and the response of sex steroid-target genes. In addition, immunoreactive SHBG has been located in the extra-vascular compartments of some tissues, where it has been reported to interact with proteins in the cell membrane, which are thought to act as endocytotic receptors, such as megalin; signaling receptors which remain to be identified; or proteins in the extra-cellular matrix, such as fibulin, with which SHBG interacts in a ligand dependent manner. These novel activities and functions of SHBG will be reviewed because they provide evidence that SHBG has activities beyond that of a simple plasma steroid transport protein.

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## S26.2

### The role of IGFbps in the tissue-specific actions of IGFs

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The IGFs are present ubiquitously at very high levels throughout the body and affect virtually every aspect of cell function. These high levels are maintained due to their association with 6 distinct binding proteins (IGFBP-1 to -6) which specifically bind IGFs (but not insulin). The IGFs are not stored within any cells but are stored in the circulation due to their binding to IGFBP-3 which then results in its association with a large glycoprotein, the acid labile subunit (ALS). This large ternary complex has limited ability to cross the capillary endothelium dramatically slowing clearance and maintaining a large circulating reservoir of IGFs which can then be made available to tissues when required by the action of specific proteases. In the tissues the 6 IGFBPs bind the IGFs with affinity greater than that of their cell surface receptors enabling them to tightly control tissue activity. Each IGFBP then has specific proteases which cleaves the IGFBP lowering the affinity with which IGFs are bound and increasing their availability to receptors. The IGFBPs can also associate with other specific proteins and proteoglycans in the matrix and on cell surfaces enabling them to concentrate IGFs in the pericellular environment and enhance IGF actions or reduce down-regulation of cell IGF receptors. Each IGFBP can also affect cell functions independently of the IGF-receptors by interacting with specific cellular proteins and modulating other signalling pathways, such as that of TGF- $\beta$  and EGF. The IGFBPs, their proteases and their other interacting proteins are present in different tissues in various combinations and are all differentially regulated. The IGFBPs can therefore confer considerable specificity on the ubiquitously present IGFs: enabling tissue-specific actions according to the local context.

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## S26.3

### The role of TTR in tissue delivery of thyroid hormone

M J Saraiva

Abstract unavailable.

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## Breaking news in adrenal malignancy

### S27.1

#### European Network for the Study of Adrenal Tumors: Adrenal Cancer Consortium (ENS@T-CANCER)

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Among patients with adrenal masses adrenocortical carcinoma (ACC) and malignant pheochromocytomas (MPH) are found with a low incidence but very unfavourable prognosis. Owing to this poor clinical outcome, concomitant hormone dysregulation and limited treatment options the two cancer entities severely impact on affected patients. However, the rarity of the tumors also impedes clinical studies which are affected by fragmentation and low cohort sizes. The European Network for the Study of Adrenal Tumors (ENS@T) has recently implemented a collection of adrenal tumor related databases and defined an associated network of Biological Resource Centers devoted to research on adrenal tumors. The concurrence of recent achievements of this evolving network, the progress in the understanding of molecular mechanisms and increasing availability of specific diagnostic and therapeutic tools for adrenal cancers

provides the unique opportunity to achieve unmatched progress in the implementation of both translation and clinical research dedicated to ACC and MPH. Specifically, the newly formed ENS@T-CANCER consortium will address the following topics: i) structuring European clinical and translational research through implementation of a virtual research environment, ii) improving clinical outcome of patients with adrenal cancer by conducting interventional trials carried out by European centers of excellence, iii) improvement of differential diagnosis and risk stratification of adrenal cancer, iv) identification and validation of tools for follow-up of patients with adrenal cancer, v) identification of novel biomarkers for treatment response.

The ultimate aim of the ENS@T-CANCER Consortium is to develop research in the field of adrenal cancers to improve diagnosis and treatment abilities. The Network will allow recruiting sufficient patients in all relevant European centers, to harmonize diagnosis criteria and to use the various technological approaches of a number of laboratories.

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## S27.2

### Transcriptome analysis and microRNAs in adrenal tumours

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The analysis of mRNA expression (transcriptome) and microRNAs represent powerful tools for elucidating the pathogenesis of tumours, establishment of biomarkers and identifying possible drug targets. Several studies have been performed to date on the mRNA expression profiles of adrenocortical tumours. Some studies on the transcriptome of pheochromocytomas and the microRNA expression patterns in adrenocortical and adrenomedullary tumours have also been recently reported. Transcriptome analysis in adrenocortical tumours has revealed markers useful for the differentiation of benign and malignant tumours (e.g. IGF2, topoisomerase 2A), markers for disease-free or total survival (DLG7, PINK1, BUB1) and several gene alterations relevant for tumourigenesis. In a recent meta-analysis of transcriptome and comparative genome hybridization studies including our own data, we have established three major pathways of adrenocortical cancer pathogenesis: i) damage of cell cycle, ii) retinoic acid signalling, iii) immune-complement changes. We have also established the damage of cell cycle as a pathway affected by microRNAs in adrenocortical cancer. Significantly differentially expressed microRNAs have been found both among normal adrenal cortex, benign and malignant adrenocortical tumours (miR-503, miR-511, miR-195 etc.), and in various sporadic benign, hereditary benign, sporadic recurrent (miR-1225-3p) and malignant pheochromocytomas (miR-483-5p, miR-15a, miR-16 etc.). As the histological diagnosis of malignancy is difficult for adrenocortical tumours, but it is not feasible in pheochromocytomas at all, these microRNA expression profiles may have major clinical relevance. Bioinformatic analysis of the potential mRNA targets of microRNAs has revealed the possible involvement of Notch-signaling in the pathogenesis of pheochromocytoma recurrence. Despite these major achievements, there are many unclear issues yet and there are major differences in the studies reported that warrant further examinations.

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## S27.3

### Management of adrenocortical carcinoma: what have we learnt from recent trials?

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Adrenocortical carcinoma (ACC) is a rare malignancy and most of the therapeutic strategies are not yet prospectively validated. However, several studies have been published in 2010 and preliminary results from two prospective trials are available now.

Open adrenalectomy is treatment of choice for resectable tumors. However, two recent retrospective studies provided evidence that a laparoscopic approach might be also a safe alternative in selected cases (especially in tumors preoperatively of uncertain malignancy). Based on a study by Terzolo *et al.* (*NEJM* 2007), adjuvant mitotane treatment is recommended for the majority of patients. However, prospective data suggest that the outcome in patients with stage II ACC is better as assumed in the past. Therefore, prospective trials like the currently running randomized ADIUVO trial on adjuvant mitotane are urgently needed.

In advanced ACC, the first ever randomised trial (FIRM-ACT study) will set the new standard in first-line cytotoxic therapy. Preliminary results showed a clear trend for improved overall survival by the combination of etoposide, doxorubicin, cisplatin, and mitotane (EDP-M) in comparison to streptocotizin and mitotane (14.8 vs 12.0 months;  $P=0.069$ ) and progression-free survival was clearly prolonged by EDP-M (5.7 vs 2.1 months;  $P<0.0001$ ). The first experience using targeted therapies is disappointing: gefitinib, sorafenib, erlotinib + gemcitabine, or bevacizumab + capecitabine exhibited only limited efficacy in pretreated patients. An interesting observation was found in the SIRAC study investigating sunitinib in refractory ACC. The preliminary analysis indicated that in 5 of 35 evaluable patients (15.3%) a prolonged stable disease for > 12 weeks was seen. Of note, concomitant mitotane did rather impair the clinical outcome, which might be related to an induction of CYP3A4 enzyme. Another important study investigating the efficacy of the IGF1 receptor inhibitor OSI-906 in a placebo controlled phase III trial, is currently still recruiting. Overall the prognosis of ACC is still poor and 5-year survival rate is about 45%. However, recent collaborative efforts (e.g. by the European adrenal network ENSAT) and ongoing international trials will further advance the field in the near future.

## Polycystic ovary syndrome: classification, genetics and therapy

### S28.1

#### Phenotypes and environmental factors in PCOS

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PCOS is a complex endocrine syndrome characterized by the combination of reproductive aberrations, namely hyperandrogenism and chronic anovulation, with metabolic defects. Insulin resistance is a major component in metabolic as well as reproductive aspects of PCOS. The pathogenesis of the syndrome remains under investigation. However, environmental factors appear to unmask a genetically determined susceptibility to PCOS and also determine the specific phenotypic expression of the syndrome. Particularly, environmental triggers acting during early stages of human development, from prenatal life to puberty, may convert a predisposed genotype to the phenotypical manifestation of PCOS. In addition, environmental determinants may modulate the clinical severity of PCOS, ranging from a mild phenotype (nonhyperandrogenic anovulatory, as determined by Rotterdam criteria<sup>1</sup>, or normoovulatory hyperandrogenic, as determined by Rotterdam<sup>1</sup> and Androgen Excess Society criteria<sup>2</sup>) to the full-blown phenotype of classic PCOS.

The environmental component in the pathogenesis of PCOS includes dietary factors and other exogenously derived substances. Overnutrition with consumption of calorie-rich diets leads to obesity which may accelerate the development or aggravate the clinical course of PCOS<sup>3-5</sup>. Beyond calorie excess, the high intake of dietary advanced glycation end products (AGEs) may also contribute to the pathogenesis and perpetuation of PCOS. In particular, increased AGEs levels were reported in lean, normoglycemic and non-insulin resistant women with PCOS<sup>6</sup> and AGEs were found to accumulate in human polycystic ovaries<sup>7</sup>. These bioactive molecules may exert actions on both ovarian compartments, since circulating AGEs levels were positively correlated with serum levels of testosterone<sup>6</sup> and Antimüllerian hormone (AMH)<sup>8</sup>, a theca cell and a granulosa cell product respectively.

Bisphenol A (BPA) emerges as another environmental contributor to the pathogenesis of PCOS. BPA belongs to endocrine-disrupting chemicals, which are substances in our environment, food, and consumer products that interfere with hormone biosynthesis, metabolism, or action resulting in a deviation from normal homeostasis or reproduction. Most importantly, data from experimental animals have demonstrated that neonatal exposure to BPA leads to PCOS development<sup>9</sup>. Extending these findings to humans, a recent study has shown that serum BPA levels are increased in lean and obese PCOS women compared to age and BMI matched controls<sup>10</sup>. Moreover, serum BPA levels were positively associated with serum androgen levels and insulin resistance indices<sup>10</sup>. The latter finding suggests a role of BPA in androgen synthesis in addition to its potential contribution to insulin resistance in PCOS.

Overall, PCOS may arise from a combination of genetic predisposition and environmental insults that lead to failure of reproductive and metabolic functions. Environmental exposure may start *in utero* and persist during early life until adolescence, when PCOS becomes clinically evident. Unravelling the specific environmental components of the pathogenesis of PCOS may help to elucidate the etiology and the ideal treatment of the syndrome.

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### S28.2

#### Genetics

S Franks

Abstract unavailable.

### S28.3

#### PCOS: treatment of infertility and prediction of success

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Polycystic ovary syndrome is a complex genetic condition, diagnosed based on oligo/anovulation, hyperandrogenemia and polycystic ovaries. Moreover, these women frequently present with obesity, insulin resistance and other signs of metabolic disease. In a gynaecology practice, the great majority of PCOS women present with anovulatory infertility. In fact, it is often suggested that around 20–30% of all infertility is due to PCOS. The medical treatment aiming to restore normo-ovulatory cycles in anovulatory women should be referred to as 'ovulation induction'. This approach is under increasing pressure in relation to the widespread use of assisted reproduction (ovarian hyperstimulation and intra-uterine insemination, or IVF) or surgical procedures such as laparoscopic ovarian cauterization.

Recently, an ESHRE/ASRM sponsored consensus workshop on ovulation induction strategies in PCOS was organised in Thessaloniki, Greece (published in both Human Reproduction and Fertility Sterility in 2008). In brief, life style and diet changes should be recommended and actively encouraged to all obese PCOS women. Clomiphene citrate remains the first line drug of choice, despite relatively low efficacy. Several large, multi-center, comparative trials showed limited efficacy of insulin sensitising agents such as metformin for ovulation induction. Despite initial positive experience with aromatase inhibitors, well powered randomized trials need to show its effectiveness and safety before this

compound can be recommended for large scale clinical use. As second line treatment both exogenous gonadotrophins or ovarian cautery can be applied depending on patient (and doctor) preference. The major shortcoming of even low-dose, step-up gonadotrophin protocols remains increased chances for multiple gestation. The drawback of the surgical approach is that surgery is needed and the relatively low efficacy, requiring additional ovulation inducing drugs is many women. However, multiple pregnancies following ovarian cautery are negligible. There is no need for IUI in addition to ovulation induction provided that sperm quality is normal.

IVF should generally be considered as third line treatment after failed ovulation induction. Ovarian hyperstimulation for IVF remains a challenge in PCOS patients, with a distinct tendency to over-respond. Overall, success rates are quite

good, with the major benefit that multiple pregnancy can be prevented by the transfer of just a single embryo. Therefore, IVF may be considered an earlier treatment option in women of more advanced reproductive age.

Overall, ovulation induction treatment is effective and singleton live birth rates of over 70% have been reported. We have worked extensively on assessing possible predictors of treatment outcomes based on initial screening characteristics. Using multi-variate prediction models we could show that factors such as body mass index, high androgen levels, female age, and insulin resistance are fairly good predictors of both ovulation and pregnancy chances. Such approaches may help to render ovulation induction more patient tailored, helping to choose the best treatment option and individualize dosing.

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# JOE/JME Prize Presentation

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**The central nervous system and the control of adipocyte and hepatic metabolism**

**JP1**

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**The central nervous system and the control of adipocyte and hepatic metabolism**

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The central nervous system (CNS) is crucial in the regulation of energy homeostasis. Apart from endocrine signaling and nutrient sensing, there is an important neuronal network that connects the CNS with peripheral metabolic processes. Many neuroanatomical studies have shown that the white adipose tissue (WAT) and the liver are innervated by the autonomic nervous system.

For instance, there is evidence that, as the levels of various peripheral signals change, specific neuronal circuits within the CNS respond by adjusting ongoing autonomic nervous system activity to a wide spectrum of organs.

During the last years, several reports have demonstrated that signals from the CNS directly control the amount of fat by modulating the storage or oxidation of fatty acids in WAT and liver. Importantly, some CNS pathways regulate these processes independent of food intake, suggesting that those signals possess alternative mechanisms to regulate energy homeostasis. Different neuronal circuits within the hypothalamus, such as leptin- ghrelin- insulin- GLP1- and resistin-responsive neurons, as well as melanocortins, neuropeptide Y exert their direct actions on lipid metabolism in peripheral tissues such as WAT or liver. Contrary, other signals including the cannabinoid system, seem to play more important functions at peripheral level. Dissecting the complicated interactions between peripheral signals and neuronal circuits regulating lipid metabolism might open new avenues for the development of new therapies preventing and treating obesity and its associated disorders.

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# Meet the Expert

## MTE1

### Pros and cons of bariatric surgery

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Bariatric surgery has become the most effective therapeutic option for morbid obesity (BMI > 40 or > 35 with major complications). All established surgical techniques are followed by significant body weight reduction as well as clear improvement/remission of main obesity-associated comorbidities such as type 2 diabetes (DM2), dyslipidaemia, hypertension, sleep apnoea and cardiac dysfunction. Mixed restrictive-malabsorptive techniques, such as Roux-en-Y-gastric bypass (RYGB), induce more weight loss, better metabolic improvement and make postoperative weight regain (WR) less likely than restrictive procedures such as vertical gastropasty or gastric banding (GB). Therefore, individualization and a complete preoperative assessment by a multidisciplinary team are needed in order to tailor bariatric surgery to patient characteristics. More recently, BS has shown to induce DM2 remission in a significant percentage of patients, which vary depending on the technique (GB; RYGB; BPD/DS). Short DM2 duration, low needs of antidiabetic medications and good metabolic control seems to be preoperative predictive factors of success. Besides acute postoperative complications, malabsorptive BS, especially biliopancreatic diversion but also RYGB, can cause mid and long-term vitamin and nutritional deficiencies, which should be prevented by providing routine iron, vitamin B12, calcium, vitamin D, and folic acid supplementation. Patients operated of BS should be strictly followed in order to prevent nutritional complications, but also to avoid WR, which can start as soon as two years following surgery leading to metabolic deterioration and partial reversal of achieved benefits. Nutritional as well as lifestyle and perhaps hormonal factors may contribute to WR.

The effect of BS on DM2 remission has set the basis of proposing different surgical techniques in patients with BMI lower than 35, or even without obesity. Although the initial experimental results are encouraging, we need more experience before establishing the involved mechanisms and the possible role of this surgical approach in DM2 management.

## MTE2

### Primary hyperaldosteronism

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It is now widely recognized that primary aldosteronism (PA) is much more common than previously thought, accounting for up to 5–10% of hypertensives with most patients normokalemic, and that aldosterone excess has adverse cardiovascular consequences that go above and beyond hypertension development. These findings have supported the concept that PA plays an important role in cardiovascular disease states and should be systematically sought and specifically treated. In recognition of this, the US Endocrine Society published in 2008 a clinical guideline for the case detection, diagnosis and management of this common, specifically treatable and potentially curable condition. Areas of recent, topical research include i) the demonstration of excess morbidity (including cardiovascular and renal) in patients with PA compared with other forms of hypertension and reduced quality of life, confirming the clinical relevance of non-blood pressure-dependent adverse effects of aldosterone excess; ii) the further demonstration that this excess morbidity is ameliorated with specific (but not non-specific antihypertensive) therapy directed against aldosterone excess, confirming the importance of detection and diagnosis of PA to enable optimal specific management; iii) an ongoing appraisal and refinement of diagnostic approaches including the recent demonstration that treatment with beta-blockers, female gender and the luteal phase of the menstrual cycle are associated with higher aldosterone/renin ratios and hence a greater risk of false positive results whereas selective serotonin reuptake inhibitors can lower the ratio and thus may increase the risk of false negatives; iv) the importance of assay reliability and the development of new, rapid-throughput, highly reliable and reproducible mass spectrometric methods of measuring aldosterone; and v) further insights into the importance and nature of causative genetic factors, the identification of which is being sought through high-throughput next generation sequencing technologies.

## MTE3

### Management difficulties in recurrences and metastases of thyroid cancer

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Patients with thyroid carcinoma are usually considered as having a good prognosis although the disease may take an aggressive course. Indeed, 5–27% of patients develop locoregional recurrences located in cervical lymph nodes (60–75%), thyroid bed (20%) and trachea or muscle (5%), thus worsening the prognosis. Distant metastases develop in 10% of patients and are located mainly in the bones and the lungs but also in the liver leading to a risk of cancer-related death.

Firstly, the most difficult complication for the clinician to manage is radioiodine refractory thyroid cancer particularly when it is progressive or advanced with distant metastases that remain difficult to treat. Local treatments, such as surgery, radiofrequency, embolisation, cement injection or external radiation therapies can be beneficial in selected patients. Cytotoxic chemotherapy with partial responses has been reported. Recently therapies that inhibit angiogenic signaling pathways have emerged as potentially effective treatment options and are reviewed in this 'meet the expert' session. However, not all patients respond well to these treatments.

Secondly, recurrences of thyroid cancer can be difficult to manage because of their small size and/or very slow development. Detected with highly sensitive tools during follow up, small recurrences raise questions regarding the benefits (versus secondary effects) of treatments and highlight the need for preserving good quality of life among patients in whom the disease can remain stable for many years. Alternative treatments for small recurrences are discussed. Very slow progression of recurrences also raises questions regarding both the frequency of follow-up and imaging examinations and the relevance of RECIST criteria in such patients with slowly progressing recurrences.

## MTE4

### Mass spectrometry in the measurement of hormones

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Endocrinology came to its third revolution in the field of hormone determination. As radioimmunoassays in the 60s paved the way to the knowledge of modern endocrinology, and as automated immunoassays in the 70s linked analytical measurement to the clinical practice, nowadays liquid chromatography – tandem mass spectrometry (LC–MS/MS) represents the evolution of the high-throughput approach, typical of automated platforms, toward the high reliability and specificity of the measurement, typical of gas chromatography (GC)–MS, and toward the power of simultaneous profiling of wide panels of hormones working on a small sample volume and with minimal pretreatment of the sample. First introduced for therapeutic drug monitoring and for neonatal screening of inborn errors, LC–MS/MS is breaking into clinical and research laboratories for steroids determination, as proved by a growing literature. Comparison studies between immunoassays and LC- or GC–MS methods, attested with analytical proof what clinicians had already perceived about the lack of specificity and the derived inconsistent results affecting immunoassays. The earlier and more targeted steroid, for the urgency of the problem in the clinical practice, was testosterone, followed by androgens, 17OHprogesterone, cortisol and 25hydroxyvitamin D3. Estrogens and aldosterone are also attracting steroids, but they require last generation devices or additional steps in the sample preparation. Furthermore, LC–MS/MS allows determination of other steroids, like dihydrotestosterone, 21- and 11deoxycortisol, corticosterone, deoxycorticosterone, essential for paediatric deficit diagnosis. The introduction of LC–MS/MS in routine requires specific site requirements and highly experienced personnel. Furthermore, it needs medium-high initial costs for the instrument installation and maintenance, but they can be rapidly amortized thanks to the low cost-per-sample. An international effort should now be made in order to standardize new LC–MS/MS methods and to produce new shared reference intervals, toward the complete replacement of immunoassays.

## MTE5

Abstract unavailable.

**MTE6****Difficult adrenal cases**

W Young

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**Objective**

To present 2 difficult adrenal cases in an interactive computer-based CPC format.

**Methods**

The history of present illness will be presented for 2 patients: i) A 24-year-old woman who is 13 weeks pregnant presenting with spells and marked hypertension; ii) A 56-year-old man with hypertension, hypokalemia, and renal insufficiency. For the details of these cases, please see the Meet-the-expert handbook.

**Results**

The attendees will use diagnostic and treatment test menus to solve the cases and direct appropriate treatment.

**Conclusions**

As the cases are discussed we will review many of the practical issues surrounding the evaluation and treatment of pheochromocytoma and primary aldosteronism.

**MTE7****When to instigate insulin therapy in DM2**

Jan Skrha

University of Prague, Prague, Czech Republic.

The main pathophysiological feature of type 2 diabetes mellitus is based on combination of impaired  $\beta$ -cell insulin secretion and insulin action in peripheral tissues. However, as progressive loss of the  $\beta$ -cells develops as a consequence of apoptosis, the insulin supply is later necessary. This picture is completely different in type 1 diabetes when insulin insufficiency is developed early in the disease and exogenous insulin is exaggerated to maintain metabolic homeostasis and patient's life as well.

The key question can be postulated: what is the proper time for insulin treatment in type 2 diabetic patient? The replies are different between specialists and frequently depend on their experience with the treatment of type 2 diabetic patients. It could be therefore important if there would exist any 'evidence based' data supporting the needs for insulin treatment in earlier or later time during the disease. Insulin role in the  $\beta$ -cell apoptosis has been evaluated with a conclusion that there is some evidence on  $\beta$ -cell protection due to insulin administration. From this point of view, this early insulin instigation would be appropriate. The problem will come when significant insulin resistance is developed, typically in obese individuals. Then higher doses of insulin administered may be deleterious for worsening of resistance and further of diabetes control. In those diabetic patients (with increasing obesity) may be used the insulin treatment in much more later stage than in the lean individuals.

Positive and negative influences of early insulin treatment in Type 2 diabetic patients are discussed in the light of individual selected patients for the appropriate therapy. It may be admitted that no single recommendation for each type 2 diabetic patient to introduce early insulin administration can be suggested because of heterogeneity in this population with diabetes.

**MTE8****Pathogenesis and management of primary ovarian failure**Luca Persani<sup>1,2</sup><sup>1</sup>Department of Medical Sciences, University of Milan, Milan, Italy;<sup>2</sup>Division of Endocrinology and Metabolic Diseases and Lab of Experimental Endocrinology, IRCCS Istituto Auxologico Italiano, Milan, Italy.

Primary ovarian failure (POF) is defined as a premature ovarian defect characterized by absent menarche (primary amenorrhea) or secondary amenorrhea lasting at least 6 months associated with a premature depletion of ovarian follicles before the age of 40 years. POF is a frequent disease affecting female fertility and well-being and generating several co-morbidities. Since the onset of POF is frequently silent and fertility defects occur in the occult phases of the disease several groups are working to generate novel tests for POF prediction. The availability of efficient tests would constitute the basis for the preservation of fertility in POF women. This search is however hampered by the present lack of knowledge on POF pathogenesis. The MTE session will focus on the current

knowledge on the pathogenesis of premature ovarian failure and on how genetic and biochemical investigations may be applied for the counselling of POF women.

**MTE9****Pleiotropic effects of vitamin D**

Marija Pfeifer

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Vitamin D is phylogenetically an old compound present in simple organisms without skeleton for millions of years. Its primary function, acting as a cytokine, has been the defence against microbial invaders. Vitamin D modulates the innate immune response by inducing the synthesis of endogenous antibiotics in monocytes-macrophages. Epithelial 'barrier' tissues in lung, gut, placenta and skin employ the same antimicrobial mechanisms. Vitamin D also interacts with dendritic cells and lymphocytes to modulate the acquired immune response. Other important biological functions of vitamin D are the maintenance of muscle strength and size, stimulation of insulin production in  $\beta$ -cells, suppression of renin secretion from kidneys, attenuation of autoimmune inflammatory processes of atherosclerosis in arterial wall. It can decrease cellular proliferation of both normal and cancer cells and induce their differentiation. The more advanced function of vitamin D is that of a hormone, reserved for species with endo-skeleton where it regulates calcium, phosphorus and bone homeostasis.

Vitamin D<sub>3</sub> is metabolized in the liver to 25-OH vitamin D, which levels determine patient's vitamin D status. 25-OH vitamin D is activated in kidneys to 1,25-(OH)<sub>2</sub> vitamin D or hormone D that binds to its nuclear vitamin D receptor and controls more than 200 genes.

There is an epidemic of vitamin D deficiency (25-OH vitamin D less than 50 nmol/l) and insufficiency (25-OH vitamin D less than 75 nmol/l) in general population. Reasons are greater sun protection and increase in population body mass index. Severe vitamin D deficiency causes rickets or osteomalacia. Less severe forms have other serious consequences: increased risk of type I and type II diabetes, multiple sclerosis, rheumatoid arthritis, hypertension, cardiovascular disease, and cancer (colon, breast). Vitamin D insufficiency and deficiency are significantly associated with all-cause and cardiovascular mortality.

Optimal vitamin D levels are 75–100 nmol/l. In the absence of sun exposure 1000 IU of cholecalciferol is required daily or 7000 IU weekly to maintain normal vitamin D levels. To achieve normal levels, 2000 IU daily are needed for a month or two. Vitamin D replacement is cheap and safe.

**MTE10****Biomarkers of thyroid malignancy**

Manuel Sobrinho-Simões

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This topic will be discussed taking into consideration the need to incorporate the continuously increasing molecular data in the old frame (imaging, cyto and histopathology and biochemistry). Using this strategy three practical issues may be addressed: diagnosis, prognosis/therapy selection and surveillance.

Out of the above mentioned issues we will focus the discussion of the usefulness of molecular markers in two steps of the management of patients with thyroid tumours:

i) In the initial evaluation of thyroid nodules together with sonography and cytopathology data; the latter obtained by fine needle aspiration biopsy (FNAB) since core biopsy methods have not proved (yet?) their value in thyroid oncology. ii) In the definitive characterization of the tumours after surgery, in conjunction with all the available clinical, imagiological and laboratorial data and with the huge amount of information that can be obtained by the thorough histopathological study of the surgical specimens.

When discussing these two 'steps' one has to be aware of the relatively few possible options from the therapeutic standpoint. Oversimplifying the issues (I am pathologist...) and avoiding the problems raised by poorly differentiated/undifferentiated (anaplastic) carcinomas, I would summarize the critical questions as follows: i) To operate or not? ii) If yes, what is the adequate extension of the thyroidectomy? iii) What to do about regional lymph nodes? iv) Radioactive iodine: Yes or no? v) What to do in radioactive iodine-refractory tumours? vi) When and how to use the new targeted biologic approach therapies?

In case there is time, we will also discuss the role molecular biomarkers may play in the new therapeutic approaches. Besides addressing the MAPK pathway (and

its networks), oncologists will also try to target the blood supply using antiangiogenesis drugs and/or HIF1 $\alpha$  upregulation, as well as to counterbalance the metabolic shift of neoplastic cells towards glycolysis. Most likely, oncologists will try to apply to thyroid cancer the new strategies that the studies of epigenetics and microRNA are generating in the oncology field.

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### MTE11

#### **Prolactin and breast tumorigenesis: a novel concept of prolactin biology**

Ph Touraine<sup>1,2</sup> & V Goffin<sup>2</sup>

<sup>1</sup>GH Pitie Salpetriere, Paris, France; <sup>2</sup>Inserm U845, Paris, France.

Prolactin was discovered 80 years ago. It is currently viewed by clinical endocrinologists as a hormone of pituitary origin, whose production is negatively controlled by dopamine, whose biological actions relate exclusively to lactation and reproductive functions, for which any genetic disorder is yet to be identified, and whose unique associated pathology is hyperprolactinemia, which is efficiently cured using dopamine agonists. Although there is no debate about the relevance of these features, experimental and clinical studies performed during the past decade have considerably widened our perception of prolactin biology: i) in addition to the endocrine (circulating) hormone, locally-produced prolactin has been documented in various human tissues such as the mammary gland and the prostate, where it acts via autocrine/paracrine mechanisms, ii) there is increasing evidence supporting the role of prolactin in human breast and prostate tumorigenesis, especially when it is locally-produced, iii) we recently reported the first gain-of-function variant of the prolactin receptor, that was to date identified in patients presenting with breast tumors (benign or cancer), iv) we have engineered human prolactin variants acting as pure competitive antagonists of the prolactin receptor, and we have demonstrated their ability to down-regulate the actions triggered by local prolactin as well as by the constitutively active receptor variant in experimental models; these compounds thus represent a novel class of molecules of therapeutic interest as potential alternative to dopamine agonists. These novel aspects of prolactin biology will be presented, with major focus on experimental models highlighting its effects on tumor growth.

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### MTE12

#### **Treatment strategies for obesity**

Dragan Micc

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Overweight and obesity have become an increasingly serious clinical and socioeconomic problem worldwide. Recent systematic analysis of health examination surveys demonstrated that in 2008 an estimated 1.46 billion adults worldwide had BMI of 25 kg/m<sup>2</sup> or greater, of these 205 million men and 297 million women were obese. Overweight and obese patients are at increased risk for developing various co-morbidities (type 2 diabetes, hypertension, dyslipidemia, cardiovascular disease, obstructive sleep apnea, hepatobiliary disease and certain cancers). The goals of treatment strategies for overweight and obesity are to reduce body weight and to maintain lower body weight over the long term. Patient should understand that, since obesity is a chronic disease, weight management will need to be lifelong. In some patients, especially in those with overweight, prevention of further weight gain rather than weight loss per se may be appropriate target. Specific components of treatment for obesity are: diet, cognitive behavioural approaches, physical activity, psychological support, pharmacological treatment and surgery. Current drug therapy is recommended for patients with a BMI  $\geq 30.0$  kg/m<sup>2</sup> or a BMI  $\geq 27$  with an obesity-related disease (such as hypertension, type 2 diabetes). The major groups of anti-obesity drugs are: centrally acting medications that impair dietary intake; medications that act peripherally to impair dietary absorption, and medications that increase energy expenditure. Currently, Orlistat is the only FDA-approved antiobesity drug on the market. Surgery should be considered for patients from 18–60 years of age with BMI  $\geq 40.0$  or with BMI between 35.0 and 39.9 kg/m<sup>2</sup> and co-morbidities in whom surgically induced weight loss is expected to improve the disorder (type 2 diabetes, cardiorespiratory disease, severe joint disease and obesity-related severe psychological problems) (according EASO European Clinical Practice Guidelines for Management of Obesity).

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### MTE13

#### **Hyponatraemia**

Chris Thompson

Academic Department of Endocrinology, Beaumont Hospital/RCSI Medical School, Dublin, Ireland.

Hyponatraemia is the commonest electrolyte abnormality in hospitalised patients, with a prevalence of 20–30% and particularly high incidence in neurosurgical, pulmonary, geriatric, intensive care and oncology units. Up to 6% of patients have severe hyponatraemia (plasma sodium <125 mmol/l). A wealth of data is available which indicates that severe hyponatraemia is associated with cerebral irritation, causing confusion, coma and seizures, and significant excess mortality. The rapidity of onset of hyponatraemia is equally important in determining the likelihood of cerebral symptoms and death. Recent papers have reported that in addition there is excess mortality in patients with mild/moderate hyponatraemia (125–135 mmol/l). In addition even mild hyponatraemia is associated with significant morbidity, including gait disturbances and falls, cognitive deficits, bone fractures and osteoporosis. The commonest cause of hyponatraemia is SIADH; it is important to distinguish SIADH from other forms of hyponatraemia, particularly hypovolaemic hyponatraemia, for instance diuretic-induced. Glucocorticoid deficiency also biochemically mimics SIADH and it is particularly important to diagnose in patients with neurosurgical conditions, in whom the cerebral insult can produce both SIADH and acute ACTH deficiency. Accurate diagnosis is essential to ensure that the correct treatment of hypoglycaemia is instituted, both for the underlying cause and the biochemical disturbance. SIADH is traditionally treated with water restriction, which patients find difficult to maintain, and which has unpredictable efficacy. Demeclocycline, the first line pharmaceutical agent, also has unpredictable action, and has side effects, such as hypernatraemia, liver and renal damage, and photosensitive skin rashes. A European licence was granted in 2009 for tolvaptan, the specific vasopressin V2 receptor antagonist, whose efficacy in treating hyponatraemia was demonstrated in the SALT-1 and SALT-2 trials. The potential use of V2 receptor antagonists will be discussed.

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### MTE14

#### **Graves' ophthalmopathy (GO)**

Wilmar Wiersinga

AMC, Amsterdam, The Netherlands.

#### **Immunopathogenesis**

Orbital fibroblasts are recognized as the prime target cells of the autoimmune attack. There is good circumstantial evidence that autoimmunity against the TSH receptor is primarily involved, but a suitable animal model of GO is lacking. Autoimmunity against the IGF-1 receptor is nonspecific for Graves' disease, but might contribute to the immune response in the orbit.

#### **Diagnosis**

Diagnosis of GO is based on: i) assessment of eye changes according to severity (lid aperture in mm, soft tissue changes, proptosis in mm, eye muscle motility, diplopia, corneal lesions, sight loss) and activity (clinical activity score); ii) Thyroid function tests (about 10% of GO patients is euthyroid, but most of them have detectable serum TSH receptor antibodies); iii) Exclusion of an alternative diagnosis (by orbital CT or MRI in unilateral or euthyroid cases).

#### **Management**

Management of GO is based on: i) To stop smoking. Smoking is associated with less favourable outcome of immunosuppression. ii) To restore and maintain euthyroidism. 131I therapy is associated with a risk on developing or worsening of GO, which can be largely prevented by a course of steroids (optimal dosage and duration are not well defined). Preventive steroids should be considered in high-risk patients (smokers, severe hyperthyroidism, active GO, or high TBII). Total thyroidectomy can be considered, as well as long-term treatment with antithyroid drugs (preferably as block-and-replace regimen). iii) To restore appearance and visual functions. In dysthyroid optic neuropathy urgent treatment is required (steroids, decompression). In moderately severe and active GO iv pulses of methylprednisolone are more efficacious and better tolerated than oral prednisone. Oral prednisone + cyclosporine or retrobulbar irradiation are useful in recurrences, and rituximab in resistant cases. In mild GO treatment with selenium has proven to be beneficial. In inactive cases rehabilitative surgery can be done.

**MTE15****Cushing's**

Susan Webb

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Cushing syndrome (CS) is associated with systemic complications, like central obesity, 'moon' face, limb atrophy, increased fat mass and reduced bone and lean body mass, excessive fatigue, purple striae, easy bruising and skin ulceration, hirsutism, hypogonadism and decreased libido. Morbidity like hypertension, insulin resistance and/or diabetes mellitus, dyslipidemia, prothrombotic state, vascular disease, atherosclerosis and increased cardiovascular risk, acquired metabolic syndrome, depression, loss of brain volume, cognitive decline and impaired health-related quality of life -HRQoL- may also occur. Mortality is higher than in age- and gender-matched subjects, due to complications directly and/or indirectly correlated with GC excess. Altogether, despite being a rare disease, difficulties in early diagnosis and residual morbidity represent a significant burden for the patients and for the health system.

Biochemical 'cure' of hypercortisolism is not followed by normalization of morbidity and mortality, which are still increased. Cardiovascular and metabolic risk, central fat and an unfavorable adipokine profile persist. Bone mass is reduced not only due to endogenous hypercortisolism, but also to duration and dose of exogenous glucocorticoid (GC) replacement therapy. Thus, therapy in operated patients with inhibition of the hypothalamic-pituitary-adrenal axis should be reduced to the lowest dose and minimum duration possible. Specific treatments should be considered in patients with decreased bone mass, aimed at reducing increased fracture incidence. Cognitive and health related quality of life impairments, described in active disease, are still present after endocrine remission. Thus, residual morbidity persists in cured CS, suggesting irreversibility of GC-induced phenomena typical of chronic hypercortisolism. Thus, the primary goal in the prevention and treatment of complications of Cushing's syndrome is correction of hypercortisolism as soon as possible. Supported by FIS080302 and ERCUSYN 800200.

**MTE16****Endocrine care in HIV-infected patients**

Vincenzo Rochira

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Several co-morbidities are associated with the HIV infection involving also the endocrine-metabolic system. Consistently, the recent development of HAART therapy ruled out a significant improvement both in the prognosis and life expectancy of HIV-infected patients, but disclosed also new serious drug-related toxicity. Of these, the lipodystrophy syndrome is the most frequent, occurring in up to 83% of HIV-infected patients under HAART. HIV-related lipodystrophy is associated with metabolic alterations consisting with severe dyslipidemia, insulin resistance, hyperinsulinism, impaired glucose metabolism, and diabetes mellitus. Among endocrine diseases, clinically relevant bone loss and male testosterone deficiency are very frequent. Depletion of bone mineral density accounts for both osteopenia and osteoporosis that are surprisingly observed more often in men with HIV infection. The finding of low circulating testosterone is observed in about 20% of HIV-infected men under HAART and seems to be more frequently associated with inappropriate low serum LH, suggesting a dysfunction of the hypothalamic-pituitary unit. As alterations in GH dynamics are also common in HIV-infected patients, it is possible to hypothesize that the hypothalamus-pituitary unit is affected in the setting of HIV, but if a true pituitary disorder or only a functional reduction of pituitary secretory pattern are involved in these hormonal alterations remains to be determined. The pituitary impairment has been mainly ascribed to the hormonal and metabolic inhibitory effect due to adipose tissue redistribution in these patients, but the impact of both HIV per se and medications on pituitary function has not studied in detail. The prevalence of thyroid diseases is similar to that observed in the general population. Owing to the complexity and the coexistence of more than one metabolic and hormonal abnormalities in a single HIV patient, in clinical practice, a multidisciplinary approach involving also the endocrinologist represents the best choice for managing HIV patients.

**MTE17****Management of pheochromocytomas and paragangliomas**

Pierre-Francois Plouin

Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, Paris, France.

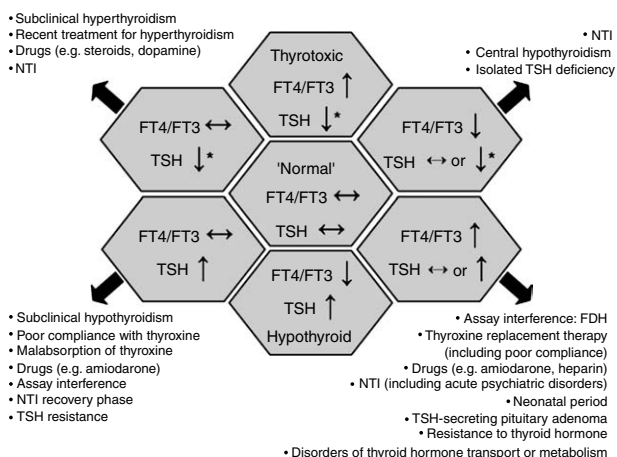
Pheochromocytomas (PH) and functional paragangliomas (PGL) are neoplasms of chromaffin tissue that synthesize catecholamines. The diagnosis of PH or functional PGL is based on the determination of metanephrines. Most catecholamine-secreting tumors arise in the adrenal glands (PH proper) and are easily detected by computed tomography or magnetic resonance imaging. Patients may also harbor extraadrenal primary tumors (PGL) or distant metastases. Adrenal imaging should therefore be combined with whole-body metaiodobenzylguanidine scintigraphy. One in four patients with PH or PGL has germline mutations conferring a predisposition to catecholamine-secreting tumors. The identification of a causative mutation should lead to presymptomatic genetic testing in the family. Patients with catecholamine-secreting tumors should be referred to centers with extensive experience in the anesthetic and surgical management of the disease. Blood pressure should be normalized before surgery, using alpha-adrenergic and possibly beta-adrenergic antagonists. Most PH and many PGL can be resected laparoscopically. Adrenal cortex-sparing surgery is feasible in patients with bilateral PH. PH and PGL may recur. Patients should be subject to life-long follow-up, with check-ups at least one yearly including blood pressure measurement and metanephrine determination.

**MTE18****Weird thyroid function tests**

Mark Gurnell &amp; Krish Chatterjee

Metabolic Research Laboratories, Addenbrooke's Hospital, Cambridge, UK.

Although overt thyroid disease is easily recognised, lesser disturbances of thyroid function can prove difficult to diagnose and be mimicked by other endocrine and non-endocrine disorders. Establishing the correct diagnosis depends on accurate interpretation of thyroid function tests (TFTs), which is usually straightforward; however, in a minority of situations, the results of FT4, FT3 and TSH measurement either do not 'fit' with the clinical picture and/or appear discordant with each other (so-called 'funny' or 'weird' TFTs) (Figure). In many such cases reassessment of the clinical context provides an explanation for the discrepant TFTs; in other instances, interference in one or other laboratory assays can be shown to account for divergent results; uncommonly, genetic defects in the hypothalamic-pituitary-thyroid axis are associated with anomalous TFTs. Failure to recognize these potential 'pitfalls' can lead to misdiagnosis and inappropriate management.



## MTE19

### Management of primary hyperparathyroidism

Jens Bollerslev

Oslo University Hospital and University of Oslo, Oslo, Norway.

The clinical presentation of primary hyperparathyroidism (PHPT) has changed dramatically after increased accessibility to biochemical auto-analysers and the diagnosis is today often made by change in patients without specific symptoms. Operative treatment is recommended in patient with markedly increased calcium levels or typical symptoms. However, the vast majority of patients in the modern clinic do not present organ related symptoms and their calcium levels are only slightly increased, or even within the upper limit of normal. It has been a matter of debate whether neuro-psychiatric symptoms is a clinical manifestation in mild PHPT and to what extent Quality of Life and cognitive function would improve following operation. Systematic randomized studies on these patients with up to two years observation time have so far not indicated benefit of surgery. Several consensus development conferences have discussed management of mild, borderline PHPT during the last twenty years.

Educational goals

Discuss indications for treatment in PHPT

- Differential diagnoses for PHPT
- Work-up also in the elderly
- Discuss the value of clinical guidelines

Target audience

- Clinicians with interest in internal medicine and endocrinology.

Teaching methods

Based on case presentations, indications for operative treatment will be presented, as will conservative management, also in the elderly. As no final answers are given due to the lack of long term prospective studies, the value of international guidelines will be discussed.

## MTE20

### Glucocorticoid replacement therapy

Kristian Lovas

Section for Endocrinology, Institute of Medicine, University of Bergen, Bergen, Norway.

The glucocorticoid replacement therapy has been virtually unchanged since the invention of cortisone 60 years ago. Studies in patients with primary adrenal insufficiency have indicated increased mortality and unfavourable metabolic effects such as reduced bone mineral density, and evidence has accumulated that these patients suffer from reduced health-related quality of life (HRQoL). Although these endpoints possibly relate to inappropriate glucocorticoid therapy, the impact of glucocorticoid replacement regimens has been little studied. Furthermore, the source of the large interindividual variation in treatment effect is largely unknown and needs to be addressed.

We will discuss how to evaluate the patient with adrenal insufficiency and a practical approach to the treatment. Recent developments in glucocorticoid replacement therapy such as modified-release formulations and subcutaneous hydrocortisone infusion will be presented.

## MTE21

### To whom to offer genetic analysis in patients with pituitary adenoma

Beckers Albert

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Genetic predisposition to pituitary adenomas is increasingly recognized and extends beyond diseases such as MEN1 and Carney complex to familial isolated pituitary adenomas (FIPA). A genetic predisposition occurs in >5% of all pituitary adenoma cases, with MEN1 and FIPA comprising the vast majority of these. The identification of the *AIP* gene as a predisposition gene for pituitary adenomas in 15–25% of FIPA kindreds and in a significant number of young patients with macroadenomas has stimulated much debate about familial screening and early diagnosis. In addition the MEN1-like (MENX or MEN4) caused by germline *CDKN1B* mutations adds to the complexity of testing choices. Clearly a systematic evaluation of familiarity and associated diseases in patients with pituitary adenomas would be helpful for clinical endocrinologists and geneticists alike.

Screening guidelines are already in place for MEN1 and the clinical features and associations of MEN1 related diseases are well established. Pituitary tumors in Carney complex are a less common disease manifestation that occur generally within the setting of more clearly diagnostic extra-pituitary manifestations.

*AIP* mutation-related pituitary adenomas are characterized by an early onset (50% before the age of 18 years) and a large predominance of somatotropinomas (80%) followed by mixed GH/PRL-secreting tumors, prolactinomas, and non-secreting adenomas. These tumors are usually large at diagnosis and appear to be more frequently resistant to pharmacological treatment.

Based on the demonstrated clinical characteristics of *AIP* mutation related pituitary adenoma patients, it is reasonable to systematically assess FIPA kindreds for *AIP* mutations. Similarly, patients with apparently sporadic aggressive adenomas diagnosed at age <30 years is a clinically well-delineated population that is enriched for *AIP* mutation positive cases. Children with pituitary adenomas should be assessed for *AIP* mutations and consideration given to MEN1 testing also. In the case of *AIP* mutations, the risk for unaffected mutation carriers remains unclear. A pragmatic approach would be to offer regular hormonal assessments and an initial MRI; the optimal interval for follow up surveillance remains to be determined.

## MTE22

### Diabetes and the genome: any use for our patients?

A M Wägner<sup>1,2</sup>

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The study of the genetics of diabetes, in its different forms, has produced a huge amount of data only very recently and new information is becoming available as this text is being written. The question, at this time, is: are they any good to our patients?

As the study of the genetics of a disease progresses, one would expect several clinical applications, the most obvious being improved prediction and diagnosis, as well as tailored treatment of the disease. In diabetes, some of these expectations have been met already and more should be met in the future. This discussion will focus on what is currently available and its clinical relevance.

Monogenic diabetes represents the paradigm of clinical applications of genetic information. Although rare as a whole, certain clinical features should make us suspect this diagnosis, with important implications for the patient. This is especially true for neonatal diabetes and beta-cell defects due to mutations in *HNF1A* and *GCK*, where identification may have a huge impact on the choice and effectiveness of treatment. Our main challenge now, as clinicians, is to identify these patients and offer the best treatment available. Non-genetic measurements are being developed to optimise patient selection and genotyping strategy and new whole-genome genotyping methods will soon provide a more efficient diagnosis.

Type 1 diabetes (T1D) is characterised by auto-immune destruction of beta-cells, which leads to the dependence on insulin to survive. Most genes associated with T1D play a relevant role in immune response. *HLA*, the major gene/region, explains about half of the genetic risk for developing the disease, and high-risk *HLA* haplotypes are currently being used to select individuals for diabetes prevention trials.

Type 2 diabetes (T2D) is characterised by a combination of beta-cell failure and insulin resistance, often associated with increasing age, inactivity and obesity. The identification of genetic variants influencing disease predisposition have highlighted the importance of beta-cell failure in the pathogenesis of this disease and will probably have an impact on the design of new treatments and preventive measures in the future. The second main potential application of the genetics of T2D, prediction of disease risk for a given individual, is not ready for clinical application yet, though recent studies show promising results.

## MTE23

### Transgenic animals in endocrinology

Matti Poutanen

University of Turku, Turku, Finland.

Mice have been used as a model system in biomedicine for several decades. As mammals their relevance to human endocrinology is substantially greater than

many other model systems such as flies, worms and yeast. Mice are also easily adapted to various experimental conditions with human relevance. Most importantly, the mouse genome is well annotated, it can be precisely engineered by several powerful ways, and international consortia have recently produced variety novel tools for efficiently manipulate the mouse genome (<http://www.knockoutmouse.org/>). By applying these tools a growing number of genetically modified (GM) mice have been produced, providing novel information about the endocrine regulation of organ systems and new insight on the molecular mechanisms of actions of reproductive hormones. It is well recognized that the function of numerous of genes and their protein products have been finally resolved using GM mouse models, while several of hypotheses drawn by studies *in vitro* have failed when tested in whole organism. Endocrinology, with several organ system interactions, in particular, is a research area where models *in vivo* are of great importance.

As the possibilities to carry out clinical research on endocrine systems in humans are limited, we need novel experimental approaches. For this, GM mice provide excellent opportunity. The techniques applied in analyzing endocrine function GM male mice include, among others, the analysis of macroscopic anatomy and histology of endocrine glands and target organs at various ages, assessment of reproductive performance and behaviour, analyses of hormones and their receptors, and analyses of the signalling pathways potentially affected. Carrying out the analyses at different periods of life, representing differentiation, development, maturation and involution allow us to obtain a global view about the putative changes in the endocrine functions during the life span. However, there are also significant differences in the structure and functions of the endocrine systems between mouse and human, and these differences are to be acknowledged when mouse models are applied.

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## MTE24

### Stem cell biology

Clara Alvarez

Department of Physiology, University of Santiago de Compostela, Santiago, Spain.

Cell therapy with Embryonic, Adult or iPSCs Stem cells? As knowledge and scientific data start to increase the answer to that question seems still far away. Although all of them have in common their pluripotentiality and their infinite growth *in vitro*, the physiological regulation of stem cells *in vivo* is not really defined. Embryonic stem cells isolated from different steps from the initial embryonic development present in culture some different properties. New studies need to be performed to clarify the importance and deepness of that heterogeneity.

We know that adult stem cells remain organized in a delicate structure called the stem cell niche. The concept of niche includes aside from the stem cells organized in a characteristic way, vascular, neural and stromal support. There are known a few niches in the body but not all have been described with similar characteristics. The niche is an essential part in the recruitment of the stem cells and also can be affected by disease. Thus it is important to study in detail the biology and physiological regulation of the niche. Recently, the role of oxygen in the regulation of the stem cell niche has been highlighted. It is also under discussion if the asymmetric division of stem cells is a rule or if they usually divide symmetrically either as stem cells or getting differentiated into progenitors.

In the endocrine organs, the pituitary and the testes present a well localized niche that seems to share some characteristics. Among them the organized expression of the RET/GFRa receptors and its ligands. We do not know if they could be markers of ectodermic stemness or be common to all stem cells. But they are useful for technical isolation of the stem cells.

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# Oral Communications



## Thyroid/Adrenal

### OC1.1 – ESE Young Investigator Award

#### Maternal first trimester thyroid hormone levels within the normal range affect birth weight: results from a large population-based cohort study

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#### Introduction

Maternal hypo- and hyperthyroidism during pregnancy have been associated with high and low birth weights, which predispose for various diseases later in life, such as cardiovascular diseases and cancer. However, the effects of variation in maternal thyroid hormone levels within the normal range on fetal growth and birth weight are largely unknown.

#### Methods

First trimester serum TSH, FT<sub>4</sub> and TPO-antibody (TPOab) levels were determined in 4950 pregnant Caucasian women from a population-based cohort study. Fetal weight was estimated in late pregnancy by ultrasound. In 4469 subjects with normal range TSH and FT<sub>4</sub> levels, the effects of TSH, FT<sub>4</sub> and TPOab levels on fetal and birth weight were studied.

#### Results

No associations of maternal TSH levels with either fetal or birth weight were detected. Higher maternal FT<sub>4</sub> levels were associated with both lower weight in late pregnancy ( $P_{\text{lin}} < 0.001$ ) and at birth ( $P_{\text{lin}} < 0.0001$ ). In mothers with FT<sub>4</sub> levels in the highest quintile (17.0–21.9 pmol/l), late pregnancy fetal weight was  $22.8 \pm 8.7$  (mean  $\pm$  s.e.m.) g ( $P = 0.009$ ) and birth weight  $75.5 \pm 21.1$  g ( $P < 0.001$ ) lower compared to the lowest quintile (10.3–12.9 pmol/l). In mothers with TPOabs in the highest quartile of the detectable range ( $> 8.9$  IU/ml), birth weight was  $55.8 \pm 20.7$  g ( $P = 0.007$ ) higher compared to the group without TPOabs, which remained significant after adjusting for TSH and FT<sub>4</sub> levels. No association with late pregnancy fetal weight was detected.

#### Conclusions

We show that high-normal FT<sub>4</sub> levels in the first trimester are associated with a substantially lower birth weight. We additionally show that higher TPOab levels are associated with higher birth weights, even after adjusting for TSH and FT<sub>4</sub> levels. These data demonstrate that even mild variation in thyroid function within the normal range can have important fetal consequences, and underline the importance of tight regulation of maternal thyroid hormone levels during pregnancy.

### OC1.2 – ESE Young Investigator Award

#### Smoking habits and early pregnancy thyroid hormone and antibody levels

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#### Introduction

Pregnancy is a stress test on maternal thyroid and pregnant women may be more susceptible to thyroid dysfunction. Consequences of this may be harmful for the mother and fetus. Smoking has been associated with alterations in thyroid hormone concentrations, but little knowledge exists on this association in pregnant women.

#### Subjects and methods

The study population is large, prospective population-based Northern Finland Birth Cohort 1986 ( $n = 9362$ ), with extensive data throughout gestation. Mothers underwent serum sampling in early pregnancy. Samples have been assayed for TSH, free thyroxine (FT<sub>4</sub>), free triiodothyronine (FT<sub>3</sub>), thyroid-peroxidase and

thyroglobulin antibodies ( $n = 5805$ ). Mothers with thyroid dysfunction diagnosed before or during pregnancy were excluded. Smoking status of mothers and fathers were asked by questionnaires during pregnancy. Subsequent maternal morbidity to hypothyroidism 20 years after the index pregnancy was evaluated using national registers.

#### Results

Mothers who smoked before pregnancy or during its first trimester had higher FT<sub>3</sub> ( $P < 0.001$ ) and lower FT<sub>4</sub> ( $P = 0.023$ ) concentrations than non-smokers. Smoking in second trimester was associated with lower TSH ( $P = 0.021$ ) and higher FT<sub>3</sub> ( $P < 0.001$ ) concentrations, but no difference in FT<sub>4</sub> concentrations compared to non-smokers. Cessation of smoking during pregnancy did not improve maternal thyroid hormone status. Thyroglobulin antibodies were less common among smoking than non-smoking mothers (2.5 vs 4.7% respectively,  $P < 0.001$ ), but the prevalence of thyroid-peroxidase antibodies was similar. Risk for subsequent hypothyroidism of the mother was similar among smokers and non-smokers. Paternal smoking did not have an independent effect on maternal early pregnancy thyroid hormone/antibody concentrations.

#### Discussion

Smoking during pregnancy was associated with alterations in thyroid hormone concentrations which may be due to smoking-induced changes in peripheral metabolism of thyroid hormones. Smoking may therefore render the diagnosis of thyroid dysfunction in pregnant women even more difficult. Along with other known health risks, smoking affects maternal thyroid function, providing more weight to cessation of smoking.

### OC1.3

#### Maternal thyroid dysfunction during pregnancy and thyroid function of the offspring at the age of 16 years

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#### Introduction

Foetus is dependent on maternal thyroid hormone production during the first trimester. Thus, maternal thyroid dysfunction and thyroid antibodies may affect thyroid function of the offspring.

#### Subjects and methods

We studied a subcohort ( $n = 2000$  mother-child-pairs) from a prospective population-based Northern Finland Birth Cohort 1986 (singleton births, total  $n = 9247$ ) covering data throughout pregnancy, childhood and adolescence. The mothers underwent serum sampling in early pregnancy and their offspring at the age of 16 years. Samples were analyzed for thyroid hormones (TSH, free thyroxine (FT<sub>4</sub>)) and thyroid-peroxidase antibody (TPO-Ab). Mothers were classified according to their thyroid hormone levels and antibody status (euthyroid  $n = 1641$ , overt and subclinical hypothyroid  $n = 84$ , overt and subclinical hyperthyroid  $n = 122$  and TPO-Ab-positive  $n = 91$ ). The offspring of mothers with thyroid dysfunction were compared to the ones of euthyroid mothers.

#### Results

The offspring of the hypothyroid mothers had higher TSH and TPO-Ab concentrations than those of the euthyroid mothers ( $P = 0.001$  and  $P = 0.02$  respectively). When the gender of offspring was taken into account, only the sons of hypothyroid mothers had higher TSH and TPO-Ab concentrations ( $P = 0.004$  and  $P = 0.054$  respectively). The children of the hyperthyroid mothers had lower TSH than those of the euthyroid mothers ( $P = 0.002$ ). The same was seen when comparing sons and daughters separately ( $P = 0.022$  and  $P = 0.041$ ). In the whole subcohort TPO-Ab-positivity was more prevalent among adolescent daughters (8.2%) than sons (4.8%). Especially daughters of TPO-Ab-positive mothers had higher prevalence of positive TPO-Ab than daughters of TPO-Ab-negative mothers (23.1 vs 7.6%,  $P = 0.003$ ), when in sons no significant difference was seen (7.7 vs 4.7%,  $P = 0.310$ ).

#### Conclusions

The thyroid dysfunction of the mother during pregnancy was associated with altered thyroid hormone levels in her adolescent offspring. TPO-antibodies were more common among the daughters of TPO-Ab-positive mothers. Maternal thyroid dysfunction and antibodies modify thyroid function of the offspring even in adolescence.

**OC1.4****Results of 1,Ortho-1,para'-dichloro-diphenyl-dichloroethane (O,p'DDD) treatment in 76 patients with Cushing's disease**

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**Introduction**

Alternative to transphenoidal pituitary surgery (TSS) may be required in Cushing's disease (CD), as first or second line treatment. 1,ortho-1,para'-dichloro-diphenyl-dichloroethane (o,p'DDD) has a potent anticortisol action. Its place in CD treatment is not well defined. The aim of this study was to further evaluate the efficacy and tolerance of o,p'DDD in CD.

**Patients and methods**

Seventy-six patients treated with o,p'DDD out of a single center cohort of 219 patients with CD diagnosed between 1993 and 2009 were retrospectively studied. Remission was defined as normalization of 24-h urinary cortisol (24 h-UC). Remission, recurrence and time-to-event were estimated by the Kaplan-Meier method, and potential predictors analyzed using Cox's proportional hazards regression models.

**Results**

Remission was achieved in 48 (72%) patients, with a median time of 6.7 months (5.2-8.2, 95% confidence limits). Plasma o,p'DDD (mean  $\pm$  s.d.) at the time of remission was 10.5  $\mu$ g/ml  $\pm$  8.9, with a mean daily dose of 2.6  $\pm$  1.1 g. A negative linear relationship was observed between plasma o,p'DDD and 24 h-UC ( $P < 0.0001$ ). Intolerance leading to treatment discontinuation occurred in 19 patients (29%). Recurrence after drug cessation occurred in 71% of patients, with a median time of 13.2 months (5.0-67.9, 95% confidence limits). Only high ACTH plasma level at the time of treatment discontinuation was statistically associated with a lower recurrence probability (HR 0.57 (0.32-1.00),  $P = 0.05$ ). A pituitary adenoma became visible during o,p'DDD treatment in 12 patients (25%) with initial negative pituitary imaging allowing subsequent TSS.

**Conclusion**

O,p'DDD is useful at different steps of CD management, either as first line when pituitary adenoma is not visible or because of the severity of hypercortisolism, or as a second line after TSS failure or recurrence, with most often easily manageable side effects. Monitoring of plasma o,p'DDD allows dosage adaptation to optimize hormonal control together with drug tolerance.

**OC1.5****Adrenal crisis and general morbidity in chronic adrenal insufficiency prospectively assessed in 472 patients**

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Adrenal crisis (AC) is a life threatening condition in chronic adrenal insufficiency (AI). Furthermore, recent data demonstrate increased mortality in this patient group.

Incidence and causes of adrenal crisis as well as general morbidity and hospitalization was assessed in 472 patients in a prospective trial. Patients were contacted via mail every 6 months and in case of AC additionally by phone to evaluate the exact circumstances of AC. Criteria for AC were predefined as no commonly accepted definition exists and grading of severity was additionally performed.

Nine hundred and twenty-three patients were contacted, 472 patients (52% primary AI, 48% secondary AI) sent questionnaires and 381 patients completed the full 2 year study period, covering 815 patient years. Five hundred and thirty cases of acute clinical impairment were reported, 62 fulfilled our predefined criteria of AC, resulting in a frequency of 7.6 per 100 patient/years, which is high

compared to results from previous retrospective analyses. 65.5% were treated as inpatients (grade II), with 9.5% receiving intensive care (grade III). Two patients died during the study period in the context of AC. Main precipitating factor was infectious disease (39% of cases), but also psychic distress, neglected hydrocortisone intake, strong pain, heat and other factors were reported. Ninety-five percent of patients possessed an emergency card but only 28% were equipped with an emergency set. Patients at work demonstrated a mean absence from work of 16 days per year. 12.7% of participants reported additional hospitalization for other reasons than AI.

In this prospective study, we demonstrate that despite patient education and established glucocorticoid replacement patients are at a substantial risk to experience AC which has been fatal in 3%. This demonstrates that there is still a need of improved strategies for prevention of AC, e.g. frequently repeated patient education and broader distribution of emergency sets.

**OC1.6****Increased risk of infections in patients with Addison's disease**

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**Context**

Previous studies have suggested that infections are an important cause of death in patients with Addison's disease, but epidemiological studies on the frequency of infections in this population are scarce.

**Design and setting**

We conducted a cohort study, using data from the Dutch PHARMO record linkage system, linking patients' demographics and medical histories to the use of prescription drugs, diagnostic and therapeutic data from hospital and general practitioner records.

**Participants**

From a cohort of oral glucocorticoid users, 390 patients with Addison's disease were identified by assessing concurrent use of glucocorticoids and mineralocorticoids using pharmacy dispensing records. A reference cohort ( $n = 1933$ ) with the same age and sex distribution was sampled from patients not using glucocorticoids.

**Outcome measure**

Incidence rates and incidence rate ratios (IRR) of infectious events and hospital admission for infection.

**Results**

The risk of infectious episodes among patients with Addison's disease was 1.5 times higher compared to controls, yielding an overall incidence rate of 59.2/100 person-years, with a crude IRR of 1.61 (95% CI 1.51-1.72). The IRR decreased slightly to 1.58 (95% CI 1.47-1.70) after adjustment for confounding co-medication and -morbidity. Regarding hospital admission for infection we also found higher rates among Addison patients (3.8/100 person-years versus 0.8/100 person-years) with a crude and adjusted IRR of respectively 5.02 (95% CI 3.66-6.87) and 4.34 (95% CI 3.04-6.22).

**Conclusion**

Patients with Addison's disease had an increased risk of infectious events and hospital admissions related to infection.

**Neuroendocrinology****OC2.1****Etoposide, doxorubicin, cisplatin, and mitotane versus streptozotocin and mitotane in adrenocortical carcinoma: preliminary results from the first international phase III trial: the FIRM-ACT study**

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**Background**

No randomized trials have been conducted in adrenocortical carcinoma (ACC) patients. Treatment recommendations for this rare but aggressive disease have been based on data from small phase II trials. We have now performed the first

randomized phase III trial, comparing etoposide, doxorubicin, cisplatin, plus mitotane (EDP-M) against streptozotocin plus mitotane (Sz-M).

#### Methods

Three-hundred and four chemotherapy-naïve patients with ACC not amenable to radical surgery were randomly assigned to receive either EDP-M or Sz-M until progression. In case of progression, the alternative regimen was offered to the patient. The primary endpoint was overall survival. Key secondary endpoints were time to progression and response to second line therapy.

#### Results

One hundred and fifty-one patients randomized to EDP-M as first-line treatment received 608 cycles (scheduled every 28 days) and 153 patients in the Sz-M group 634 cycles (every 21 days). Forty-seven patients experienced 87 serious adverse events (SAE) during first-line treatment with EDP-M in comparison 37 patients with 68 SAEs in the Sz-M group ( $P=0.199$ ). Median overall survival in the EDP-M and Sz-M groups (107 and 124 deaths, respectively) were 14.8 and 12.0 months, respectively (HR 0.79, 95% CI, 0.61 to 1.02,  $P=0.069$ ). Median time to progression was 5.0 vs 2.1 months (HR 0.54, 0.42 to 0.68,  $P<0.001$ ). Patients treated with EDP-M after failure of Sz-M ( $n=83$ ) had a median time to progression of 6.2 months. In contrast, time to progression in 75 patients treated with Sz-M as second line was only 2.1 months. In both groups, patients pretreated with mitotane had a similar outcome compared to mitotane-naïve patients.

#### Conclusions

Although a trend towards better overall survival in patients with advanced ACC treated with EDP-M as first-line therapy was observed, overall survival was not significantly different between groups. However, EDP-M significantly prolonged time to tumor progression compared with Sz-M. Thus, new treatment regimens for advanced ACC should be tested against EDP-M.

## OC2.2

### Gonadotropin responses to kisspeptin are modulated by changes in aminoacidergic and neuropeptidergic neurotransmission: studies in peripubertal male rats

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#### Introduction

Kisspeptins, the products of the *Kiss1* gene that act via the receptor, GPR54 (or Kiss1R), are pivotal elements in the neuroendocrine control of GnRH neurons and, thereby, gonadotropin secretion. Extensive efforts have been devoted recently to elucidate the pharmacological effects and major putative regulators of the kisspeptin system. Yet, our knowledge on the potential interactions of kisspeptins with other key neurotransmitters involved in the central regulation of the gonadotropin axis remains superficial.

#### Design

We present herein a series of hormonal analyses addressing the impact of pharmacological manipulations (either activation or inactivation by the use of suitable agonists or antagonists) of major central aminoacidergic (glutamate, GABA) and neuro-peptidergic (neurokinin-B, dynorphin) pathways on the patterns of gonadotropin responses to kisspeptin-10 (Kp-10) in peripubertal male rats.

#### Results

Blockade of ionotropic glutamate receptors decreased (to a variable extent) LH responses to a sub-maximal dose of Kp-10, whereas activation of both ionotropic and metabotropic receptors, which enhanced LH release *per se*, failed to evoke further increases in LH responses to Kp-10. Conversely, selective activation of GABA-A receptors decreased Kp-evoked LH secretion, while central inhibition of GABA-A transmission elicited robust LH secretory peaks, and protracted responses to Kp-10 when combined with GABA-B receptor blockade. In addition, antagonization of dynorphin ( $\kappa$ -opioid) receptors enhanced LH responses to Kp-10, while activation of dynorphin or NKB signaling evoked mild inhibitory effects in peripubertal male rats.

#### Conclusion

Our present data document the roles of glutamate-, GABA-, dynorphin- and NKB-neurotransmission as putative modifiers of gonadotropin responses to kisspeptin. These studies, together with on-going analyses of gonadotropin secretory profiles following activation or inactivation of aminoacidergic and neuropeptidergic pathways in models of genetic or pharmacological blockade of kisspeptin signaling, will aid to dissect out the central interactions and eventual hierarchy of different neuroendocrine pathways with key functions in the control of the gonadotropin axis.

## OC2.3

### Distribution of serotonin transporters in the human hypothalamus

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#### Background

Serotonergic signalling has been implicated in numerous hypothalamic functions including circadian rhythmicity, feeding and the modulation of neuroendocrine systems. In spite of the importance of these functions, the neuroanatomy of the serotonergic system in the human hypothalamus is still unknown.

#### Aim and methods

To increase insight in hypothalamic serotonin signalling in humans, we investigated the distribution of serotonin transporters (SERT) by means of immunohistochemistry, using a monoclonal antibody raised against human SERT. The distribution of SERT immunoreactivity was systematically examined over the entire rostro-caudal axis in each 50th 6  $\mu$ m coronal section of six post-mortem human hypothalami (Netherlands Brain Bank).

In addition, we performed immunofluorescent double-labelling of SERT with respectively the neuropeptides VIP, AVP, NPY, AgRP and  $\alpha$ MSH to identify cell types showing basket-like staining of SERT-immunoreactive fibers.

#### Results

SERT-immunoreactivity was ubiquitously expressed in fibers throughout the hypothalamus. A dense track of fibers was seen in the perifornical area and in close proximity to the anterior commissure. The ependymal lining of the third ventricle showed also strong SERT-immunoreactivity.

The suprachiasmatic nucleus and infundibular nucleus contained a high density of SERT-immunoreactive fibers. Clusters of SERT-immunoreactive fibers outlining capillaries and basket neurons were present in these areas, highly suggestive of synaptic endings. A minority of these basket cells were VIP-, AVP-, NPY-, AgRP- and  $\alpha$ MSH-immunoreactive.

#### Conclusion

We report the distribution of SERT in post-mortem human hypothalamus. The strong immunoreactivity in the suprachiasmatic and infundibular nucleus suggests a modulatory role for serotonin in a number of hypothalamic functions including circadian rhythmicity and the regulation of feeding behaviour.

## OC2.4

### Genetic and molecular cytogenetic investigations in Turner syndrome patients with spontaneous pubertal development

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Spontaneous puberty occurs in a subset of Turner syndrome (TS) patients with significant 45,X/46,XX mosaicism. This observation leads to the belief that haploinsufficiency of still unidentified genes on the X chromosome is the cause of the accelerated follicle atresia in TS. Examination of particular X chromosome rearrangements/deletions led to the identification of 2 Xq and 1 Xp loci associated to the ovarian defect in TS. The availability of new generation genetic and cytogenetic investigations leads us to reexamine this issue on 39 TS patients aged 12–46 years. Spontaneous menarche (SM) was registered in 6 of them and 33 had primary amenorrhea (PA). Conventional techniques assigned the 45,X karyotype to 2/6 with SM and 28/33 with PA. Array CGH analysis was performed on blood DNA of 6 SM cases and 6 PA, used as negative controls, and revealed the unique case of a 45,X TS woman with SM harboring a duplication containing entirely the only BMP15 gene. The BMP15 duplication was confirmed by FISH analysis and Taqman RT-PCR excluded significant 45,X/46,XX mosaicism in vaginal and buccal epithelia. Interestingly, this 45,X TS patient with a double dose of the only BMP15 gene had regular menses for >4 years. Array CGH and FISH analyses revealed the presence of mosaicism at variable levels in the other 5 TS cases with SM and at very low levels (1–10%) in those with PA, including those previously karyotyped as 45,X. Therefore, our data indicate that likely all TS patients are indeed mosaics but only those with a 46,XX cell line above 10% in circulating leukocytes are likely to have spontaneous puberty. A double dose of the only BMP15 gene at Xp11.2 appears sufficient to support spontaneous menarche in a TS patient, but double dose of other X-linked genes is indeed required to sustain prolonged ovarian function.

**OC2.5****Risk of cardiac valve regurgitation with dopamine agonist use in Parkinson's disease and hyperprolactinemia**

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**Background**

There is growing evidence that ergot dopamine agonists (DA) may induce cardiac valve regurgitation (CVR) in persons with Parkinson's disease (PD). It is unclear whether the CVR risk is increased with ergot-DA use in persons with hyperprolactinemia, in whom the dose is much lower.

**Objective**

To assess the association between ergot and non-ergot DAs and CVR in patients with hyperprolactinemia and PD.

**Methods**

Nested case-control studies were separately conducted in two cohorts: i) DA-naïve patients and new users of DA with hyperprolactinemia; ii) PD patients who were newly treated with DA or Levodopa. The cohorts were identified from three general practice databases in Europe: THIN (UK), Health Search (Italy) and IPCI (NL). Cases of CVR were manually validated and matched to controls by age, gender, index date and database. Relative risks and 95% confidence intervals (CI) of CVR were estimated through conditional logistic regression for different DAs using non-use as reference in hyperprolactinemia cohort and levodopa in PD cohort.

**Results**

In the cohort of 21 044 patients with hyperprolactinemia, 37 cases of CVR were identified but no association between individual Ergot DAs and CVR was observed. In the cohort of 19 656 PD patients, 85 incident cases of CVR were identified during follow-up. Compared to levodopa the class of ergot-DAs was associated with CVR. Among individual drugs only cabergoline was associated with significantly increased risk of CVR (OR: 4.6; 95% CI: 2.4–8.8).

**Conclusions**

Increased risk of CVR was confirmed in PD patients who were treated only with ergot-DAs, mostly due to cabergoline. On the other hand, ergot-DA use in patients with hyperprolactinemia was not associated with increased risk of CVR.

**OC2.6****The spectrum and causes of gonadal dysfunction in adolescents and adults with Prader-Willi syndrome (PWS)**

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**Introduction**

Hypogonadism is a cardinal feature of PWS and was generally attributed to hypothalamic dysfunction. Recently, however, primary gonadal defects were also documented. We characterized the spectrum and causes of hypogonadism in PWS adolescents and adults.

**Methods**

We measured reproductive hormonal profiles of 19 males (m) and 16 females (f) ages 15 to 32 years with genetically confirmed PWS. Puberty was assessed by Tanner staging; blood was sampled for gonadotropins, sex-steroids and the gonadal-specific peptides, inhibin B (INB) and anti-Müllerian hormone (AMH).

**Results**

We found four distinct hormonal profiles based on INB and FSH levels: Group A (m:f; 8:1): hypergonadotrophic (primary gonadal) hypogonadism with elevated FSH levels (>15 IU/l) and undetectable inhibin B. Group B (m:f; 4:4): hypogonadotrophic hypogonadism with FSH <0.5 IU/l and inhibin B <7 pg/ml. Group C (m:f; 3:5): partial gonadal and hypothalamic function with inhibin B >20 pg/ml and FSH 2–10 IU/l. Group D (m:f; 4:6): mild hypothalamic and severe gonadal dysfunction (FSH 0.5–10 IU/l and INB <20 pg/ml). There were significantly more males in Group A versus C or D ( $P < 0.05$ ). Mean breast Tanner stage and testosterone levels were highest in Group C ( $P < 0.03$ ), mean LH

was highest in Group A ( $P < 0.001$ ), and mean AMH was highest in Group B ( $P < 0.005$ ). No differences were found in genetic subtype, age and BMI among the four groups.

**Conclusion**

We characterized four distinct phenotypes of hypogonadism in PWS ranging from primary gonadal to hypothalamic hypogonadism; the minority had gonadotropin deficiency. Determining individual reproductive hormone patterns, including inhibin B, may be important for assessing the possibility of fertility in women and for recommending the need for contraception or hormonal replacement therapy.

**Bone/Reproduction****OC3.1****Mild hyponatremia as a risk factor for fractures: the Rotterdam study**

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**Introduction**

Recent studies suggest that mild hyponatremia is associated with fractures, but prospective studies are lacking.

**Aim**

Our aim was to study whether hyponatremia is associated with fractures, falls and/or bone mineral density (BMD).

**Methods**

Five thousand two hundred and eight elderly men and women with serum sodium assessed at baseline were included from the prospective, population-based Rotterdam Study. The following data were analyzed: BMD, vertebral fractures (at baseline and after mean follow-up of 6.4 years), non-vertebral fractures (mean follow-up of 7.4 years), recent falling, co-morbidity, medication, and mortality.

**Results**

Hyponatremia was detected in 399 subjects (7.7%,  $133.4 \pm 2.0$  mmol/l). Subjects with hyponatremia were older ( $73.5 \pm 10.3$  vs  $70.0 \pm 9.0$  years,  $P < 0.001$ ), had more recent falls (23.8 vs 16.4%,  $P < 0.01$ ), higher type 2 diabetes mellitus prevalence (22.2 vs 10.3%,  $P < 0.001$ ), and more often used diuretics (31.1 vs 15.0%,  $P < 0.001$ ). Hyponatremia was not associated with BMD, but was associated with increased risk of incident non-vertebral fractures (HR = 1.39, 95% CI 1.11–1.73,  $P = 0.004$ ) after adjustment for age, sex and body mass index. Further adjustments for disability index, use of diuretics or psycholeptics, recent falls and prevalent diabetes did not modify results. Subjects with hyponatremia also had increased risk of vertebral fractures at baseline (OR = 1.78, 95% CI 1.04–3.06,  $P = 0.037$ ) but not at follow-up, after adjustment for all covariates. Finally, all-cause mortality was higher in subjects with hyponatremia (HR = 1.21, 95% CI 1.03–1.43,  $P = 0.022$ ).

**Conclusion**

Mild hyponatremia in the elderly is associated with increased risk of prevalent vertebral fractures and incident non-vertebral fractures but not with BMD. Increased fracture risk in hyponatremia was also independent of recent falls, pointing towards a possible effect on bone quality. When our findings are replicated in an independent population, screening for hyponatremia should be advised for elderly patients with fractures.

**OC3.2****Changes in serum magnesium levels during cinacalcet therapy for primary hyperparathyroidism**

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The calcium receptor (CaR) participates in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  metabolism at the parathyroid gland and the kidney. Cinacalcet, a calcimimetic, increases the sensitivity of CaR and has been introduced for the treatment of patients with primary hyperparathyroidism (PHPT). However, there are no data for the influence of cinacalcet on serum  $\text{Mg}^{2+}$  levels in the literature.

**Aim**

To evaluate the effect of cinacalcet treatment on magnesium levels in patients with primary hyperparathyroidism.

**Methods**

Sixteen patients, aged  $65 \pm 11$  years with primary hyperparathyroidism receiving cinacalcet therapy were enrolled in the study. Six patients were diagnosed with parathyroid adenoma and ten patients with parathyroid hyperplasia. Median daily cinacalcet dose was 60 mg (range 30–90 mg). Patients were evaluated for a period of 2–8 months. Adverse effects of the drug were reported and serum calcium and magnesium were determined.

**Results**

During cinacalcet therapy thirty eight percent of patients reported cramps with normal CPK, 12% myalgia, and 12% atrial arrhythmia. Mean serum calcium levels were reduced to the normal range ( $P < 0.0001$ ) within the first 2 weeks of treatment and remained constant throughout the study in all patients. The reduction was dose-dependent ( $P < 0.0001$ ). Serum magnesium concentrations were significantly reduced in 14 patients (88%). ( $P = 0.03$ ). The reduction was also dose-dependent ( $P = 0.006$ ). In contrast to Ca levels a time-dependent fluctuation of  $Mg^{2+}$  at steady state was observed.  $Ca^{2+}$  and  $Mg^{2+}$  serum levels changes were highly correlated at all doses ( $r = 0.9$ ;  $P = 0.037$ ).

**Conclusions**

Cinacalcet treatment in patients with primary hyperparathyroidism results in normal serum  $Ca^{2+}$  levels but reduces the  $Mg^{2+}$  serum levels below normal values in the majority of the patients. Hypomagnesemia may cause symptoms like cramps, myalgia, and arrhythmia. It has been stated that activation of the CaR in the thick ascending limb of loop of Henle leads to reduced reabsorption of  $Ca^{2+}$  and  $Mg^{2+}$  and activating CaR mutations result in hypomagnesemia in some patients. Cinacalcet is responsible for the reduction both calcium and magnesium levels and may cause the reported adverse effects.

**OC3.3****Evolution of neurobehavioral and cognitive symptoms in patients with primary hyperparathyroidism belonging to the Swiss primary hyperparathyroidism cohort**

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**Objective**

To describe the evolution of neurobehavioral and cognitive symptoms in patients with primary hyperparathyroidism as measured by mini-mental state (MMS), Hamilton's anxiety and depression scale (HADS) and Clock tests. Scores were also compared with biochemical data.

**Methods**

The Swiss primary hyperparathyroidism (PHPT) Cohort Study is an ongoing, prospective, non-interventional national project collecting clinical, biochemical and outcome data in patients with PHPT. Newly diagnosed patients were enrolled. All three tests applied were interpreted centrally. Thereafter, follow-up data were recorded every 6 months as recommended by the NIH guidelines at the time of study onset. The tests were re-administered on a yearly basis. Patients with parathyroidectomy had one follow-up visit 3 to 6 months after surgery. We applied a multiple regression model comparing test results with serum calcium, serum parathyroid hormone (PTH) and serum 25-hydroxyvitamin D, adjusting for age and gender.

**Results**

From June 2007 to December 2010, 253 patients (mean age  $68.8 \pm 14.5$  years; 77.5% female) were included. To avoid the effect of age, we limited our analysis to the 100 subjects aged 65 years old and less. Fifty-one had completed at least one of the neuropsychological tests. We found a positive correlation between PTH levels and the HADS anxiety scores ( $r = 0.23$ ,  $P = 0.01$ ) and a negative one with MMS scores ( $r = -0.13$ ,  $P = 0.03$ ). A Wilcoxon matched-pairs signed-rank test showed an improvement in MMS ( $P = 0.04$ ), anxiety ( $P = 0.01$ ) and depression ( $P = 0.02$ ) scores in the 16 operated patients.

**Conclusion**

The severity of PHPT as evaluated by serum markers is correlated with impaired cognitive functions, increased anxiety and depression. These alterations may be reversible upon treatment by parathyroidectomy.

**OC3.4****Low doses of bisphenol A promote human seminoma cell proliferation *in vitro* by activating PKA and PKG via the non classical membrane G protein-coupled estrogen receptor (GPER)**

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Fetal exposure to environmental estrogens may contribute to hypofertility and/or to testicular germ cell cancer. However, many of these endocrine disruptors have only a weak affinity for the classical ERs and other mechanisms have been suggested. Bisphenol A, a chemical pollutant, used as monomer to manufacture polycarbonate plastic, released from lining canned food or beverages or from dental sealants, was able to promote human testicular seminoma cell (JKT-1) proliferation *in vitro* at very low environmental relevant concentrations ( $10^{-9}$  to  $10^{-12}$  M), (seminoma cells are supposed to derive from germ stem cells (fetal gonocytes or adult undifferentiated spermatogonia). BPA activated both PKA and PKG pathways and triggered a rapid (15 min) phosphorylation of the transcription factor CREB and the cell cycle regulator Rb. This non genomic activation, did not involve classical ERs since it could not be reversed by ICI182780, an ER antagonist, nor reproduced either by  $E_2$  or by DES a potent synthetic estrogen which triggered instead, a suppressive effect. It was only reproduced by  $E_2$  coupled to BSA, unable to enter the cell. As  $E_2$ -BSA, BPA promoted JKT-1 cell proliferation through a G protein coupled receptor (GPCR) involving a  $G\alpha_s$  and a  $G\alpha_i/G\alpha_q$  subunit as shown by the reversible effect observed by the corresponding inhibitors NF449 and Pertussis toxin. This promoting effect on JKT-1 cells was completely inhibited by G15, a specific antagonist of the G protein coupled estrogen receptor (GPER) (also known as GPR30) and by invalidation of GPER by siRNA and reproduced by G1, a GPER agonist.

This GPRE-mediated non genomic action represents a new basis for evaluating BPA, which could at low doses and with a high affinity for this GPER, interfere, when crossing the placenta, with the developmental programming of fetal germ cell proliferation and/or differentiation.

**OC3.5****IGF1 promotes survival of mast cells and regulates their number in the developing mammary gland**

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We found that mast cells were present in pubertal mammary glands, and hypothesized that they participate in mammary morphogenesis and are sensitive to IGF1, which is essential for mammary development.

As independently reported in another model of mast cell deficiency (*Developmental Biology* 2010 **337** 124), mast cell-deficient  $W/W^c$  mice had significantly less terminal end buds and ducts and smaller gland areas than controls at 3, 6, and 9 weeks of age ( $P < 0.01$ ).

In 75-day-old oophorectomized Ames dwarf mice (deficient in GH, PRL, and TSH) IGF1 +  $E_2$  for 5 days significantly increased the number of tryptase-positive periglandular and stromal mast cells as compared with vehicle ( $P < 0.01$  and  $< 0.02$  respectively). Mast cell increase coincided with mammary development. Mammary mast cell dependence on IGF1 was confirmed by treatment with pasireotide, which blocks IGF1 action in the mammary gland probably by inducing IGF1 binding protein 5. Pasireotide for 7 days reduced periglandular and stromal mast cell density in 28-day-old intact CD rats ( $P < 0.02$  and  $< 0.05$  respectively), and prevented GH +  $E_2$  from increasing mammary mast cell number in hypophysectomized, oophorectomized 28-day-old CD rats ( $P < 0.02$ ). To determine whether IGF1 also had an effect on mast cells elsewhere in the body, we cultured bone marrow-derived mast cells from C57Bl/6 mice. Formazan-producing viable mast cells were significantly increased in number in response to 100 ng/ml IGF1 added to serum-free culture medium during prolonged serum starvation ( $P < 0.05$  after 24 h and  $< 0.01$  after 48 h). IGF1 effect was associated with activation of the anti-apoptotic AKT/PKB pathway. PQ401, an IGF1 receptor phosphorylation inhibitor, blocked AKT activation and prevented IGF1 from rescuing serum-starved mast cells, while somatostatin-14 had no effect.

Our results support that mast cells are involved in mammary development. They further suggest that growth of mammary mast cell may depend, in part, on IGF1.

### OC3.6 – ESE Young Investigator Award

#### Materno-fetal cardiovascular complications in Turner syndrome after oocyte donation: a French multicenter retrospective study

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#### Context

Recombinant human GH treatment and oocyte donation (OD) have improved the quality of life in women with Turner syndrome (TS). However, life expectancy is reduced, mainly due to cardiovascular complications. Pregnancy may itself increase that risk and be associated with hazardous materno-fetal outcome.

#### Objective

The objective of this study was to evaluate the materno-fetal outcome of ongoing pregnancies beyond 20 weeks of gestation obtained by OD in TS.

#### Design

This was a multicenter retrospective study including all assisted reproductive technology centers affiliated with the French Study Group for Oocyte Donation.

#### Results

Among 93 patients, only 37.6% were prescreened with echocardiography or thoracic magnetic resonance imaging. Maternal outcome was dominated by 37.8% of pregnancy-associated hypertensive disorders including preeclampsia in 54.8% and severe eclampsia in four patients. Prematurity occurred in 38.3% and was correlated with pregnancy-associated hypertensive disorder ( $P=0.01$ ). The frequency of *in utero* growth retardation was 27.5%. One fetal demise was linked to eclampsia. Two patients died from aortic rupture after cesarean section in a context of aortic root dilatation. Only 40% of pregnancies were associated with an absolutely normal materno-fetal outcome.

#### Conclusions

OD pregnancies in TS who have not been managed following recent specific recommendations were at high risk for maternal death by aortic dissection and for preeclampsia and its complications (fetal distress and *in utero* growth retardation). These recommendations include previous echocardiography, thoracic magnetic resonance imaging, and overnight blood pressure monitoring associated with a tight follow-up during pregnancy. Until future assessment of these recent recommendations, pregnancies obtained in TS after OD must be still considered as very high-risk pregnancies.

## Diabetes/Thyroid

### OC4.1

#### Obestatin improves *in vitro* generation of novel insulin and glucagon secreting islet-like cell clusters from islet-derived non differentiated cells

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#### Introduction

The ghrelin gene products ghrelin and obestatin (Ob) protect mice from STZ-induced  $\beta$ -cell death, inflammation and STZ-induced diabetes. A possible event is ghrelin/Ob-induced regeneration of damaged  $\beta$ -cell population through the recruitment of pancreatic progenitor cells (PPC). We hypothesized that Ob affects proliferation and endocrine commitment of PPC within the islets of Langerhans.

#### Methods

Islets of Langerhans were isolated from pancreas of 6 months old mice and plated for enrichment. Enriched islets were cultured to permit adhesion and stimulate islet-derived cell proliferation (emersion phase). Part of these islets were chronically treated with 100 nM Ob. After a week, culture medium was changed into a serum-free medium supplemented with leukaemia inhibitory factor and

fibroblast growth factor 2 to allow proliferation of undifferentiated cells. The expression of staminal and endocrine markers was studied for the next 15 days. During this period cells proliferated (proliferation phase) and aggregated into islet-like cell clusters (ILCC) (differentiation phase).

#### Results

During the emersion phase Ob treatment increased proliferation of  $\beta$ -cells, but not that of  $\alpha$ -cells and of cells expressing Nestin, Ngn3, PDX1, Oct4. At the end of the proliferation phase, Ob-treated cells expressing Nestin or Oct4 highly proliferated, while cells clustering into ILCC expressed more Ngn3 with respect to control. At the end of the differentiation phase Ob-ILCC expressed higher levels of insulin and glucagon. Furthermore, glucose-induced insulin release was increased. Ob effect depended on its modulation of specific signalling molecules essential to endocrine pancreas development.

#### Conclusions

Ob efficiently stimulated islet-derived cell proliferation and precursor cell differentiation into novel insulin and glucagon secreting ILCC. Thus, *in vivo*, Ob might ameliorate diabetic conditions through stimulation of PPC. Ob may also improve *in vitro* islet generation for transplantation purpose.

### OC4.2

#### Hepatic insulin resistance in rats on free-choice high-fat diets

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#### Background

Recently, we showed that rats on a free-choice high-fat high-sugar (fcHFHS) diet became rapidly obese and developed glucose intolerance within a week. Interestingly, rats on a free-choice high-fat diet (fcHF), although equally obese and hyperphagic as rats on a fcHFHS diet, did not develop glucose intolerance. Glucose tolerance was also unaffected in rats on a free-choice high-sugar diet, although they consumed more liquid sugar than rats on a fcHFHS diet. Hence, the diet-induced glucose intolerance is specific for rats on a fcHFHS diet. We investigated whether changes in (hepatic) insulin sensitivity contribute to the observed glucose intolerance.

#### Methods

Male Wistar rats received a jugular vein and carotid artery catheter. After recovery, rats were subjected to either a fcHFHS diet, a fcHF diet or a chow (control) diet for one week. We subsequently performed a hyperinsulinemic-euglycemic clamp, with insulin concentrations two times basal insulin values, combined with stable isotope dilution to measure the decrease in endogenous glucose production (EGP) as a measure of hepatic insulin sensitivity.

#### Results

Rats on a fcHF and fcHFHS diet increased caloric intake significantly and gained more fat mass compared to rats on chow. EGP suppression by hyperinsulinemia in rats on a fcHFHS diet and on a fcHF diet was more than 50% less compared to suppression in rats on a control chow diet. Moreover, rats on a fcHFHS diet showed a decreased rate of disappearance for glucose compared to chow controls (90 vs 67  $\mu\text{mol}/\text{kg min}$  respectively), which was not observed in rats on a fcHF diet.

#### Conclusions

These data suggest that both obesogenic diets resulted in hepatic insulin resistance whereas a fcHFHS diet also reduced peripheral insulin sensitivity.

### OC4.3

#### Association of genetic polymorphisms in the organic cation transporters OCT1, OCT2 and multidrug and toxin extrusion 1 transporter protein with the metformin intolerance and weight lowering in patients of type 2 diabetes

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#### Objective

Metformin is one of the most widely used drugs in primary therapy for the treatment of type 2 diabetes (T2D). Despite its overall efficiency in T2D treatment

metformin shows significant proportion of patients suffering from number of side effects. So far number of polymorphisms mainly in genes coding for various metformin transporter proteins have been described in association with metformin efficiency. However, no information exists on influence of genetic variation of metformin transporters on metformin side effects. OCT1, OCT2 and MATE1 transporters are involved in pharmacokinetics of metformin. In this study, we assessed whether 5 SNP and 2 indel polymorphisms are associated with metformin side effects in Latvian patients of type 2 diabetes.

#### Methods

We used information from Genome Database of Latvian Population and METFOGENE study to identify all metformin users and their response to metformin intake. Polymorphisms in SLC22A1 – rs12208357, rs34059508, rs628031; rs36056065 and rs72552763; SLC22A2 – rs316019; SLC47A1 – rs2289669 were compared between 74 type 2 diabetes patients with metformin intolerance and 236 metformin users without symptoms of metformin intolerance. Results

We found a statistically significant association between A allele of rs628031 ( $P=0.008175$ , OR=0.558, CI 95% (0.3848–0.8703)) as well as 8 bp insertion (rs36056065) ( $P=0.008527$ , OR=0.546, CI 95% (0.3929–0.8749)) and presence of metformin intolerance. Additionally carriers of rs2289669 A allele (SLC47A1) displayed significantly lower ( $P=0.002114$ ) mean BMI (32.67 kg/m<sup>2</sup>) compared to wt GG genotype (36.49 kg/m<sup>2</sup>) among regular metformin users.

#### Conclusion

Genetic variation in OCT1 encoded by SLC22A1 may predispose to increased incidence of metformin intolerance in patients with type 2 diabetes. Our results may also suggest that rs2289669 located in SLC47A1 gene coding for MATE1 transporter may be associated with increased efficiency of metformin effect on the weight lowering, which is in accordance with MATE1 function in hepatocytes.

## OC4.4

### HLA-G polymorphism and papillary thyroid cancer histological aggressiveness

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#### Introduction

Human leukocyte antigen-G (HLA-G) is a non-classical HLA-class Ib molecule with multiple immunoregulatory properties. Its main role is the maintenance of tolerance between maternal immune system and semiallogenic fetus. The expression of HLA-G antigen has been reported in several cancers and seems to play an important role in the escape of tumour cells from immune surveillance. A 14 bp insertion/deletion polymorphism in exon 8 of the 3' untranslated region of the HLA-G gene has been reported to be associated with HLA-G mRNA stability.

#### Aim

To evaluate the prevalence of the 14 bp insertion (+14 bp) and deletion (–14 bp) polymorphism in the HLA-G gene in patients affected by papillary thyroid carcinoma (PTC) as well as autoimmune thyroiditis. The possible association between HLA-G 14 bp polymorphism and thyroid cancer aggressiveness was also assessed.

#### Patients and methods

We studied 182 patients (147 F) with PTC; 120 patients (104 F) with autoimmune thyroiditis and 245 healthy subjects (191 F), matched for sex and age, served as control groups. HLA-G polymorphism was studied by PCR techniques.

#### Results

The frequency of the 14 bp insertion polymorphism was significantly higher in patients with PTC and autoimmune thyroiditis as compared to healthy controls (0.48 and 0.46 vs 0.38,  $P=0.0003$  and  $P=0.01$ , respectively). Among PTC patients, a significant correlation was found between +14 bp allele and advanced TNM stage ( $\rho: 0.37$ ,  $P<0.0001$ ), as well as higher tumour aggressiveness, expressed as lymph node and/or multifocality and/or thyroid capsule invasion ( $\rho: 0.39$ ,  $P<0.0001$ ). A positive correlation between the +14 bp allele and cancer dimension was also observed ( $\rho: 0.15$ ,  $P<0.05$ ).

#### Conclusions

Our preliminary data show, for the first time, an increased frequency of the 14 bp insertion polymorphism in PTC patients, suggesting a potential role of the HLA-G polymorphism in PTC immune escape and aggressiveness. The polymorphism may be also involved in the reported increased prevalence of PTC in patients with autoimmune thyroiditis.

## OC4.5

### Comparison of four strategies of radioiodine ablation in patients with thyroid cancer with low-risk of recurrence: the randomized, prospective ESTIMABL study

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#### Objective

The objective of this clinical trial is to compare four strategies of management of postoperative radioiodine ablation in a 2×2 factorial design, each strategy combining a method of TSH stimulation and an activity of I<sup>131</sup>I. The primary endpoint is the rate of thyroid ablation.

#### Patients and methods

This multicentric, randomized, controlled, phase III trial involved 24 French centers, and compared 4 strategies for postoperative radioiodine ablation in a 2\*2 factorial design: a method of TSH stimulation (either thyroid hormone withdrawal (THW) or rhTSH (Thyrogen, Genzyme)) and an activity of I<sup>131</sup>I (either 1.1 or 3.7 GBq). Study patients met the following criteria: age > 18 years; total thyroidectomy for differentiated papillary or follicular (no aggressive histology) thyroid carcinoma, between 30 and 120 days before randomization, treatment with L-T<sub>4</sub> for at least one month; TNM stage pT1 < 1 cm, N1 or Nx, pT1 > 1 cm (any N) or pT2, N0; absence of distant metastasis, no iodine contamination. Thyroid ablation was assessed at 6–10 months after radioiodine ablation with rhTSH stimulated Tg determination and neck-US; whole-body scan was performed in case of positive Tg antibodies (TgAb). The comparison between the four strategies is based on equivalence framework, with two-side  $\alpha=0.05$ .

#### Results

Seven hundred and fifty-three patients who gave written informed consent were included in the study between April 2007 and February 2010 and currently data on radioiodine ablation are available for 693 patients (92%) who form the basis of the present report: 79% were females, mean age was 49 years, and 90% had papillary cancer; 30% of tumors were pT1N0, 17% were pT1N1, 39% were pT1, Nx and 12% were pT2, N0. Mean time between thyroidectomy and randomization was 50 days, and mean time between randomization and radio-iodine ablation was 39 days. Data on the follow-up control are currently available for 477 patients. Neck-US was normal in 444 patients (93%) and suspicious or abnormal in 33 (7%). Stimulated Tg level was >1.0 ng/ml in 25 (5%) patients and 31 patients had TgAb. Thyroid ablation was considered complete in 417 patients (87%), incomplete or doubtful in 58 patients (12%) and non-evaluable in 2 patients.

#### Conclusion

These data will be updated for the ECE meeting, and results of ablation will be presented.

## OC4.6

### No causal relationship between Yersinia enterocolitica infection and autoimmune thyroid disease: evidence from a prospective study

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#### Objective

To evaluate prospectively the relationship between Yersinia Enterocolitica (YE) infection and the development of overt autoimmune hypo- or hyperthyroidism (study A) and the *de novo* occurrence of thyroid antibodies (study B).

#### Subjects and methods

Prospective cohort study of 790 euthyroid women who were 1st or 2nd degree relatives of AITD patients. Follow-up was 5 years, with annual assessments.

Study A. Nested case-control study in which YE serological status was measured between cases (subjects who developed overt hypothyroidism (TSH > 5.7 mU/l and FT<sub>4</sub> < 9.3 pmol/l) or overt hyperthyroidism (TSH < 0.4 mU/l and FT<sub>4</sub> > 20.1 pmol/l)) and matched controls.

Study B. Three hundred and eighty-eight euthyroid women without thyroid antibodies at baseline were enrolled. The YE serological status was compared

between subjects who developed TPO-Ab and/or Tg-Ab at 4-year follow-up and those who remained negative.

#### Results

Study A. The proportion of subjects positive for YOP-IgG or YOP-IgA did not differ between cases and controls at baseline. One year before the development of overt hypo- or hyperthyroidism, the proportion of subjects with YOP-IgG was not different between cases and controls, but YOP-IgA were less prevalent in cases. Study B. *De novo* occurrence of TPO (or TPO-Ab and/or Tg-Ab) did not differ between subjects in whom at baseline YOP-IgG were positive or negative. Neither persistence nor emergence of YOP-IgG at 4-year follow-up was associated with the occurrence of TPO-Ab or Tg-Ab. Similar results were observed with respect to YOP-IgA.

#### Conclusions

*Yersinia enterocolitica* infection does not contribute to an increased risk of thyroid autoimmunity.

## Hormone metabolism and action

### OC5.1 – ESE Young Investigator Award

#### HDL receptor SR-BI dysfunction is associated with a diminished adrenal glucocorticoid output in mice and man

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Adrenal-derived glucocorticoids originate from their precursor cholesterol. Scavenger receptor BI (SR-BI) is a major high-density lipoprotein (HDL) receptor that mediates the selective uptake of cholesteryl esters (CE) from this lipoprotein in the circulation. In our present studies in SR-BI knockout (KO) mice and human carriers of a functional mutation in the extracellular domain of SR-BI (P297S), we determined whether SR-BI-mediated uptake of HDL-CE is required for adrenal glucocorticoid production.

Impaired uptake of HDL-CE in SR-BI knockout mice induced depletion of adrenal lipid stores and adrenal hypertrophy. Basal steroidogenesis was, however, not affected by murine SR-BI deficiency. In contrast, fasting- and lipopoly-saccharide (LPS)-induced plasma corticosterone levels were markedly lower in SR-BI KO mice compared to controls, which coincided with fasting hypoglycemia and an increased susceptibility to inflammation. A compensatory 2-fold increase in hepatic and plasma corticosteroid-binding globulin (CBG) and >2-fold higher ACTH levels were detected. 'Humanizing' SR-BI KO mice through transgenic expression of human cholesteryl ester transfer protein (CETP; mice are naturally CETP deficient) partially restored CE delivery to the adrenals due to transfer of CE from HDL to apoB-containing lipoproteins for uptake via the adrenal LDL receptor. However, CETP was unable to rescue the glucocorticoid insufficiency phenotype in SR-BI KO mice. In parallel, human heterozygotes for the SR-BI P297S mutation exhibited a diminished urinary excretion of most steroids in the context of higher plasma HDL-cholesterol and CBG levels. ACTH (tetracosactin) stimulation tests revealed unchanged baseline plasma levels of free cortisol, while the percentage increase in free cortisol levels following tetracosactin stimulation was significantly lower in carriers (127 vs 223% in controls).

In conclusion, our studies show that disruption of SR-BI function is associated with a diminished adrenal glucocorticoid output in mice and man and suggest that HDL fulfils a thus far unanticipated role in human adrenal steroid synthesis.

### OC5.2

#### Site specific overexpression of urocortin 2 results in modulation of steroidogenesis *in vivo*

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Urocortin 2 (Ucn2) is a recently identified neuropeptide of the CRF-Family, involved in homeostatic mechanisms for stress response and control of anxiety. To further elucidate Ucn2 effects on steroidogenesis, we developed a mouse model with Ucn2 overexpression in the adrenals, gonads and parts of the hypothalamus, by crossbreeding SF1-Cre<sup>+/-</sup> mice with R26<sup>stop</sup> UCN2<sup>stop</sup> UCN2 mice. Following genotypes were obtained: SF1-Cre<sup>-/-</sup>/R26<sup>+stop</sup> UCN2 (controls) and SF1-Cre<sup>+/-</sup>/R26<sup>+stop</sup> UCN2 (Ucn2OE). Adrenal glands, ovaries, testes and trunk blood of two-month-old animals were collected. Plasma

concentration of corticosterone and Ucn2 were measured with commercially available immunoassays. Expression levels of Ucn2 and steroidogenic genes were quantified by real-time PCR analysis.

A 3.5-fold elevated plasma Ucn2 ( $P < 0.001$ ) and a 20-fold increased Ucn2 expression in adrenals ( $P < 0.001$ ) of male and female Ucn2OE could be documented. A lower expression of StAR ( $P < 0.05$ ), cyp11a1 ( $P < 0.001$ ) and cyp11b1 ( $P < 0.05$ ) could be demonstrated in adrenals of female Ucn2OE, together with reduced plasma corticosterone values ( $P = 0.05$ ). In contrast, 6-fold higher Ucn2 expression in ovaries was associated with significant increase of StAR ( $P < 0.05$ ) and cyp11a1 ( $P < 0.05$ ). On the contrary, steroidogenic enzymes' expression in male adrenals and plasma corticosterone levels did not differ from controls (StAR  $P = 0.29$ , cyp11a1  $P = 0.70$ , cyp11b1  $P = 0.99$ , corticosterone  $P = 0.47$ ). Surprisingly, Ucn2 expression in testes was comparable to controls and no effects on steroidogenesis could be documented.

These data demonstrate that Ucn2 overexpression in the female mouse results in downregulation of steroidogenesis in the adrenal, suggesting a stress coping behaviour and increased steroidogenesis in the ovary, an observation not confirmed in male gonads. The gender specific discrepancies will be further investigated.

### OC5.3

#### Identification of melanocortin 2 receptor structures responsible for specific membrane expression and ACTH binding specificity

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Membrane expression of ACTH receptor MC2R is specifically limited to adrenal cells. In addition, unlike the other members of the evolutionary related MCRs that recognize different melanocortin peptides, the MC2R solely binds ACTH. We used cassette substitutions and mutagenesis of individual amino acids for systematic construction of 30 chimeric and mutant MC2R/MC4R receptors to identify the receptor structures determining the selectivity of MC2R in membrane trafficking and ACTH binding as well as interaction with MC2 receptor accessory protein (MRAP). We developed a new analysis method to quantify the localization of the recombinant receptors fused with enhanced green fluorescent protein in a cell membrane from the results of confocal fluorescent microscopy. NDP-MSH and ACTH mediated cAMP response was measured for all receptors. We have found that substitution of MC4R N-terminal part with homologues part from ACTHR significantly decreased the membrane trafficking of receptor. We have also identified the signal localized in TM3 and TM4 regions that is responsible for trapping MC2R in the endoplasmic reticulum (ER) of the cell. Replacement of two sets of amino-acids (3aa each) in TM3 and one set of four amino-acids in TM4 with the corresponding amino acids from MC4R enabled the membrane transport of MC2R in ordinary BHK cell line without the MRAP. For control purposes all constructs were also co-expressed with MRAP protein in the same cell line. Results indicate that a specific structural conformation formed by TM2 and TM4 rather than primary aa sequence is important for ER arrest of MC2R. We have also found that the TM4 and TM5 in the MC2R are involved in MC2R binding selectivity. Bypassing of these arrest signals involves MRAP protein. In summary we have identified presence of 'dual protection' system that helps to avoid the membrane expression of MC2R involving N-terminal part and specific TM3-TM4 interaction.

### OC5.4

#### Adrenal melanocortin 2 receptor associated protein (MRAP) expression is regulated by ACTH and angiotensin II, whereas MRAP2 levels are associated with ACTH responsiveness

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#### Introduction

ACTH stimulates adrenocortical steroid production through the melanocortin 2 receptor (MC2R). MC2R trafficking and signaling depend on the melanocortin 2 receptor accessory protein (MRAP). The MRAP-homolog MRAP2 also transports the MC2R but prevents effective activation. We investigated expression of these components of the MC2R complex in human adrenal tissues in order to uncover novel regulatory mechanisms and their contribution to ACTH responsiveness.



#### Methods

Expression levels of MRAP, MRAP2 and MC2R were studied in adrenal tissues from 37 patients and related to clinical data. Primary adrenal cultures from 40 patients were stimulated with ACTH, forskolin, angiotensin (Ang)II or PMA in order to study the regulation of expression of these mRNAs. Induction of ACTH-responsive genes was related to MRAP, MRAP2 and MC2R mRNA expression levels.

#### Results

MRAP and MRAP2 levels were lower in adrenocortical carcinomas (ACCs) than in adrenal ACTH-dependent or -independent hyperplasia or adenoma tissues ( $P < 0.001$ ), whereas MC2R levels were comparable in all groups. The expression of MRAP, MRAP2 and MC2R did not differ between ACTH-dependent or -independent hyperplasia. Patient plasma ACTH and serum cortisol levels were positively associated with MRAP and MC2R levels in adrenal hyperplasia samples ( $P < 0.05$ ), but not in tumors. *In vitro*, ACTH and forskolin both potently induced expression of MRAP and MC2R in all adrenal tissue types ( $P < 0.0001$ ), whereas AngII augmented these mRNAs in all tissue types as well ( $P < 0.001$ ), with the exception of ACCs. MRAP2 expression was reduced by PMA ( $P = 0.001$ ). The ACTH induction of CYP21A2 relative to that by forskolin was inversely related to MRAP2 levels ( $r = -0.84$ ,  $P = 0.038$ ), whereas there was no association between MRAP or MC2R expression and ACTH responsiveness.

#### Conclusions

MRAP and MC2R are controlled by ACTH and AngII, but expression levels do not directly relate to ACTH responsiveness. MRAP2 levels are negatively associated with the effects of ACTH.

### OC5.5

#### The effect of MCT8 on the biological activity of T<sub>3</sub>

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#### Introduction

The biological activity of thyroid hormone (TH) is largely mediated by binding of T<sub>3</sub> to nuclear T<sub>3</sub> receptors (TRs). Before TH can mediate its effects, it needs to be transported across the plasma membrane. Several TH transporters, such as MCT8, facilitate this transport. Inactivating mutations in MCT8 lead to a severe phenotype of psychomotor retardation. In addition to transport into the cell, MCT8 is also able to transport TH out of the cell. Aim of this study was to investigate if MCT8 does not only increase cellular T<sub>3</sub> uptake, but also increases the availability of T<sub>3</sub> for its nuclear receptor.

#### Method

JEG3 cells, transiently transfected with TRβ1 and a construct with a T<sub>3</sub> response element (TRE)-dependent luciferase reporter and a control renilla reporter (pdV-L1; gift from Dr W S Simonides, VUMC), were used as a model. μ-crystallin (CRYM), an intracellular TH binding protein, was used to block efflux of TH. In addition to TRβ1 and the reporter construct, cells were transfected with CRYM and/or MCT8. Forty-eight hours after transfection, cells were incubated for 10, 30 or 60 min or 24 h with 0, 0.5 or 1 nM T<sub>3</sub>.

#### Results

After 30 min with 1 nM T<sub>3</sub>, cells transfected with TRβ1, reporter and CRYM had a significantly higher luciferase/renilla ratio after co-transfection with MCT8 than without MCT8 ( $0.60 \pm 0.07$  vs  $0.20 \pm 0.01$ ,  $P < 0.001$ ). Similar differences were seen after the other incubation times, and with 0.5 nM T<sub>3</sub>. However, in cells transfected with TRβ1 and reporter, but without CRYM, co-transfection with MCT8 had no effect on the luciferase/renilla ratio at any of the incubation times or ligand concentrations.

#### Conclusion

MCT8 contributes to both influx and efflux of TH. Our data suggest that in cells with high intracellular binding capacity, MCT8 increases the availability of T<sub>3</sub> for its receptor, and has a positive effect on T<sub>3</sub>-mediated gene transcription.

### OC5.6 – ESE Young Investigator Award

#### A novel non-radioactive assay detects PTU-sensitive enzymatic deiodination of natural and synthetic iodinated compounds

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Enzymatic 5'- and 5-deiodination represent key pathways for local and systemic and local activation and inactivation of iodothyronines and iodothyronamines. Expression of the three respective deiodinases (DIO) is regulated by thyroid parameters, local and systemic inflammatory status as well as by selenium supply. In addition, the DIO isoenzymes are intended but also unanticipated target proteins of pharmacological, nutritive and environmental substances that might interfere with their proper function (endocrine disruptors). Therefore, determination of tissue-specific DIO activity is pertinent both for *in vitro* screening tests and for mammalian animal models which intend to systematically address safety issues with respect to the thyroid hormone axis. The classical radioactive DIO activity assay depends on the availability of <sup>125</sup>I-labeled substrates (e.g. <sup>125</sup>-rT<sub>3</sub>) and monitors the release of <sup>125</sup>-iodide. Therefore, an easily accessible, photometric method for determination of iodide release based on the Sandell-Kolthoff-reaction was combined with the classical deiodination assay. The protocol was optimized to minimize handling efforts, reaction volume and time. Since this method is not depending on radioactively labelled components, it expands the spectrum of the classical assay regarding the substrates to additional iodinated compounds. Comparison of DIO1 activities in tissue samples from hypo- and hyperthyroid mice and concentration-response experiments with PTU, an established DIO1 inhibitor, yielded the expected results. Incubation of iopanoic acid, a well-known, non-selective pan-inhibitor of all three DIO, with active DIO1 preparations revealed a significant release of iodide that was suppressible by PTU and exhibited features of an enzymatic reaction. We conclude that iopanoic acid itself might serve as substrate for DIO1, thereby contributing to the mobilization of iodide from the X-ray contrast agent and its re-utilization by the thyroid gland. This reaction might enhance the persistent iodide overload observed after administration of iodinated X-ray contrast agents.

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# Poster Presentations

**Adrenal cortex****P1****Pituitary–adrenal axis in male rats after exposure to high ambient temperature**F Percinic-Popovska<sup>1</sup>, V Ajdzanovic<sup>2</sup>, S Trifunovic<sup>2</sup>, M Jordanova<sup>3</sup>, V Ileski<sup>1</sup>, L Pendovski<sup>1</sup> & V Milosevic<sup>1</sup><sup>1</sup>Faculty of Veterinary Medicine, Skopje, the Former Yugoslav Republic of Macedonia; <sup>2</sup>Institute for Biological Research, 'Sinisa Stankovic', Belgrade, Serbia; <sup>3</sup>Faculty of Natural Sciences, Institute of Biology, Skopje, the Former Yugoslav Republic of Macedonia.

The global warming and its impact on the summer average temperature elevation, in the south-eastern parts of Europe, represent an inevitable stress for living organisms in this region. Heat stress was shown to be the strongest one, when compared to immobilization or cold stress. Activation of the hypothalamo-pituitary–adrenal (HPA) axis during exposure to various types of stressors is well-known physiological concept. The effect of 24 h exposure to heat stress on the morphology of pituitary adrenocorticotropes (ACTH cells) and adrenal cortex, especially on *zona fasciculata* (ZF), in adult male Wistar rats, was examined. The experimental animals ( $n=7$ ) were exposed for 24 h to high ambient temperature ( $35 \pm 1$  °C). The control group ( $n=7$ ) was kept at room temperature ( $20 \pm 2$  °C). The pituitary and left adrenal glands were fixed in Bouin's solution and serially cut (5  $\mu$ m). The ACTH cells were identified by the peroxidase-antiperoxidase (PAP) immunohistochemical procedure, while the cells of adrenal cortex were stained with hematoxylin–eosin. The volume of ACTH- and ZF- cells and their nuclei were estimated using the multipurpose test system M<sub>42</sub>. The body weight in heat stressed rats was decreased ( $P<0.05$ ) by 15.3%, compared to the controls. The relative pituitary weight was significantly increased ( $P<0.05$ ) by 16.5%, in comparison with the corresponding control. The ACTH cell volume, as well as the volume density, were significantly decreased ( $P<0.05$ ) by 12.3 and 26.7% respectively, in comparison with the controls. Absolute volumes of the adrenal gland, adrenal cortex, ZF, as well as the ZF cells were also decreased ( $P<0.05$ ) by 10.7, 8.6, 15.2 and 3.3% respectively, compared to the controls. These findings suggest that one day exposure of adult male rats to high ambient temperature ( $35 \pm 1$  °C) has the inhibitory effect on the morphological characteristics of ACTH- and ZF-cells in adult male rats.

**P2****Relativ adrenal failure in trauma patient: therapy approach**Eduard Circo, Marian Beciu & Razvan Circo  
<sup>1</sup>Ovidius' University, Constanta, CT, Romania.**Introduction**

Relative adrenal insufficiency is considered a life threatening event, especially when associated with a serious event like trauma. Recognition and treatment of relative adrenal insufficiency is a challenge for clinical practitioner.

**Objectives**

We wanted to study adrenal dysfunction, diagnostic methods corresponding to the emergency situation for a trauma patient, adaptative capability of hypothalamic–pituitary–adrenal axis and therapeutic methods for trauma patients.

**Materials and methods**

We studied 15 patients brought to the Emergency Department. We determined basal serum cortisol and after 1  $\mu$ g tetracosactide (Synacthen) stimulation test – we measured cortisol at 15 min and then at 60 min. We appreciated survival rate function basal and stimulated cortisol values.

**Results**

For 9 patients (group A) basal cortisol levels (7.37  $\mu$ g/dl) and stimulated cortisol levels (11.30  $\mu$ g/dl) were lower than normal. For 3 patients (group B), basal cortisol levels were less than normal (8.11  $\mu$ g/dl), but after the stimulation test – levels were normal (20.71  $\mu$ g/dl). For 3 patients (group C), basal (14.53  $\mu$ g/dl) and stimulated (25.05  $\mu$ g/dl) cortisol levels were both normal.

Survival rate was 22% for group A, 33% for group B and 100% for group C. All patients received i.v. hydrocortisone 100–200 mg daily.

We observed a high mortality rate for those patients with low cortisol values and adaptative cortisol dysfunction as for those with adequate basal and stimulated cortisol values. It still remains the problem of using a cortisone dose for treatment that is lower than it should be for the adaptative capability.

**Conclusions**

Relative adrenal insufficiency is a stress-induced situation. A high clinical suspicion, a correct diagnosis and cortisone replacement could higher the survival rate.

**P3****MicroRNAs as endogenous modulators of glucocorticoid receptor expression in the adrenal gland after ACTH stimulation test**A Hill<sup>1</sup>, O Issler<sup>2</sup>, A Spyrogliou<sup>1</sup>, S Haramati<sup>2</sup>, A Chen<sup>1</sup> & F Beuschlein<sup>1</sup>  
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MicroRNAs (miRs) are a subset of small RNA molecules that regulate gene expression post-transcriptionally. Little is known about the expression and the role of miRs in the adrenal gland. Thus, we determined the miRs expression pattern under baseline conditions and 10, 30 and 60 min after ACTH stimulation test in mice adrenal glands using miRs microarray. The miRs expression profile of adrenals obtained from the array was bioinformatically analyzed to identify miRs that putatively target important genes. After selecting 20 miRs, with a significant change in their expression level upon ACTH stimulation test, it was found that four of these miRs putatively target the glucocorticoid receptor (GR, Nr3c1): miR96, miR101a, miR142-3p, miR433, in addition to miR18a, which is already known from previous studies to influence GR expression in the brain. The array data could be confirmed with real-time PCR analysis showing an upregulation of miR96 10 min after ACTH test (baseline:  $100 \pm 27\%$ , 10 min post ACTH:  $244 \pm 18\%$ ,  $P<0.05$ ), and also of miR433 in all investigated timepoints (baseline:  $100 \pm 22\%$ , 10 min:  $197 \pm 24\%$ , 30 min:  $168 \pm 14\%$ , 60 min:  $163 \pm 9\%$ ,  $P<0.05$ ). Furthermore, a decrease of miR142 expression was found 30 min ( $100 \pm 29\%$ , 30 min:  $20 \pm 8\%$ ,  $P<0.05$ ) and of miR101 10 min after ACTH stimulation test (baseline:  $100 \pm 20\%$ , 10 min:  $20 \pm 1\%$ ,  $P<0.05$ ). GR expression measured with real-time PCR was elevated 10 min after ACTH stimulation ( $144 \pm 19\%$ ) in comparison to the control group ( $100 \pm 10\%$ ,  $P<0.05$ ). Targeting predictions of these four miRs GR 3'UTR was further tested by *in vitro* luciferase assays. Preliminary data show that miR96, miR101a and miR142 target the GR 3'UTR region and result in a 20–40% decreased mRNA level of GR 3'UTR. Thus, ACTH stimulation influences short term expression of different miRs and the GR; at least, three miRs, miR96, miR101a and miR142, regulate the GR expression post-transcriptionally. The impact of this regulation in physiological stress response awaits further investigation.

**P4****Effects and kinetics of mitotane in *in vitro* and *in vivo* models of adrenocortical carcinoma**M Luconi<sup>1</sup>, G Poli<sup>1</sup>, M Mangoni<sup>1</sup>, P Pezzati<sup>2</sup>, M Francalanci<sup>1</sup>, S Gelmini<sup>1</sup>, L Canu<sup>1</sup>, G Cantini<sup>1</sup>, M Serio<sup>1</sup> & M Mannelli<sup>1</sup><sup>1</sup>University of Florence, Florence, Italy; <sup>2</sup>AOU Careggi, Florence, Italy.

Adrenocortical carcinoma (ACC) is a rare aggressive tumor with a poor prognosis. At present, early diagnosis followed by total surgical tumor resection is the only valuable option for ACC cure and mitotane (MTT) is the only specific drug available for pharmacological treatment. However, its effects seem to be mainly due to its adrenocortical cytotoxicity and its bioavailability and cellular mechanism of action are still unknown, making it difficult to develop less toxic multidrug strategies for ACC treatment. We investigated MTT metabolism and effects on cell activity and proliferation in the human ACC line H295R and in an *in vivo* xenograft model obtained by s.c. injection of H295R cells in athymic mice. MTT and its metabolite DDA dose-dependently accumulated intracellularly in H295R with a peak at 24 h administration, resulting in a dose- and time-dependent inhibition of cell proliferation, evaluated by MTS assay (inhibition:  $50.0 \pm 2.1\%$  at 48 h,  $85.0 \pm 2.5\%$  at 5 days,  $P<0.001$ , 20  $\mu$ M MTT) and cell count (inhibition:  $54.0 \pm 3.2\%$  at 48 h,  $89.0 \pm 2.2\%$  at 5 days,  $P<0.001$ , 20  $\mu$ M MTT). We demonstrated that MTT interferes with MAPK signalling pathways. *In vivo* oral/i.p. administration of MTT resulted in a significant accumulation of MTT in adrenals and in the ACC xenograft model determined a significant inhibition of tumor growth versus the untreated control animals (tumor growth fold decrease following 21 day treatment:  $0.74 \pm 0.20$ ,  $P<0.01$ ,  $n=10$ ), accompanied with a reduced expression of angiogenic, vascular and proliferation genes in tumors of MTT treated mice. In prevention experiments where MTT was started contemporarily with H295R inoculation, we observed an even greater reduction in tumor growth compared with the untreated control animals (tumor growth fold decrease following 21 day treatment:  $0.38 \pm 0.10$ ,  $P<0.001$ ,  $n=10$ ). In conclusion, we contributed to elucidated MTT kinetics *in vitro* and *in vivo* highlighting the antiproliferative effects of such drug.

**P5****The utility of FDG-PET CT in differentiating malignant from benign adrenal lesions**Basak Oguz<sup>1</sup>, Sayid Shafi Zuhur<sup>2</sup>, Ozcan Karaman<sup>2</sup>, Selvinaz Velet<sup>2</sup> & Yuksel Altuntas<sup>2</sup><sup>1</sup>Internal Medicine, Sisli Etfal Training and Research Hospital, Istanbul, Turkey; <sup>2</sup>Sisli Etfal Training and Research Hospital, Endocrinology and Metabolism Clinic, Istanbul, Turkey.**Background**

With the increasing use of USG, MRI and CT, adrenal lesions are frequently found in the daily practice. These lesions may be primary or secondary in origin. However, it is important to resolve whether these lesions are benign or malignant.

**Purpose**

The aim of this case detection study was to compare FDG-PET CT with MRI in differentiating benign from malignant adrenal mass lesions.

**Patients**

All patients were admitted to our out-patient clinic between 2006 and 2010 for evaluation of adrenal mass lesions. FDG-PET CT and MRI were performed to all patients. We analyzed the laboratory data of the patients and compared FDG-PET CT results with MRI results.

**Results**

The study included 21 patients (13 female, 8 male, mean age 55 years old (between 26 and 71)) with 29 adrenal lesions (mean size  $2.77 \pm 1.07$  cm; 16 bilateral and 13 unilateral). Five out of 29 lesions were malignant according to MRI. Three of malignant lesions on MRI were also malignant according to FDG-PET CT ( $SUV_{max} > 2.5$ ). Of the 3 malignant lesions on FDG-PET CT, 1 diagnosed as tuberculosis, 1 as primary adrenal carcinoma and 1 as atypical lipoma, according to histopathological/microbiological analysis. Six out of the 29 lesions were interpreted as benign by MRI but malignant according to FDG-PET CT ( $SUV_{max} > 2.5$ ). With further investigation, 1 of them diagnosed as adrenocortical adenoma, 1 as lung carcinoma metastasis, 1 as kidney carcinoma metastasis and 1 as tuberculosis.

In 3 patients with benign MRI appearance and  $SUV_{max} < 2.5$  on FDG-PET CT, there were pathological FDG up-take in other locations as lung, epigastric and axillary area, in whom further investigations revealed inflammatory processes.

**Conclusion**

According to our study results, FDG-PET CT is not better than MRI for differentiating malignant from benign adrenal lesions. FDG-PET CT leads to unnecessary invasive investigations in some patients. However, it could be used in detecting metastases in patients with known malignancies.

**P6****CT imaging in patients with adrenal incidentaloma**J Brunova<sup>1</sup> & J Bruna<sup>2</sup><sup>1</sup>Institute for Clinical and Experimental Medicine, Diabetes Centre, Prague, Czech Republic; <sup>2</sup>Diagnostic Radiology Department, Second Medical Faculty Hospital, Charles University, Prague, Czech Republic.

Adrenal masses which are found unexpectedly with US or CT examination of abdomen are called adrenal incidentalomas. The aim of study was to evaluate the contribution of imaging characteristics of incidentally discovered adrenal mass to the differential diagnosis.

We have studied 60 patients with adrenal mass (21 M, 39 F, age 34–85 years) who had CT scan of abdomen for various diagnoses but they were not suspected to have adrenal pathology. The right adrenal gland was solitary affected in 20 cases, left in 28 cases and both adrenals in 12 cases. To determine the endocrine status of adrenal mass the usual tests for Cushing's syndrome and pheochromocytoma were performed. The aldosterone/PRA ratio was measured in hypertensive patients. The benign character of adrenal mass was confirmed operatively or by follow up of tumor size for > 1 year and lately washout test was used.

**Results**

None of our patients had Cushing's syndrome or primary hyperaldosteronism. The non-functioning adrenal adenoma was confirmed in 45 patients, subclinical hypercortisolism had 5 patients, metastases to adrenal glands were found in 5 patients, two patients had lymphoma, myelolipoma 2 patients and one patient had asymptomatic pheochromocytoma. The density of metastases on plain CT images was > 20–45 HU and myelolipomas had density minus 70 HU. The adrenal adenomas had low precontrast density in 100% cases. Most of adenomas showed low contrast enhancement but 8/50 adenomas (16%) enhanced over 55 HU (55–90 HU). Adenomas larger than 3 cm were found in 17/50 cases (34%).

**Conclusion**

From the imaging point of view the low density of lesion seems to be more important sign for the determination of the nature of lesion (myelolipoma,

adenoma, malignancy) than the size of adrenal mass. We recommend CT scans in long-term followed-up patients for 3 years minimally once a year.

**P7****Self-management in patients with adrenal insufficiency**J P van Eck<sup>1</sup>, R J Gobbens<sup>2</sup>, J Beukers<sup>1</sup>, W Geilvoet<sup>1</sup>, A J van der Lely<sup>1</sup> & S J C M M Negers<sup>1</sup><sup>1</sup>Erasmus MC, Rotterdam, The Netherlands; <sup>2</sup>School of Health Care Studies, University of Applied Sciences, Rotterdam, The Netherlands.**Objective**

The objective of this study was to assess the quality of self-management in patients with primary and secondary adrenal insufficiency and to find patient-related factors that influence quality of self-management.

**Methods**

The quantitative research was assessed by a questionnaire. Mainly focused topics were i) demographic data ii) daily medication intake iii) en route precautions and iv) dose adaptation during medical emergencies. One hundred and ninety-eight patients with primary and secondary adrenal insufficiency were assessed by the survey.

**Results**

Sixty percent ( $n=116$ ) of the patients completed the questionnaire. For the propositions about daily medication intake, the score was 3.5 of 4 (mean: max). On the propositions about the way patients are prepared en route patients scored 3.4:5. Knowledge about dose adaptation in stress and strain was 1.9:3 assessed by propositions. Sex, age and education level influence the quality of self-management in the investigated subjects. The number of years patients receive long-term glucocorticoid replacement therapy or membership of a patient self-help group does not affect quality of self-management.

**Conclusion**

Patients demonstrate a high level of self-management according to daily medication intake. En route precautions and dose adaptation during medical emergencies need to be improved to prevent life threatening situations. Therefore, patients must be educated continuously to improve these self-management aspects that come with life-long substitution therapy. Involvement of endocrine nurses and nurse practitioners is likely to be a key factor in the effectiveness of patient education and training.

**P8****Validation of the disease-specific quality of life questionnaire AddiQoL in European Addison patients**M Øksnes<sup>1,3</sup> & K Løvås<sup>1,2,3</sup><sup>1</sup>University of Bergen, Bergen, Norway; <sup>2</sup>Haukeland University Hospital, Bergen, Norway; <sup>3</sup>Euradrenal SP7, Bergen, Norway.**Introduction**

Patients with Addison's disease (AD) self-report impairment in specific dimensions on well-being questionnaires. An AD disease-specific quality of life questionnaire (AddiQoL) was developed to aid evaluation of patients. We here aimed to validate translated questionnaires in terms of internal consistency and reliability and to improve the construct of the scale.

**Methods**

After translation by a multistep approach, the final versions were tested in AD patients from Norway ( $n=107$ ), Sweden ( $n=101$ ), Italy ( $n=165$ ) and Germany ( $n=200$ ). Internal consistency was examined by exploratory factor analysis (EFA) and Rasch analysis, aiming at unidimensionality and fit to the Rasch model. Cronbach's  $\alpha$  and person separation index (PSI) were calculated for reliability. Longitudinal reliability was tested by differential item functioning (DIF, or item bias for time) in stable patient subgroups in Norway, Sweden and Italy.

**Results**

EFA identified four sub-dimensions of AddiQoL; fatigue (8 items), emotions (8), symptoms (11) and miscellaneous (sleep, sexuality and impact of intercurrent disease, 6). Three items concerning nocturia, weight gain, and dry skin did not fit any dimension and were discarded. The sub-dimensions fatigue, emotion and miscellaneous showed non-significant  $\chi^2$  interactions, implying fit to the Rasch model, also supported by the overall fit residual statistics. The symptoms sub-dimension initially displayed misfit, hence three items concerning salt cravings, light headedness and sweating were removed. The 30 remaining items, rearranged in four subtests by sub-dimensions, fitted the Rasch model; supported by a non-significant  $\chi^2$  interaction of 0.65 and no significant pattern deviation among the fit

residuals. Cronbach's  $\alpha$  was 0.94, and PSI was 0.86, indicating good reliability. Longitudinal reliability was excellent, as no significant DIF between separate time points was detected.

#### Conclusion

The Norwegian, Swedish, Italian and German AddiQoL versions have high internal consistency and reliability, and can be fitted to the Rasch model for optimisation of psychometric properties.

## P9

### Diagnostic value of urinary steroid profiling for detecting adrenocortical carcinoma

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The finding of an adrenal incidentaloma warrants a careful diagnostic work-up to assess hormonal activity and potential malignancy. Current diagnostic algorithms depend largely on imaging studies, which is time-consuming, expensive and carries a health risk. Urinary steroid profiling using gas chromatography/mass spectrometry (GC/MS) might be a valuable diagnostic test for differentiating benign from malignant adrenal lesions, as adrenocortical carcinomas (ACC) often cause abnormalities in steroidogenesis. The value of this tool was retrospectively studied in 44 patients referred for analysis of an adrenal tumor or adrenal incidentaloma. The final diagnosis was histologically confirmed ACC ( $n=14$ ), liposarcoma ( $n=1$ ), or benign adrenal tumor ( $n=29$ ). In the latter group, the diagnosis was either histologically confirmed ( $n=6$ ) or based on radiological features and the absence of metastases during clinical follow-up ( $n=23$ ). Patients were monitored with regular imaging studies (median follow-up 20 months, range 8–38 months). Patients were not treated with drugs interfering with adrenal steroidogenesis. The concentrations of 22 steroids were measured in 24-h urine samples. Differences in steroid metabolite excretion between patients with and without ACC were analyzed using the Mann-Whitney  $U$  test. We found that by taking the sum of four steroid metabolites (etiocholanolone, dehydroepiandrosterone, pregnanediol, tetrahydrocortisol) it was possible to distinguish ACC from other adrenal tumors with 100% sensitivity and 93% specificity. Our analysis shows that the performance of a urinary steroid profile seems to be highly predictive for differentiating benign incidentalomas from ACC. Compared to repeated radiological studies, a single urinary GC/MS at baseline has the advantages of being considerably less expensive, less time consuming and without any health risks. The diagnostic value of this tool needs to be prospectively validated.

## P10

### Surgery in adrenocortical carcinoma; importance of national cooperation and centralized surgery

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#### Introduction

The low incidence rate of adrenocortical carcinoma requires a multidisciplinary approach in which specialised surgery has an essential role as complete resection of the primary tumour is the only chance of cure.

In order to improve patient care, insight into surgical results within the ACC population is essential. In 2007, a Dutch Adrenal Network Registry has been created encompassing care and outcome of patients treated for ACC in The Netherlands since 1965. Using this database a study was performed with the following objectives: i) to gain insight into surgical performance in The Netherlands, ii) to compare surgical data with international literature.

#### Patients and methods

The data of 175 patients treated from 1965 until January 2008, were studied. The following data were collected; age, gender, functionality of the tumour, stage at diagnosis (ENS@T staging), surgical procedure, completeness of surgery, disease recurrence, adjuvant mitotane therapy, recurrence free survival and overall survival.

#### Results

One hundred and forty-nine patients were operated. Patients with complete resection had significantly longer survival than patients with incomplete resection

( $P=0.01$ ). Patients operated in a Dutch Adrenal Network centre had significantly longer survival in both univariate ( $P=0.01$ ) and multivariate analysis ( $P=0.01$ ). Significant longer survival was observed in operated stage IV patients compared to non-operated patients ( $P=0.00$ ).

#### Conclusion

Our data confirm the relevance of national cooperation and centralized surgery in ACC. In selected patients with stage 4 disease surgery can be beneficial in extending survival. On the basis of the retrospective analysis surgery in The Netherlands will and can be improved.

## P11

### Arthritis after removal of adrenal adenoma in Cushing's syndrome

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Endogenous hypercortisolism is a well-known immunosuppressive condition. Such endogenous cortisol secretion could suppress the clinical presentation of an ongoing autoimmune process.

A 49-year-old woman was admitted in our service in August 2010 for Cushing clinical signs, arterial hypertension and hypokalemia. She had high plasma cortisol not suppressed by 1 and 8 mg dexamethasone overnight, low ACTH. Abdominal computed tomography demonstrated a tumor (3.0×2.7×2.8 cm) in the right adrenal gland. Waiting one month for the surgical cure we initiated treatment with Ketokonazole 800 mg/day but she developed adrenal insufficiency under treatment so we interrupted it. The patient underwent a right adrenalectomy and the diagnosis of a cortisol secreting benign adenoma was histological confirmed. Blood pressure declined and cushingoid features regressed, but 2 months after the operation and while the patient was on replacement, she complained of pain on motion, marked tenderness and swelling of fingers, wrists, elbows, knees and foot joints. We founded normal tests for cortisol, no positive inflation testes except high level of transaminase and positive serology for toxoplasma. Two months of treatment with Rovamicyne normalized transaminases and lowered the titer for toxoplasma antibodies but has no effect at articular level. Rheumatological exam suggest a rheumatoid arthritis with negative rheumatoid factor and treatment with low doses of medrol have a very good effect at joint level.

In summary, we report a patient with Cushing's syndrome due to an adrenal adenoma, in which joint symptoms were triggered after curing the hypercortisolism; there are two possibilities of diagnosis: rheumatoid arthritis with negative rheumatoid factor revealed after Cushing therapy or reactive arthritis after toxoplasma infection with no amelioration after antibiotics treatment and no interaction with Cushing disease (but no certain date still now about reactive arthritis and toxoplasmosis).

## P12

### Influence of drospirenone on renin-angiotensin-aldosterone system evaluation

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#### Introduction

Drospirenone is a synthetic progestin usually found in combination with ethinylestradiol in oral contraceptive formulas. In 2007, this compound was also used in hormonal replacement therapy during menopause. Drospirenone shows antimineralocorticoid effect due to an analogy of structure with aldosterone. This effect counteracts the estrogen stimulating action of renin-angiotensin-aldosterone (RAA) system, lowering water retention symptoms due to classic oral contraception. On the other hand, primary aldosteronism (PA) accounts for 6% of hypertension. Screening of PA consists in evaluation of PAC levels and ARR after withdrawal of several antihypertensive drugs that can interfere with this dosage.

#### Aim of the study

To evaluate the interference of drospirenone with plasma aldosterone concentration (PAC), plasma renin activity (PRA) and potassium levels in the general population. Blood sample was obtained in 25 patients taking an oral contraception (11 in drospirenone group – 14 in non drospirenone group). Comparison of PAC/PRA ratio (aldosterone to renin ratio – ARR) was also performed as this ratio is used for screening of primary aldosteronism.

**Results**

Age was comparable in both groups ( $P=0.29$ ). PAC was significantly more elevated in patients taking drospirenone ( $P=0.001$ ) while potassium levels was not significantly different ( $P=0.2$ ). Interestingly, PRA levels were not different between both groups ( $P=0.07$ ). In consequence, PAC/PRA was significantly increased in drospirenone group ( $P=0.017$ ). Systolic pressure was significantly higher in drospirenone group while diastolic pressure was not different ( $P=0.02$  and  $P=0.098$  respectively). Median value of ARR in drospirenone group was 22.63.

**Conclusion**

Drospirenone is an effective oral contraception with antiminerlocorticoid effect with the advantage of lowering water retention symptoms. In consequence of this effect, we found a significantly higher PAC and ARR levels in drospirenone group. Drospirenone should then be withdrawn before screening a patient in case of primary aldosteronism suspicion.

**P13****Redefinition of age and sex specific reference intervals of eight serum steroids by liquid chromatography: tandem mass spectrometry (LC-MS/MS)**

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The use of LC-MS/MS quantitative methods in clinical and research laboratories is becoming a well established reality that may overcome specificity and accuracy limits of immunoassays. Nevertheless, among different LC-MS/MS methods an agreement on the values that are to be considered normal or pathologic has not been reached yet, and this is mainly due to differences in LC-MS/MS methods, to the poor or absent availability of reference material and to the selection and ethnicity of healthy subject. We developed and validated a simultaneous LC-MS/MS method for the measurement of cortisol, corticosterone, 11deoxycortisol, androstenedione, testosterone, 17-OH progesterone, dehydroepiandrosterone and progesterone in serum and we proved the accuracy of the method against GC-MS certified material for cortisol, testosterone, 17-OH progesterone and progesterone, while no reference material is available for the other steroids. After given informed consent, 423 Caucasian healthy, normal weight ( $BMI \leq 25.0$ ) and drug free subjects, age 18-90, were recruited among blood donors and among volunteers selected by telephonic interview. Blood withdrawal was between 0800 and 1000 h in non stressed conditions. We estimated non parametric reference intervals as the 2.5th and the 97.5th centiles in 4 age groups in males (18-25; 26-40; 41-60; >60) and in 6 age/fertility groups in females (18-25; 26-54 premenopausal; 18-54 follicular phase (day 1-10); 45-60 postmenopausal; >60). We found a general tendency for androgens to decrease with age mostly after age 40 for testosterone and androstenedione, to a greater extent in females compared to males. In both sexes, dehydroepiandrosterone has the highest rate of decrease, being its median concentration halved after age 40 compared to the youngest group. Our LC-MS/MS method generated with proper reference intervals is now ready to be applied to the study of specific endocrine dysfunctions.

**P14****Autosomal recessive pseudohypoaldosteronism: a rare cause of life-threatening electrolyte imbalance**

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A well-term baby born to consanguineous healthy Yemenese parents was observed in the hospital as parents had lost previous three babies (both male and female) within 10 days of age (in Yemen). These babies who were initially well were reported to have developed a rash and then collapsed and died. Post-mortems were not done. This baby who appeared well at birth, also developed a facial rash on day seven and was found to have hyponatremia and severe hyperkalemia, albeit a normal hemodynamic status. Further investigations revealed normal cortisol of 322 nmol/l and 17-OH progesterone 1.3 nmol/l but markedly elevated plasma renin activity of 480 pmol/l and aldosterone of 29 000 nmol/l consistent with a diagnosis of pseudohypoaldosteronism.

The baby was aggressively treated with i.v. sodium and sodium resonium rectally resulting in improvement in electrolyte balance and facilitation of an early discharge home on very high doses of oral sodium supplements and continued sodium resonium rectally with regular monitoring of electrolytes at home. She remains well at 6 months of age with acceptable electrolytes (see Table).

Age (days)	5	7	8	9	11	16	35	93	182
Serum Na <sup>+</sup> (mmol/l)	139	127	128	130	139	136	139	134	133
Serum K <sup>+</sup> (mmol/l)	5.5	9.4	7.2	8.1	6.8	6.3	5.6	6.3	6.0
Oral NaCl (mmol/kg per day)		4-7	10-20	28	24	15	20	20	24

Genetics investigations have demonstrated four variants in the SCNN1 gene (coding for epithelial sodium channel) of which three are thought to be polymorphisms but one of which is likely to be functionally important. Further investigations into this are underway.

**Conclusion**

Our case highlights the importance of instituting early and continued use of potassium chelator (sodium resonium) along with aggressive electrolyte management in cases of pseudohypoaldosteronism.

**P15****Interstitial cortisol levels obtained by adipose tissue microdialysis in mechanically ventilated septic patients: correlations with total and free serum cortisol**

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**Aim**

To measure interstitial cortisol obtained by adipose tissue microdialysis (MD) as well as serum total and free cortisol in 31 mechanically ventilated patients upon sepsis onset (days 1 and 2). MD tissue glucose, pyruvate, lactate, and glycerol were also measured (as markers of altered or disrupted local energy production).

**Methods**

The Acute Physiology and Chronic Health Evaluation (APACHE II) on day 1 and the sequential organ failure assessment (SOFA) scores were calculated for all the patients. MD cortisol was determined with enzyme immunoassay, serum total cortisol was measured with immunochemiluminescence and free serum cortisol was measured with liquid chromatography-tandem mass spectrometry (only day 2). Statistics were done with Student's *t*-test and Pearson's correlation coefficient (statistical significance was set at  $P < 0.05$ ).

**Results**

Serum total cortisol and cortisol measurements in the interstitial fluid obtained with MD remained fairly constant. On day 2 mean serum free cortisol was  $2.052 \pm 1.769 \mu\text{g/dl}$  ( $n=16$ ); serum free cortisol was higher than MD (free) cortisol on day 2 ( $P=0.010$ ). Serum free cortisol on day 2 (for  $n=16$ ) correlated well with serum total cortisol ( $r: +0.879$ , 95% CI:  $+0.721$  to  $+0.950$ ,  $P < 0.0001$ ) and MD cortisol ( $r: +0.863$ , 95% CI:  $+0.654$  to  $+0.949$ ,  $P < 0.0001$ ). There were also significant correlations between interstitial MD cortisol and APACHE II, SOFA and MD pyruvate levels.

**Conclusions**

Interstitial cortisol measurements represent an additional tool to investigate the adrenal axis in septic, critically ill patients. In these patients tissue cortisol availability, as reflected by cortisol levels obtained by adipose tissue MD, correlates well with total and free serum cortisol levels.

## P16

### Potential role of mast cells in the physiopathology of aldosterone-producing adenoma

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We have shown that 5-hydroxytryptamine (5-HT) receptor type 4 (5-HT4R) agonists stimulate aldosterone production in patients with aldosterone-producing adenoma (APA). Moreover, 5-HT-positive cells were observed in APA tissues and 5-HT4R mRNAs were markedly overexpressed in APAs in comparison with normal adrenals. All these results suggested that 5-HT produced by adrenal mast cells could be involved in the physiopathology of APAs. The aim of this work was to investigate the role of mast cells in the physiopathology of APA. Mast cells were characterized by specific immunohistochemical stainings using antibodies against tryptase. A high density of mast cells was observed in peritumoral adrenal tissue surrounding adenomas or in the tumor tissues. Tryptase labelling was also detected in some steroidogenic cells in the vicinity of mast cells, suggesting interactions between these two cell types. In addition, staining of APA tissues with antibodies anti-tryptophan hydroxylase, the key enzyme of 5-HT biosynthesis, revealed the presence of immunoreactivity in both mast cells and a subpopulation of steroidogenic cells. Interactions between mast cells and adrenocortical cells were explored using the LAD2 human mast cell line and the human adrenocortical cell line H295R. Administration of LAD2 cell culture supernatant to H295R cells induced a significant increase in aldosterone production which was potentiated when LAD2 cells were pre-incubated with the mast cell degranulator, compound 48/80. On the other hand, we observed that 5-HT stimulated H295R cells steroidogenesis via 5HT4R activation suggesting that the effect of mast cells supernatant could be at least in part due to 5-HT. These studies will be completed by co-cultures of mast cells and adrenocortical cells. Altogether, our results show that APA tissues contain numerous mast cells which may influence aldosterone secretion through local release of soluble regulatory factors.

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## P17

Abstract withdrawn.

## P18

### Bilateral primary adrenocortical carcinomas developing severe muscle weakness

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#### Introduction

Primary adrenocortical carcinoma (ACC) is a rare adrenal tumor and bilateral tumors are reported in 10% of total patient with ACC in Japan. We present a case of bilateral ACCs accompanied by severe muscle weakness.

#### Case report

A 63-year-old male patient with bilateral adrenal masses was referred to our department for the examination and treatment of progressive muscle weakness and appetite loss lasting over 2 months. He was not able to stand up by himself because of loss of muscle power. Computed tomography showed bilateral huge adrenal tumors (110 mm in diameter each) extending into the inferior vena cava. No significant uptake was found in I-131 MIBG scintigraphy. Plasma levels and 24-h urinary excretion of catecholamines and their metabolites were normal. Serum cortisol levels were slightly low and plasma ACTH levels were slightly high. These findings suggested presence of primary adrenocortical insufficiency and we began replacement therapy with low doses of hydrocortisone. His symptoms such as appetite loss and muscle weakness were immediately improved after replacement therapy and it enabled him to walk by himself. To establish the diagnosis, we performed open biopsy and histological findings revealed adrenocortical carcinoma. In spite of medical therapy with mitotane, the development of ACCs was aggressive and he died from multiple organ failure.

#### Conclusion

To the best of our knowledge, a case of ACC accompanied by serious muscle weakness is very rare. Bilateral ACCs can cause primary adrenal insufficiency by their invasion to both adrenal glands. It is known that muscle weakness can be developed in patients with adrenocortical insufficiency but its exact mechanism is still unknown. Marked and immediate improvement of muscle power after the hydrocortisone replacement therapy strongly suggests that cortisol deficiency plays a critical role in developing severe muscle weakness in this case.

## P19

### Polymorphisms of the HSD11B1 gene as phenotype modifiers in women with hypercortisolism

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#### Objective

Several studies including ours have reported significant associations between polymorphisms (SNPs) of the HSD11B1 gene and bone metabolism. The aim of the present study was to explore systematically the potential associations between SNPs of the HSD11B1 gene and bone mineral density (BMD) in women with endogenous Cushing's syndrome (CS) and healthy postmenopausal women.

#### Patients and methods

A complex bioinformatical including haplotype analysis was performed for identification of SNPs located in the HSD11B1 gene and its 15 kb promoter sequence. Of the 65 polymorphisms found in this region 7 (rs11576775, rs17389016, rs4844880, rs84910, the previously explored insA, rs12086634, and rs11807619) were selected for further analysis. Sixty-two women with endogenous hypercortisolism (35 patients with ACTH-producing pituitary adenomas: Cushing's disease, CD; 27 patients with cortisol-producing adrenal tumors, ACS) and 94 healthy control women were examined. Genotyping was performed by real-time PCR and direct DNA sequencing. BMD was measured by dual-energy X-ray absorptiometry.

#### Results

No differences in polymorphic allele frequencies between patients and controls were detected. The insA, rs12086634 and rs11807619 were in linkage disequilibrium. Haplotype containing insA, polymorphism rs12086634 and wild-type rs11807619 was associated with better BMD in healthy women and in patients with ACS but not in patients with CD. Serum OC was higher in carriers for this haplotype. The rs4844880 located in the promoter region was associated with higher BMD, and in patients with CD with higher BMI, OC and cortisol measured morning, midnight and after low dose dexamethasone suppression compared to non-carriers.

#### Conclusions

Our results suggest that SNPs of the HSD11B1 gene may modulate the glucocorticoid effects on bone metabolism and represent genetic modifiers of BMD. In patients with endogenous hypercortisolism these SNPs may contribute to the severity of the disease.

**P20****Clinical presentation of Cushing's syndrome in a large series from the European Registry on Cushing's syndrome (ERCUSYN)**

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The European Registry on Cushing's syndrome (ERCUSYN) is designed to collect prospective and follow-up data at EU level on patients with Cushing's syndrome (CS). Baseline data on 481 CS patients (390 females, 91 males; mean age ( $\pm$  s.d.):  $44 \pm 14$  years) collected from 36 centres in 23 countries. This cohort included new patients since 2008 and retrospective cases since 2005. Patients were divided into four major etiologic groups: pituitary-dependent CS (PIT-CS) (66%), adrenal-dependent CS (ADR-CS) (27%), CS from an ectopic source (ECT-CS) (5%) and CS from other etiologies (OTH-CS) (2%). Proportion of men in the ECT-CS group was significantly higher than in the other etiologic groups ( $P < 0.05$ ). Patients in the ADR-CS group were significantly older than those in the PIT-CS group ( $P < 0.05$ ).

Prevalence of hirsutism and diabetes in ECT-CS patients was 92 and 74%, respectively, which was significantly higher as compared with the other etiologic groups ( $P < 0.05$  for hirsutism and  $P < 0.01$  for diabetes). Patients with PIT-CS had significantly more skin alterations, menstrual irregularities and hirsutism than those with ADR-CS ( $P < 0.01$ ). Reduced libido was more prevalent in men than in women ( $P < 0.01$ ). Prevalence of osteoporosis at spine was significantly higher in men as compared with women ( $P < 0.05$ ), and males had significantly more vertebral and rib fractures than females (52 vs 18% for vertebrae;  $P < 0.001$ ; 34 vs 23% for ribs;  $P < 0.05$ ). Patients with ECT-CS consulted a diabetologist more frequently than those with ADR-CS ( $P < 0.05$ ), while a gynecologist was consulted more often by women with either PIT-CS or ADR-CS than those in the ECT-CS group ( $P < 0.05$ ). Overall, weight gain was significantly more common in women as compared with men ( $P < 0.05$ ). EuroQoL-VAS score in PIT-CS was significantly better than in ADR-CS ( $P < 0.05$ ), while CushingQoL score did not differ between groups. The ERCUSYN project allows an analysis of the heterogeneous clinical presentation and course of CS at a European level, depending on gender and etiology.

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**P21****Mortality and morbidity in Cushing's syndrome: a single centre experience**

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Cushing's syndrome (CS) is a severe disease caused by an endogenous glucocorticoid excess and it is correlated to an increased morbidity and mortality especially for sepsis and cardiovascular diseases, while hypertension, glucose intolerance or diabetes mellitus, and dyslipidemia are the most common systemic complications. The aim of this retrospective study was to evaluate the mortality and morbidity of patients with CS admitted at the Department of Endocrinology and Oncology of 'Federico II' University of Naples during the period from 1980 to 2010. Excluding patients with adrenal carcinomas, data were available for 66 patients with CS (15 males and 51 females), with the following distribution of etiology: 53 (80%) patients with pituitary tumors, seven (11%) with adrenal adenomas, six (9%) with ectopic CS due to bronchial, thymic or pancreatic carcinoids. The overall mortality amounts to 8/66 (12%); in particular, the

mortality accounted for 7.5% (4/53) in patients with pituitary tumors and for 50% (3/6) in patients with ectopic CS. One single case of adrenal adenoma died. With the exception of unknown causes (2/8, 25%), heart stroke (2/8, 25%) and heart failure (2/8, 25%) represented the most frequent causes of death, followed by renal failure (1/8, 12.5%) and sepsis (1/8, 12.5%). As far as systemic complications were concerned, dyslipidemia (38/66, 57%) was the most common, immediately followed by hypertension (37/66, 56%), coagulopathy and glucose intolerance (35/66, 53% and 33/66, 50%, respectively), and followed at fair distance by heart diseases (especially ischemic events) (23/66, 35%), psychiatric diseases (mainly depression) (22/66, 33%), severe infections (11/66, 17%) and brain diseases (4/66, 6%). Hypertension was present in 8/8 (100%) dead patients, glucose intolerance or diabetes mellitus, coagulopathy and heart disease in 7/8 (87%), dyslipidemia in 6/8 (75%) and severe infections, psychiatric diseases and brain diseases in 4/8 (50%), 2/8 (25%) and 1/8 (12.5%), respectively. In conclusion, the results of this retrospective single centre study confirmed that CS is associated to an increased morbidity especially for metabolic and cardiovascular diseases, and that mortality of these patients are generally consequence of cardiovascular accidents or septic complication of severe infections, strongly suggesting that these condition predisposing to death needs to be carefully monitored.

**P22****Cardiac structure and function in patients with adrenal incidentaloma with and without subclinical Cushing's syndrome**

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Hypercortisolism has been reported to affect cardiac structure and performance. Subclinical Cushing's syndrome (SCS) is increasingly being reported in incidentally discovered adrenal tumors; its hallmark is a mild autonomous cortisol hypersecretion without specific clinical syndrome of glucocorticoid excess. The aim of this study was to compare cardiac structure and function in patients with adrenal incidentaloma. Thirty patients (10 men, 15 women, 32–74 years) and 30 sex- and age-matched healthy controls entered the study: among patients, 11 had SCS and the remaining 14 had normal cortisol secretion. All patients and controls were submitted to Doppler echocardiography, with evaluation of left ventricular (LV) mass index (LVMI), ejection fraction (EF), main parameter of systolic function, and early (E) to late or atrial (A) peak velocity (E/A), main parameter of diastolic function, together with the measurement of systolic (SBP) and diastolic (DBP) blood pressure. SBP ( $P < 0.01$ ) but not DBP, was higher in patients than in controls. At Doppler echocardiography, EF and E/A were significantly reduced in patients compared to controls. However, no significant difference was found in LVMI. In particular, both patients with and without SCS had reduced EF and E/A compared to controls. A slight but not significant increase in LVMI was found in patients with but not in patients without SCS. No significant difference in SBP and DBP, and only slight difference in cardiac parameters, were found between patients with and without SCS. In conclusion, patients with adrenal incidentaloma have a mild impairment of cardiac performance, represented by both a systolic and diastolic dysfunction independently on the presence of SCS. These findings suggest that patients with incidentally discovered, or clinically non-functioning adrenal tumors need to be monitored for cardiac performance during their follow-up.

**P23****Multicenter study on the prevalence of sexual symptoms in men affected by Addison disease**

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**Introduction**

Male sexual behaviour is deeply influenced by the endocrine system. However, few data are available on low cortisol serum levels and sexuality.

**Subjects and methods**

In order to investigate the sexual function in 12 men before and during replacement treatment for Addison disease (AD), we performed sexual and psychological evaluations before replacement therapy ('baseline') and at least



8 weeks after complete recovery from cortisol deficiency ('recovery phase'), by collecting blood and urine for hormonal assays and by filling the International Index of Erectile Function (IIEF) questionnaire, the Beck Depression Inventory (BDI), the Spielberger State Anxiety Inventory (SSAI) and the Spielberger Trait Anxiety Inventory (STAD).

#### Results

IIEF showed a significant improvement of libido and erectile function during the 'recovery phase'. BDI, SSAI and STAI showed a significant improvement of depression and both trait and state anxiety in the 'recovery phase' compared to 'baseline'. Testosterone and LH serum levels did not show a significant difference comparing the two phases of the study.

#### Discussion

Low cortisol serum levels could indirectly impair sexuality and mood through arterial hypotension and asthenia. For what concerns the high ACTH serum levels, it is well known that LH receptors on Leydig cells are down-regulated by ACTH, so it is possible that AD leads to a relative hypogonadism which can contribute to sexual dysfunction. Moreover, a relative hypogonadism could be due to GnRH serum levels lowered by CRH.

#### Conclusions

Several data and our results suggest the presence of mood disturbances in AD patients even if it is not clear which factor linked with cortisol has the prominent role in affecting sexual life, sexual behaviour and mood in hyposurrealism.

## P24

### Hormones failing the heart

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Cardiovascular disease and diastolic hypertension are major causes of morbidity in Cushing's although left ventricular failure is more rarely described. There are three other cases of documented dilated cardiomyopathy with subsequent left ventricular failure.

Thirty-five year old lady who presented with obesity and striae was found to have a suppressed ACTH, elevated cortisol (914 post dexamethasone suppression test) and a right adrenal adenoma (on CT), confirming a diagnosis of Cushing's syndrome. She was started on metyrapone 250 mg TDS and was referred for a right adrenalectomy. During the next 2 months, she was admitted to hospital with acute dyspnoea. Her CT pulmonary angiogram revealed no pulmonary embolus. She was discharged on oral antibiotics for a chest infection. She was readmitted a week later with left ventricular failure (LVF) and type 1 respiratory failure requiring high flow oxygen and diuretics. She clinically had moderate mitral regurgitation confirmed on echocardiogram, which also suggested a diagnosis of dilated cardiomyopathy with severe LVF (ejection fraction – EF 26%). Her cardiomyopathy screen (autoantibodies, virology and thyroid) was negative but her cortisol day curve had values over a thousand suggesting a steroid induced cardiomyopathy. She gradually responded well to doubling of her metyrapone to 500 mg TDS and supportive care in the form of oxygen, ACE inhibition, beta blockade and diuretics. She had an angiogram a month later that ruled out any ischaemic causes for her cardiomyopathy and showed an improving LVEF of 40%. She had her adrenalectomy (right) 3 months later. This revealed a benign cortical adenoma (55×45×28 mm). Her post operative echocardiogram showed an EF of more than 45% and her 3 month post operative echo showed reversal of her cardiomyopathy.

Monotherapy (metyrapone blockade) of her Cushing's with subsequent curative surgery resulted in reversal of her cardiomyopathy which would have otherwise had a significant effect on her morbidity.

## P25

### Cushing's syndrome in pregnancy due to LH/hCG receptor positive adrenocortical carcinoma: case report

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#### Introduction

Cushing's syndrome is rare during pregnancy perhaps because hypersecretion of glucocorticoids suppresses pituitary gonadotrophin secretion resulting in

subfertility. When present, the syndrome is usually due to adrenal disease and less commonly to pituitary adenomas or ectopic ACTH production. In the contrary, Cushing's syndrome outside pregnancy is usually due to pituitary adenomas whereas non ACTH-dependent Cushing's is less frequent.

#### Case report

We describe the case of a 27-year-old female primigravida, who was admitted during the 22nd week of pregnancy with hypertension and probable preeclampsia. On admission, blood pressure was 130/90 mmHg on oral nifedipine and methylpoda. Clinical examination revealed signs of florid Cushing's syndrome. Laboratory investigations: plasma glucose 103 mg/dl, routine biochemical testing within normal range. Urine collection revealed proteinuria of 5.6 g/24 h. Serum cortisol: 49.7 µg/dl (5.0–23.0) with loss of diurnal variation and low ACTH (5.2 pg/ml). DHEAS was normal, total testosterone raised at 4.5 ng/ml (0.3–1.4) due to raised SHBG. Serum cortisol following overnight dexamethasone (1 mg) suppression testing was raised (> 50 µg/dl). Abdominal ultrasound showed a 5.6 cm hypoechoic mass in the left adrenal. The patient underwent laparoscopic left adrenalectomy; a 5.9 cm mass was excised. Histological and immunohistochemical findings were consistent with adrenocortical carcinoma (Weiss score 5/9). In addition, immunohistochemistry showed expression of LH/hCG receptors in the tumour tissue. Postoperatively, the patient's condition improved with normalization of the blood pressure and gradual improvement of the proteinuria (0.9 g/24 h). The patient underwent caesarian section (36th week of pregnancy), giving birth to a healthy male infant weighing 1800 g.

#### Conclusion

This is the second report of adrenocortical carcinoma presenting with Cushing's syndrome during pregnancy. The presence of aberrant LH/hCG receptors in adrenal tumour tissue might have contributed to the development of Cushing's syndrome in pregnancy and possibly to tumour growth in this case.

## P26

### Predictive value of acute cortisol response to cabergoline administration on the responsiveness of cortisol secretion to short and long term treatment with cabergoline

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Cabergoline (CAB), a potent dopamine agonist, is one of the most effective neuromodulatory drug in the second line treatment of patients with Cushing's disease (CD). The aim of the current study was to estimate the predictive value of the acute CAB administration on short and long term responsiveness to CAB treatment. Twenty CD patients (6 males, 14 females, 12–60 years) entered the study. The acute CAB test was performed evaluating serum cortisol every hour for 6 h after 1 mg CAB administration. Serum cortisol and urinary cortisol (UC) levels were evaluated at baseline and after three (short-term) and six (long-term) months of treatment with CAB (dose 1–9 mg weekly). Patients were considered responsive when UC levels were ≤50% compared with baseline after 3 months or normalized after 6 months of treatment. A ROC curve was performed to establish the cut-off of the percentage of serum cortisol response reduction (%ΔC) which guaranteed the highest sensitivity (SS) and specificity (SP) in predicting the responsiveness after short and long-term CAB treatment. The positive and negative predicting values (PPV and NPV) were estimated at both 3 and 6 months of treatment. Ten of the 20 patients (50%) showed responsiveness after either 3 or 6 months. A direct correlation was found between %ΔC and the percentage of reduction of UC at 3 months ( $r=0.467$ ;  $P=0.038$ ), while no direct correlation was found between %ΔC and the percentage of reduction of UC at 6 months. The 18% (SS=100%, SP=67%) and 20% (SS=100%, SP=50%) were considered as the best %ΔC cut-offs of the acute test response to predict the chronic responsiveness at 3 and 6 months, respectively; using these values we obtained a PPV of 83% and a NPV of 100% after 3 months and a PPV of 67% and a NPV of 100% after 6 months of treatment. In conclusion, the acute CAB test is useful to predict short-term but not long-term responsiveness; on the other hand it is completely reliable to select patients non responders to chronic CAB treatment.

**P27****Do mitotane levels impact on the outcome of patients treated adjuvantly following radical resection of adrenocortical cancer (ACC)?**

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We have demonstrated that adjuvant mitotane prolonging recurrence free survival (RFS) in patients with radically resected ACC. Aim of the present study was to correlate mitotane levels with patient outcome in an adjuvant setting in 3 different referral centres in Europe. There were 120 patients (45 W, 75 M, median age 44 years, range 16–76) radically resected for ACC who were treated adjuvantly with mitotane from 1996 to 2010. ACC was stage I in 10 cases, II in 73, III in 31, IV in 6; 62 patients had secreting ACC. Forty patients were treated with 8–10 g/daily and 80 patients with 1–6 g/daily. Target mitotane concentrations of 14–20 mg/l were attained within 3 months from treatment start in 49 patients (41%), within 6 months in additional 61 patients (51%), while 10 patients (8%) never got them. Mitotane levels > 14 mg/l were maintained in more than 75% of determinations in 64 patients (53%). Median duration of treatment was 25.5 months (range: 4–165) and median duration of follow-up was 36 months (6–165). Only 11 patients (9%) experienced severe adverse events (CTC 3), but none of the patients discontinued mitotane definitively for toxicity. At the last follow-up, 58 patients (48%) relapsed and 33 patients (27%) died. RFS was significantly longer in patients who attained target mitotane concentrations within 3 months ( $P=0.01$ ) and in patients who maintained consistently levels > 14 mg/l during follow-up ( $P=0.004$ ). In a multivariate analysis (including sex, age, stage, number of mitoses or Ki-67 value) maintenance of target mitotane concentrations was an independent factor influencing RFS (HR=0.41, 95%CI 0.21–0.77;  $P=0.006$ ). The effect on overall survival was significant only in univariate but not in multivariate analysis (HR=0.61, 0.26–1.43). The present data demonstrate for the first time that target mitotane concentrations may have a therapeutic impact in an adjuvant setting. This finding supports indirectly the efficacy of adjuvant mitotane treatment.

**P28****Impact of long-term cabergoline treatment on clinical, metabolic and hormonal features in patients with Cushing's syndrome**

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Cabergoline (CAB) was demonstrated to be effective in normalizing cortisol secretion in a subset of patients with Cushing's disease (CD). The aim of the study was to evaluate the impact of chronic treatment with CAB on clinical, metabolic and hormonal parameters in CD patients. Twenty-six patients with CD (11 males, 15 females, 12–72 years) entered the study. Patients were divided into two groups, responders and non responders, according to the achievement or not of normalization of urinary cortisol (UC) levels. The evaluation of clinical, metabolic and hormonal parameters were performed before and after 6 months treatment with CAB, administered at the dose of 1–9 mg weekly. The clinical parameters included weight, BMI, waist, systolic and diastolic blood pressure, Beck Depression Inventory-II, International Index of Erectile Function (IIEF) and Female Sexual Function Index (FSFI) scores, the metabolic parameters included serum glucose, triglycerides, cholesterol levels, whereas hormonal parameters included plasma ACTH and serum cortisol, androstenedione, DHEAS, renin and aldosterone levels. After 6 months of treatment, 15 patients were responsive (UC decrease=63.2%) whereas 11 patients were not responsive (UC decrease=44.3%). In responsive patients, a significant decrease of plasma ACTH and serum cortisol, systolic and diastolic blood pressure, serum glucose, androstenedione, DHEAS, renin and aldosterone ( $P<0.05$ ) and a significant increase of IIEF and FSFI ( $P<0.05$ ) was found. Conversely, in non responsive patients, a significant decrease of plasma ACTH, serum cortisol and androstenedione as well as blood pressure levels ( $P<0.05$ ) was found, whereas weight and waist significant increased ( $P<0.05$ ). In conclusion, long-term CAB treatment is able to ameliorate blood pressure, metabolic and hormonal parameters as well as sexual function in responsive patients, but is also able to

improve blood pressure and decrease androgen levels in non responsive patients. These results demonstrated that long-term CAB treatment has a relevant impact of the clinical syndrome and complications of patients with CD.

**P29****TP53 and adrenocortical cancer: analysis of germline mutations and polymorphic changes in 140 patients**

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Adrenocortical carcinoma (ACC) is part of the Li-Fraumeni tumour syndrome which is due to germline mutations in TP53. Recent studies demonstrate low penetrance mutations leading to later tumour manifestation. Furthermore, in ACC mutations outside the hotspot region have been found. TP53-polymorphisms have also been described to impact on p53 function. We, therefore investigated TP53 sequence alterations in a large cohort of adult patients with ACC.

**Methods**

Germline DNA of 140 ACC-patients was extracted from leucocytes. Mutation and polymorphism analysis was performed by amplification and sequencing of exons 2–11 of TP53. Corresponding tumour DNA of mutation carriers was also investigated and LOH analysis was performed.

In 103 evaluable cases 5 TP53 germline mutations were found in 4 patients (4%). Three mutations have not been described previously. Three mutation carriers were diagnosed at young age (<40 years), had a short survival time (<3 years) and 2 had a family history of cancer. One young female was found to have 2 mutations and a family history of ACC (mother and uncle) indicating an adrenal specific phenotype. Furthermore, the Brazilian hot spot mutation R337H was found in a male patient diagnosed at high age and with a current survival time of 10 years. LOH of one TP53 allele in the tumour was detected in all cases with loss of the mutated allele in one patient. Analysis of 11 TP53-polymorphisms revealed significantly different distributions for 3 polymorphisms in ACC compared to controls. However, correlation with clinical outcome did not reveal a prognostic impact of these polymorphisms in multivariate analysis.

TP53 germline mutations can also be found in a significant but lower percentage of adult ACC patients and may appear outside the hot spot region (2 of 5 mutations in exon 10). TP53-polymorphisms seem to be of minor relevance in ACC.

**P30****Is plasma mitotane level > 30 mg/l a serious adverse event in patients with adrenocortical carcinoma (ACC)?: a retrospective analysis of the French COMETE network**

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**Background**

Mitotane antitumor efficacy is related to plasma levels. Objective responses have been associated with mitotane plasma levels > 14 mg/l. However, high plasma levels > 20 or > 30 mg/l are at higher risk of toxicity, especially neurotoxicity. National-based survey is lacking to evaluate the frequency and severity of high mitotane plasma levels. Our aim was to retrospectively describe serious adverse events (SAE).

**Methods**

Mitotane plasma levels, SAE and grade 3–4 toxicities were recorded in ACC patients, followed between 09/2006 and 05/2010 in the French COMETE

network. Plasma mitotane levels were monitored by the Lysosafe service. Grading of toxicity was done with NCI NCTAE (version 3) criteria. SAE is defined by: death, life threatening, hospitalization, persistent disability, congenital anomaly / birth defect or other medically important events.

#### Results

A mitotane plasma levels >30 pg/ml was observed in 15 out of 310 (4.8%) patients. Fifteen assays > 30 mg/l were identified among 2057 analysed (0.72%). The median mitotane plasma level was 35.76 mg/l, after a median time of 8 months from mitotane initiation, corresponding to a median mitotane dosage of 4.5 g/day. Grade-3 toxicity was observed in 8/15 (53.3%) patients (vertigo, nausea, anorexia, alopecia, hypotension and gynecologic bleeding) and a grade 4 toxicity in 6/15 (40%) patients (asthenia, vigilance trouble, anorexia, abdominal pain and aplasia). Hospitalization was required in 6/15 patients (40%). A life threatening SAE was observed in 1/15 (6.7%) due to severe sepsis in aplasia, related to mitotane therapy. No patient died or suffered from sequelae, and no unexpected SAE was observed.

#### Conclusion

Mitotane plasma levels > 30 mg/l is a rare event. A SAE was observed in 8/15 (53%) patients and 10/15 (66.7%) experienced a grade 3–4 toxicity. We therefore suggest that the plasma mitotane cut-off of 30 mg/l should not be exceeded until a better understanding of the mechanisms that drive clinical toxicity.

### P31

#### 18-Oxocortisol measurement in adrenal vein sampling as a biomarker for subclassifying primary aldosteronism

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#### Context

18-Oxocortisol (18-oxoF) is a derivative of cortisol that is produced by aldosterone synthase (CYP11B2). The potential for this steroid, as a biomarker for differentiating patients with aldosterone-producing adenoma (APA) from those with idiopathic hyperaldosteronism (IHA), has not been examined.

#### Objectives

We measured 18-oxoF, aldosterone (A) and cortisol (F) in plasma from adrenal vein sampling (AVS) patients. We compared 18-oxoF and 18-oxoF/F and A/F levels for their potential to differentiate APA from IHA.

#### Design, setting, and subjects

We measured 18-oxoF, F and A in AVS samples obtained from unilateral APAs (14 cases) and bilateral IHAs (7 cases, 14 samples total), using liquid chromatography–mass spectrometry (LC/MS/MS) and RIA analyses, and performed in Tohoku University Hospital.

#### Results

The levels of 18-oxoF and the ratios of 18-oxoF /F, before and after ACTH stimulation, were significantly higher in blood-draining APAs than in those from the contralateral adrenal gland and from adrenal glands with IHA.

#### Conclusions

The 18-oxoF levels and ratios of 18-oxoF/F in AVS samples can be a clinically useful biomarker for differentiating APA from IHA and for determining the localization or lateralization of APA in patients with primary aldosteronism.

### P32

#### Mission study: an international observational study on the mortality in Cushing's syndrome

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Cushing's syndrome (CS) is a rare but severe disease associated with an increased mortality especially due to cardiovascular diseases, and associated with various

systemic complications, including hypertension, glucose intolerance or diabetes mellitus, dyslipidemia and coagulopathy. Few studies have systematically evaluated the mortality of CS in a large and worldwide population of CS. The MISSION study is a retrospective study aimed at assessing the mortality in a large population of patients with all types of CS followed-up in many clinical centres around the world. This report describes the preliminary results of the study. The total number of patients registered in the database was 5092, (79% females and 21% males), with the following distribution of race: 83% Whites, 9% Oriental, 4% Indians, 3% Hispanics and 1% Blacks. Pituitary tumors (66%) represent the most frequent etiology, followed by adrenal diseases (21%), and ectopic disease (5%); a certain number of patients with non definitive diagnosis were classified as ACTH-dependent diseases (5%), ACTH-independent diseases (2%) and disease of unknown origin (1%). The overall mortality accounts for 7% (356 deaths) with a statistically significant difference between patients with active and with inactive disease ( $P < 0.01$ ) and between males and females ( $P < 0.01$ ). The mortality among patients with pituitary adenoma is 5% (168/3661) and it's the same of the patients with adrenal adenoma whereas as expected for patients with adrenal carcinoma and ectopic tumor, the mortality is significantly higher than pituitary and adrenal adenoma, being 43.5% (89/206) and 26% (66/255) respectively. Excluding the unknown causes, and the caused associated with tumor progression (21/356, 6%), severe infections or sepsis represent the most frequent (60/356, 17%), while heart stroke (32/356, 9%) and brain stroke (21/356, 6%) are the second and third causes of death. Hypertension represents the most common complication (72%), followed by dyslipidemia (44%) and glucose intolerance (39%), psychiatric diseases (17%), heart diseases (16%), infections (12%), coagulopathy (12%) and brain diseases (6%). In conclusion, the preliminary results of this study including more than 5000 patients confirms that CS is associated with an high mortality rate due to sepsis and cardiovascular accidents, and associated with cardiovascular, metabolic and infective complications.

### P33

#### Response of human adrenocortical carcinoma cell lines to cytostatic drugs and mitotane and the role of the multidrug resistance pump MDR1

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#### Introduction

In case of inoperable disease or tumor recurrence, therapy with mitotane (MIT), sometimes in combination with doxorubicin (DOX), etoposide (ETO), cisplatin (CIS) or streptozocin (STREP), is frequently applied in patients with adrenocortical carcinoma (ACC). The multidrug resistance pump MDR1 is responsible for resistance of tumor cells to cytostatic drugs. The activity of MDR1 is inhibited by the calcium-channel blocker verapamil. There are equivocal data in the literature regarding the efficacy of cytostatic compounds in the treatment of ACC.

#### Aim of the study

To evaluate the effects of MIT and verapamil, in combination with cytostatic drugs, on the growth of human ACC.

#### Methods

The human ACC cell lines H295, HAC-15 and SW13 were treated with increasing concentrations of cytostatic drugs (range: 10 nM–100 μM), with or without MIT (IC<sub>50</sub>) or verapamil (10 μM). After 144 h, cells were harvested for determination of cell number. MDR1 mRNA expression was measured in a series of adrenal tissues by qPCR.

#### Results

H295, HAC-15 and SW13 cell lines showed a dose-dependent inhibitory response to DOX (EC<sub>50</sub> range: 0.01–0.09 μM), ETO (EC<sub>50</sub> range: 0.62–0.79 μM), CIS (EC<sub>50</sub> range: 0.06–0.53 μM) and MIT (EC<sub>50</sub> range: 5.81–10.00 μM), but not to STREP. The effects of the cytostatic drugs and MIT were only partially additive. MDR1 expression was detected in H295 and HAC-15, but not in SW13 cells. MDR1 mRNA was detected in normal adrenal, adrenal hyperplasia, adrenal adenoma, and at a significantly lower level in ACC ( $P < 0.005$ ). Verapamil increased the sensitivity of H295 and HAC-15 to DOX (10- and 13-fold respectively) and ETO (3- and 3.5-fold respectively).

#### Conclusions

The cytostatic drugs, except STREP, used for treatment of patients with ACC have an inhibitory effect on growth of human ACC cell lines. The effects of the cytostatic drugs are only partially additive with the effects of MIT. Inhibition of MDR1 by verapamil increases sensitivity to DOX and ETO, suggesting that MDR1 plays a role in the sensitivity of ACC to these cytostatic drugs.

**P34****Norwegian patients with congenital adrenal hyperplasia have a high frequency of adrenal myelolipomas and adrenal hypoplasia, and testicular adrenal rest tumours was only detected in men with the saltwasting form**I N Nermoen<sup>1,2</sup>, J R Rørvik<sup>3,4</sup>, S H H Holmedal<sup>5</sup>, D L H Hykkerud<sup>5</sup>, K J Fougner<sup>6,11</sup>, J S Svartberg<sup>7,8</sup>, E S H Husebye<sup>9,10</sup> & K L Løvås<sup>9,10</sup>

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**Background**

Increased frequencies of adrenal tumours and testicular adrenal rest tumours (TART) have been reported in congenital adrenal hyperplasia (CAH).

**Objective**

To investigate the frequency of adrenal abnormalities and TART in an unselected adult population of CAH due to 21-hydroxylase deficiency (21-OH) and whether adrenal and testicular pathology correlate with disease categories, current hormone levels and treatment.

**Patients, methods and design**

From a cross-sectional population-based study of 101 adult Norwegian patients with classical CAH, sixty-three men ( $n=23$ ) and women ( $n=40$ ) participated in this study (age range 18–75, response rate 62%); thirty-three were salt-wasting (SW) and 30 were simple virilising (SV). The patients were investigated with adrenal computed tomography (CT), testicular ultrasound, and morning hormones were measured after overnight medication fast.

**Results**

Adrenal tumours were detected in seven (11%) patients (bilateral tumours in two); four were myelolipomas and one was a pheochromocytoma. Adrenal hypoplasia was found in eight (13%) patients, whereas 36 (57%) had hyperplasia, and 17 (27%) normal adrenal size. Abnormal adrenal CT scans were significantly more frequent in SW compared to SV. Males had significantly larger adrenal volumes and more tumours than females. TART was present in 33% of the men and occurred only with SW. Morning ACTH levels correlated positively with total adrenal volume and the frequency of TART.

**Conclusions**

In this population-based survey of patients with classical CAH, we found a high frequency of adrenal tumours, particularly myelolipomas, hypoplasia and hyperplasia. TART was frequently found in CAH men with SW.

**Methods**

Total RNAs was extracted from 10 CSA and from 6 surrounding adrenal tissues. Quantitative real-time PCR was used to measure LXRalpha and LXRbeta transcripts. Primary culture of adrenal cells were set up from 2 CSAs and treated with 1  $\mu$ M T09 or solvent as control. Cultures were harvested after 6 h (for expression study), and after 24–48 h (for cortisol measurement by ELISA assay).

**Results**

LXRalpha and LXRbeta transcripts were found in CSA and surrounding adrenal tissue samples, and in primary cultured cells. LXRalpha resulted less abundant than LXRbeta in all samples; moreover, LXRalpha mRNA levels were lower in CSA than in surrounding adrenal tissues. LXRalpha expression increased after T09 treatment ( $P<0.01$ ) in cultured adrenal cells. T09 induced also a 35% decrease of cortisol level after 48 h.

**Conclusion**

We demonstrate that LXRs are expressed in CSAs, with a lesser evidence of LXRalpha. LXR agonist modulates positively LXRalpha level and negatively cortisol secretion in primary cultured adrenal cells derived from CSAs. These data suggest a role of LXR mediated pathway in the pathogenesis of CSAs, and a potential use of LXR ligands in the control of cortisol secretion.

**P36****Radiosurgery with application of protone beam in patients with Cushing's disease: a hormonal monitoring of the long-term results**

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Patients with Cushing's disease were treated with proton beam surgery (pituitary irradiation) and the long-term results were studied. Hormone levels in 197 patients with the verified Cushing's disease (161 woman and 36 men) were monitored after proton irradiation. Age of patients before irradiation is 17–54 years. Duration of supervision is 1–24 years.

In peripheral blood, the concentration of pituitary hormones (ACTH, prolactin, LH, FSH, TSH, GH), and hormones of peripheral glands (cortisol, estradiol, testosterone, free thyroxine) and free cortisol in daily urine were measured. ACTH and cortisol were measured at 0800 and 2300 h in order to follow the daily dynamics of their secretion.

Full remission of the basic disease as judged by the hormonal parameters was found in 83.6% ( $1.5 \pm 1.8$  years after the therapy). Remission without the subsequent decrease of pituitary functions was observed in 21.8%. Multiple side effects of irradiation were observed in 50.1%, whereas single side effects were registered in 27.4%. The main side effects were: adrenal insufficiency – 29.7%, secondary hypothyroidism – 33.8%, hypogonadism – 47.4%, hyperprolactinemia – 36.7%.

We conclude that the first side effect to appear is adrenal insufficiency ( $2.5 \pm 2.3$  years), then increase of prolactin and decrease of gonadotropins (consecutively,  $3.0 \pm 2.9$  and  $3.0 \pm 3.1$  years). The last side effect to appear is secondary hypothyroidism ( $5.0 \pm 3.2$  years).

Biochemical hormonal monitoring is essential requirement of the dynamic follow-up of patients with Cushing's disease during lifelong period of time for timely revealing of hypopituitarism and appointments of corresponding therapy.

**P35****Presence of liver X receptors alfa (LXRalpha) and beta (LXRbeta) in cortisol-secreting adrenal adenomas**G Bellastella, D Visconti, G Conzo, V Rossi, V Palumbo, A De Bellis, A Bellastella & A A Sinisi  
Clinical and Experimental Medicine and Surgery, Second University of Napoli, Napoli, Italy.

Liver X receptors (LXRalpha and LXRbeta) act on the cholesterol homeostasis in several tissues, including adrenals. In particular, LXRalpha regulates the expression of several genes involved in the cholesterol efflux and storage and modulates glucocorticoid synthesis in mice. The level of the expression of LXRs in the human adrenal adenomas is unknown. We evaluated LXRalpha and LXRbeta mRNA levels in cortisol-secreting adenomas (CSA) and in the surrounding normal tissue, obtained from patients with Cushing syndrome underwent unilateral adrenalectomy; moreover, we studied the effects of the LXR agonist T09 on LXRs expression level and on cortisol release in adrenal cell primary cultures derived from CSA.

**P37****Thoracic and gastroenteropancreatic (GEP) neuroendocrine tumors (NETs) and the ectopic ACTH syndrome (EAS)**K Kamp, A Alwani, R A Feelders & W W de Herder  
Erasmus MC, Rotterdam, The Netherlands.**Introduction**

The ectopic ACTH syndrome (EAS) is associated with a variety of malignancies, mostly of (neuro-)endocrine origin. Several series report on the relative contribution of EAS in the spectrum of Cushing's syndrome. However, information on the incidence/prevalence of EAS in the setting of patients diagnosed with thoracic or gastroenteropancreatic (GEP) neuroendocrine tumours (NETs) is virtually absent.

#### Study design

In our tertiary academic referral centre for NET patients and also for patients with Cushing's syndrome, a large consecutive series of thoracic NET and GEP NET patients was screened for the presence/occurrence of EAS.

#### Patients

Four hundred and ninety-one patients, diagnosed with thoracic or GEP NETs, referred between 1-1-2000 and 1-12-2009, were studied.

We differentiated between previous, synchronous and metachronous occurrence of EAS. Synchronous EAS was defined as occurring between 6 months before and 6 months after the date of first diagnosis of the thoracic or GEP NET.

Patients with MEN-1 were excluded. Small cell lung cancer was also excluded.

#### Results

This series comprised of 491 patients with thoracic and GEP NETs (258 males, 233 females; female to male ratio, 1.1:1; mean age, 58.1 years).

Ten patients (2.0%) had EAS (5 males, 7 females; mean age, 53.1 years). All patients had synchronous EAS. Five patients had a bronchial NET, three had a thymic NET and two had a pancreatic NET.

#### Conclusions

The incidence of EAS in patients with thoracic and GEP NETs is very low (2.0%). In line with the literature thoracic NETs (thymis and bronchial carcinoids) were the most frequent cause of EAS. Pancreatic NETs are the most common GEP-NETs causing EAS.

#### Aim

To investigate QOL of patients in long-term remission of CS treated in our hospital (1969–2007) and to investigate the influence of the etiology of CS, treatment strategy, coexistent hypopituitarism and glucocorticoid deficiency on the QOL.

#### Patients and methods

Seven validated questionnaires to evaluate QOL were sent to 182 patients in remission of CS. One hundred and twenty-four patients (107 women, age  $52.2 \pm 12.0$  years, 80% Cushing's disease (CD) and 20% adrenal CS) completed the questionnaires. Mean duration of remission was  $13.3 \pm 10.4$  years (range 2–39). Questionnaire results of the patient group were compared with those of an age and sex matched control group of 105 healthy subjects. Furthermore, the results of different subgroups of patients were compared.

#### Results

The average QOL scores in the total patient group and each patient subgroup were significantly worse on all scales of all questionnaires compared to healthy controls ( $P < 0.05$ ). The subgroup analysis revealed no difference between the QOL scores in the different patient groups. In particular, there was no significant difference in QOL between patients without any hormonal deficiencies and patients with one or more hormonal deficiencies. No other patient and disease related factors significantly influenced the QOL.

#### Conclusion

Our patients in long-term remission of CS experience an impaired QOL compared to healthy control subjects, regardless of the etiology of CS, treatment strategy, coexistent hypopituitarism or glucocorticoid deficiency. Based on these results we hypothesize that the previous longstanding exposure to high cortisol levels may have irreversible effects resulting in impaired QOL, although the previous experience of a severe disease may also influence QOL.

### P38

#### The role of insulin tolerance test (ITT) and ACTH/cortisol ratio in chronic fatigue patients

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#### Rationale

In chronic fatigue syndrome (CFS) there is a great controversy about the role of the ACTH/cortisol axis. To examine this phenomena we performed ITT tests in CFS patients with low fasting cortisol ( $< 20 \mu\text{g/dl}$ ). A group of CFS patients with other hypofysal dysfunction were also examined.

#### Methods

One hundred and one patients who underwent classical fasting ITT were registered and analyzed: glucose, cortisol, ACTH and GH were measured and patients were divided in five groups according to the cortisol response or the presence of other hypofyse deficiency. ACTH/cortisol in groups was measured with the peak values during ITT.

#### Result/conclusion

Sixty-seven patients have an elevated ACTH/cortisol ratio: 8 and 29 had a normal ratio of 1.9. Patients with peak cortisol response above 30 show no ACTH increase. The elevated ACTH/cortisol ratio is a very useful tool in the early detection of adrenal insufficiency.

Therefore, ITT testing is preferred in CFS patients, instead of synacthen testing, because the phenomenon of ACTH compensation can be measured and reflected in the ACTH/cortisol ratio.

### P39

#### Quality of life in patients in long-term remission of Cushing's syndrome is impaired, regardless of etiology, treatment strategy or remaining hormonal deficiencies

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#### Background

Impaired health related quality of life (QOL) in patients in long-term remission of Cushing's syndrome (CS) has previously been reported. However, the major determinants of the impaired QOL have not yet been established.

### P40

#### Sunitinib in refractory adrenocortical carcinoma: results of a phase II trial

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#### Background

Adrenocortical carcinoma (ACC) is a rare solid tumor with poor prognosis in advanced stages. The adrenolytic drug mitotane and cytotoxic chemotherapies are current treatment options with limited clinical efficacy. Animal experiments pointed to an adrenotoxic effect of sunitinib suggesting potential antineoplastic activity in ACC.

#### Study population

Thirty-eight patients with advanced ACC progressing after mitotane and 1–3 cytotoxic chemotherapies were included. Mitotane treatment was ongoing in 20 patients.

#### Primary endpoint

Response defined as progression free survival of  $\geq 12$  weeks.

#### Results

Three patients were unevaluable for response due to withdrawal of consent, noncompliance with the study procedures or a serious adverse event. Of the 35 patients analyzed for response, 5 patients (14%) experienced stable disease (median time to progression 5.8 months, range 5.6–11.2), 24 had progressive disease according to RECIST criteria and 6 patients died of the disease before the first evaluation. Median time to progression was 2.8 months (0.3–5.7). In total, 36 serious adverse events were recorded of which 10 were possibly treatment related. Only 41 treatment-related adverse events were documented (mostly CTC grade 1+2). Surprisingly, the response rate was higher in patients not receiving mitotane (4 out of 15 vs 1 out of 20) suggesting that concomitant mitotane treatment may negatively affect clinical outcome (HR for progressive disease 6.9 (95% CI 0.7–69.9)). Therefore, we hypothesize that mitotane may lead to decreased sunitinib plasma concentrations, probably by induction of metabolizing enzymes. This could also explain the low frequency of adverse events which was much lower than expected from other sunitinib trials.

#### Conclusion

Sunitinib has a modest single-agent activity in ACC. Drug interaction with mitotane may abrogate a more impressive anti-tumor effect of sunitinib. Therefore, a clinical trial of sunitinib in mitotane naïve patients might be warranted.

**P41****Adrenal insufficiency: etiopathogeny and diagnostic aspects**Corina Crista<sup>1</sup> & Corina Laura Niederl<sup>2</sup><sup>1</sup>Clinic of Endocrinology, University of Medicine and Pharmacy 'Victor Babes', Timisoara, Romania; <sup>2</sup>Student in the VIth year, University of Medicine and Pharmacy 'Victor Babes', Timisoara, Romania.

The diagnosis of the adrenal insufficiency requires complex clinical, laboratory and imagistic investigations. The study group was represented by 59 cases of adrenal insufficiency hospitalized in the Clinic of Endocrinology Timisoara, Romania, during the period 2000–2010 (age = 42.10 ± 16.30 years; F/M ratio = 43/16). The patients were divided in two groups: primary adrenal insufficiency (42.37%) and secondary adrenal insufficiency (57.63%). In the group of primary adrenal insufficiency, the autoimmune Addison's disease represented 84% cases while the pituitary tumors had the highest incidence (44.12%) between the causes of the secondary adrenal insufficiency followed by Sheehan's syndrome (29.41%). Forty-eight percent cases of autoimmune Addison's disease associated different autoimmune disorders, like: chronic autoimmune thyroiditis (66.67% cases), Graves' disease (25% cases), gonadal failure, vitiligo, rheumatoid arthritis. Two patients with autoimmune Addison's disease presented subclinical adrenal insufficiency and were diagnosed by using tetracosactrin stimulation test. Only 6 cases of secondary adrenal insufficiency presented a normal rise of the cortisol serum levels at 24–48 h after depot tetracosactrin administration. The patients with secondary adrenal insufficiency and associated central hypothyroidism presented a significant lower serum FT<sub>4</sub> level than the patients with autoimmune Addison's disease associated with primary hypothyroidism ( $P < 0.01$ ).

**Endocrine disruptors****P42****Evaluation of the effect of L-thyroxine therapy on endothelial functions in patients with subclinical hypothyroidism**Fatma Alibaz Oner<sup>1</sup>, Selen Yurdakul<sup>2</sup>, Ender Oner<sup>3</sup>, Ayse Kubat Uzum<sup>4</sup> & Mecdi Erguney<sup>5</sup><sup>1</sup>Marmara University Faculty of Medicine, Rheumatology, Istanbul, Turkey; <sup>2</sup>Cardiology Division, Istanbul Florence Nightingale Hospital, Istanbul, Turkey; <sup>3</sup>Coronary Intensive Care Unit, Istanbul Education and Research Hospital, Istanbul, Turkey; <sup>4</sup>Istanbul Education and Research Hospital, Endocrinology and Metabolism, Istanbul, Turkey; <sup>5</sup>Education and Research Hospital, Clinic of Internal Medicine, Istanbul, Turkey.**Objectives**

Subclinical hypothyroidism (SH) is characterized by normal serum free T<sub>4</sub> (FT<sub>4</sub>), free T<sub>3</sub> (FT<sub>3</sub>) levels and increased serum TSH levels. Endothelial dysfunction that is accepted an early step of the atherosclerosis, has been reported in patients with subclinical hypothyroidism. The aim of this study was to evaluate endothelial functions and the effect of L-thyroxine (L-T<sub>4</sub>) therapy on endothelial functions in SH.

**Methods**

Twenty-seven patients with SH and 22 healthy controls were evaluated by brachial ultrasonography for endothelial functions. After restoring euthyroidism in SH, measurements were repeated.

**Results**

The patients with subclinical hypothyroidism and healthy control group were similar in terms of baseline and nitrate induced diameters of brachial artery. Compared to the control group, the patients with SH showed significantly reduced FMD. The baseline and nitrate induced diameters of brachial artery were significantly high after L-T<sub>4</sub> in SH group. Besides, FMD significantly increased in patients with SH.

**Conclusions**

Hypothyroidism accelerates atherogenesis through modification of atherosclerotic risk factors and direct effects on the blood vessels. In the present study, we found significantly improvement in FMD with L-T<sub>4</sub> therapy. This improvement in endothelial function could translate into reduction in cardiovascular mortality and morbidity.

**P43****Evaluation of the incidence of disorders glycoregulation in some diseases endocrine**I Bistriceanu<sup>1</sup>, M E Preda<sup>1</sup>, M Bistriceanu<sup>2</sup>, L Putinelu<sup>3</sup>, S Bondari<sup>2</sup>, A Covei<sup>1</sup>, A Turcu<sup>2</sup> & A Voican<sup>2</sup><sup>1</sup>Department of Endocrinology, Emergency Hospital, Craiova, Romania; <sup>2</sup>Department of Endocrinology, University of Medicine and Pharmacy, Craiova, Romania; <sup>3</sup>Department of Geriatry, Chronics Affections and Geriatric Hospital's, Stefanesti Arges, Romania.

Glycoregulation disorders in endocrine are frequently, hormone excess or deficit resulting for impaired metabolism of glucose by different mechanisms.

**Methods**

The study was conducted on 118 patients with hyperthyroidism, acromegaly and hypercortisolism. The inclusion criteria were: patients nondiagnostics that have not followed the treatment with drugs that could induce hyperglycemia (epinephrine, oral contraceptives, glucocorticoids, mineralocorticoids etc.), no-collateral family history of diabetes mellitus. Making glucose tolerance test with 75 g glucose pulvis with glucose assessment at 1 and 2 h after glucose ingestion, allowed in varying proportions the detection of diabetes mellitus in the study.

**Results**

Glycoregulation disorders were present in 58% of hyperthyroidism, 46% of patients with acromegaly, 53% of those with hypercortisolism. Altered carbohydrate metabolism was directly proportional to the excess hormone level, on the types of diseases studied.

**Conclusions**

Differential treatment specific endocrine disease, improves the disorder of glycoregulation occurred in the context of hormonal dysfunction.

**P44****Real-idiopathic precocious puberty: therapeutic approach**I Bistriceanu<sup>1</sup>, M E Preda<sup>1</sup>, M Bistriceanu<sup>2</sup>, L Putinelu<sup>3</sup>, S Bondari<sup>2</sup>, A Covei<sup>1</sup>, A Turcu<sup>2</sup> & D Bondari<sup>2</sup><sup>1</sup>Department of Endocrinology, Emergency Hospital, Craiova, Romania; <sup>2</sup>Department of Endocrinology, University of Medicine and Pharmacy, Craiova, Romania; <sup>3</sup>Department of Geriatry, Chronics Affections and Geriatric Hospital's, Stefanesti Arges, Romania.

Early sexualisation defines the appearance of any sign of sexual maturation at a younger age than 2 s.d. from the mean, namely <8 years at girls and 9 years at boys. The really precocious puberty is always isosexual, sexuality program runs in line with genetic sex and gonads of the subject. In making her part of the whole system gonadal function: the hypothalamus–pituitary–gonad–receptors, and gonads is fully enabled not only hormonogenetic, both also gametogenetic.

**Methods**

The study was conducted on six girls, aged 6–8 years whose sexual characters were installed early (thelarche – 4 years) and one of them (MF – 7 years), menarche at 6 years with the cyclical trend (monthly). Diagnostic algorithm that we applied was represented by: assessing LH and FSH to GnRH test. As MRI brain imaging was performed.

**Results**

LH and FSH values were increased (>190 mUI/ml) and the GnRH test, the response was normal. MRI brain showed no signal changes or gadolinium signal the pituitary parenchyma, which is why the cases were labeled as the treatment of precocious idiopathic puberty. Is established analogues of LH–RH agonists (Triptorellin 3.75 mg/month) to reduce low-level regulation of gonadotrophin receptors and LH–RH. After 6 months of treatment, her periods have been suspended from patient MF – 7 years, and leap stature was only 1 cm.

**Conclusions**

Early puberty causes two formidable complications: i) a stature and bone age advance, which compromise the final size by early fusion of growth cartilage; ii) The emotional disturbance resulting from development of sexual characteristics in the absence of adequate intellectual maturation and somatic; consequently, response to treatment should be monitored and will appreciate the opportunity to amend the therapeutic regimen.

**P45****Haematological and reproductive toxicity of nicotine and protective role of green tea extract in male rats**Mosbah Rachid<sup>1</sup>, Haroun Nacera<sup>1</sup>, Boulakoud Mohamed Salah<sup>2</sup> & Yousef Mokhtar Ibrahim<sup>3</sup><sup>1</sup>Department of Biology, Faculty of Sciences/University of Boumerdes, Boumerdes, Algeria; <sup>2</sup>University of Annaba, Annaba, Algeria; <sup>3</sup>University of Alexandria, Alexandria, Egypt.

Nicotine (N) is known as the main toxic and addictive component of cigarette smoke. By its ability to generate reactive oxygen species (ROS) and oxidative damage in organism, it can induce major public health problems such as cancer, cardiovascular diseases and infertility. Green tea extract (GTE) is an important source of polyphenols catechins; it has attracted a great deal of attention around the world because of its potential antioxidants role in scavenging free radicals. This research was conducted to determine the potential role of green tea extract in attenuating the toxic effects that may be caused by nicotine on haematological and reproductive parameters in rat. Thirty-two adult Wistar male rats were randomly divided into four groups/8 for each. Group I served as control. Group II rats received orally GTE (2% w/v) as the sole drinking fluid. Group III rats were injected i.p. by nicotine (1 mg/kg bw). Group IV received i.p. nicotine (1 mg/kg bw) and GTE (2% w/v) concomitantly. After 2 months of treatment, blood samples were collected for measuring haematological parameters. Then, after necropsy, reproductive organs (testes, epididymes, and seminal vesicles) were removed, weighed and used for measuring semen quality and histology. The results show a decrease in red blood cells (RBC) count, haemoglobin and haematocrit with an increase in VGM in all treated groups as compared to control; while leucocytes (WBC) count has increased in nicotine group, diminished in GTE group and adjusted to normal values in GTE plus nicotine treated animals. Furthermore, nicotine decreases significantly the body weight gain and relative weights of reproductive organs; this toxic effect was removed by GTE coadministration. Sperm analysis and histological examination of testes revealed that nicotine reduced significantly sperm count, spermatid number, sperm daily production, motility, percentage of normal sperm morphology as well as induced histological alterations pronounced by atrophy, degenerative aspect of the seminal epithelium in some seminiferous tubules, large interstitial spaces with small number of Leydig cells, perturbation in spermatogenesis leading to absence of spermatozoa and presence of cell debris in the lumen of some seminiferous tubules. All these adverse reproductive effects of nicotine were attenuated by GTE coadministration. It is concluded that GTE beverage produces a deleterious synergistic effect with nicotine on hematological parameters and plays protective role against nicotine-induced reproductive effects to improve semen quality and histological damage of testis.

**P46****Bone remodeling parameters and bone mass in patients with schizophrenia treated with long-acting injectable risperidone**M Doknic<sup>1</sup>, N Maric Bojovic<sup>2</sup>, S Pekic<sup>1</sup>, D Britvic<sup>2</sup>, A Damjanovic<sup>2</sup>, M Jasovic Gasic<sup>2</sup> & V Popovic<sup>1</sup><sup>1</sup>Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center Serbia and School of Medicine, University of Belgrade, Belgrade, Serbia; <sup>2</sup>Institute of Psychiatry, Clinical Center Serbia and School of Medicine, University of Belgrade, Belgrade, Serbia.**Introduction**

Studies suggest that schizophrenia and prolactin raising antipsychotics, one of them being risperidone, may be associated with low bone mass. On the other hand, weight gain as a consequence of antipsychotics in these patients may be the protective factor against osteoporosis.

**Objective**

Determine bone remodeling parameters and bone mass in patients with schizophrenia treated with long-acting injectable risperidone (LAIR) under naturalistic conditions.

**Patients**

Twenty-six out-patients with controlled schizophrenia (age  $31.3 \pm 1.3$  years; BMI  $28.1 \pm 1.0$  kg/m<sup>2</sup>) on maintenance therapy with LAIR for mean  $18.0 \pm 1.6$  months (range 6–36 months) with mean dose  $38 \pm 2$  mg. Thirty-five subjects matched by sex, age, BMI and education served as healthy controls.

**Methods**

Serum osteocalcin, C-terminal telopeptide of type I collagen (CTX), vitamin D, prolactin, sex steroids and PTH were assessed. Bone mineral density (BMD) was measured by dual-X ray absorptiometry at lumbar spine (LS) and femoral neck (FN). Results

Osteocalcin did not differ between patients with schizophrenia compared with healthy subjects, while serum CTx was significantly higher in patients with schizophrenia ( $P=0.023$ ). Patients with schizophrenia had lower LS BMD and LS Z-scores than healthy subjects, but not statistically significant ( $P=0.094$ ,  $P=0.072$  respectively) while at femoral neck (FN) there was no difference between two groups. Vitamin D deficiency was prevalent in patients with schizophrenia. Hyperprolactinemia was detected in 85% of patients, more commonly in females than males (all females and 67% males). Serum estradiol levels were lower in female patients than in healthy female subjects ( $P=0.001$ ), while serum testosterone in males showed no significant differences ( $P=0.675$ ). The percentage increase in body weight for all patients with schizophrenia on LAIR therapy was  $4.7 \pm 1.5\%$ .

**Conclusion**

Despite weight gain long-term administration of LAIR increased bone resorption marker CTx possibly through hyperprolactinemia-induced hypogonadism and/or possibly by decreasing brain serotonin activity. Brain serotonin has been recently shown to increase bone accrual and influence weight.

**P47****Prognostic factors of non-alcoholic steatohepatitis associated with obesity**

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Non-alcoholic fatty liver disease (NAFLD), currently considered a major problem of public health, includes fatty liver alone, steatohepatitis, fibrosis, cirrhosis. Obesity increases the risk of NAFLD in the patients having BMI  $> 30$  kg/m<sup>2</sup>, where the liver involvement is a common aspect.

**Aim**

To find non-invasive methods for the selection of patients with risk of progression of liver disease.

**Materials and methods**

We selected a group of 54 patients (31 female, 23 male) and compared the non-invasive methods – anthropometric measurements, biochemical exploration, adipokines (leptin and adiponectin) and ultrasound exploration – with the histological specimen obtained through liver biopsy (Matteoni classification).

**Results**

The histological alterations correlated with the elevated serum levels in triglycerides and ALAT and the BMI ( $P=0.008$ ). Aminotransferases, GGT, triglycerides and leptin had elevated levels in all patients. Adiponectin had negative correlation to the histological classification, whereas leptin correlated positively with the abdominal circumference, BMI and ultrasound aspect of the liver.

**Conclusions**

In our study, the anthropometric measurements, biochemical markers and liver ultrasound did not prove they can replace the liver biopsy. However, we recorded some association between BMI, high levels of triglycerides and BAAT score, low level of adiponectin that represent non-invasive markers for the selection of patients who require liver biopsy.

**P48****The role of progestins in the interplay of estradiol and genistein: influences on the steroid hormone receptor expression patterns in distinct uterine compartments**F Möller<sup>1</sup>, P Hönschied<sup>1</sup>, O Zierau<sup>1</sup>, P Diel<sup>2</sup> & G Vollmer<sup>1</sup><sup>1</sup>Technische Universität Dresden, Institute for Zoology, Chair for Molecular Cell Physiology and Endocrinology, Dresden, Germany; <sup>2</sup>Department of Molecular and Cellular Sports Medicine, Institute of Sports Medicine, German Sports University Cologne, Cologne, Germany.

Ovariectomized (OVX) rats are commonly used for the investigation of estrogenic compounds *in vivo*. We previously showed that estrogen treated

OVX animals apparently respond differently to Genistein (GEN) treatment than hormonally intact animals. An observation, that indicated that progestins might interfere in the estradiol (E<sub>2</sub>)-GEN interaction.

As the molecular effects of GEN seem to be modified by progesterone (P<sub>4</sub>), its role should be determined in presence and absence of E<sub>2</sub>. Therefore, we performed an animal experiment with adult Wistar rats which were either OVX or sham operated. These animals were s.c. treated with E<sub>2</sub> (1 µg/kg BW and d), P<sub>4</sub> (40 mg/kg BW and d) or GEN (10 mg/kg BW and d) either as single compounds or as their respective mixtures. As readout, we assessed the mRNA and protein expression pattern of the progesterone receptor (PGR) as well as of both estrogen receptor subtypes.

Performing immunohistochemical staining of OVX rat uterus cross-sections, we generally found higher PGR protein expression levels in the luminal epithelium compared to the stroma. Following E<sub>2</sub> treatment, the PGR expression level was increased in both luminal epithelium and stroma respectively. The comparison of the two uterine tissue compartments showed, that the expression increases predominantly in the stroma (twofold increase). In contrast to the other combinatorial treatments involving E<sub>2</sub> ('E<sub>2</sub>-P<sub>4</sub>' and 'E<sub>2</sub>-P<sub>4</sub>-GEN'), the E<sub>2</sub>-free combination of 'P<sub>4</sub>-GEN' showed no effect on overall PGR expression, although there might be a shift from epithelial to the stromal expression.

In conclusion, our results indicate that E<sub>2</sub> triggered estrogenic responses on PGR expression are detectable throughout all uterine tissue compartments. With regard to the fact that they are more pronounced in the stroma, compared to the luminal epithelium, the importance of the stroma as a hormone response regulating tissue is accentuated.

## P49

### Serum procalcitonin measurement as a marker of sepsis

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Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin. Procalcitonin is synthesized by a large number of tissues and organs in response to invasion by pathogenic bacteria, fungi and some parasites. Its a new and innovative parameter which reacts with high sensitivity and specificity to generalized infection and sepsis.

#### Objective

The aim of the study was to assess PCT value for the diagnosis of systemic infection.

#### Methods and materials

PCT was measured in 67 patients evaluated in our service during May-December 2010. We used Elycsys BRAHMS PCT test electro chemiluminescent immunoassay (ECLIA) for the determination of procalcitonin. In Albania, PCT has been used as a serum marker for bacterial sepsis since May, 2010. We used levels of  $\geq 2$  ng/ml as PCT positive for sepsis.

#### Results

Mean age was 44.53 years old  $\pm 17.32$  s.d., range 15-75. Female 52.3% and males 42.7%. 67.1% of patients were admitted to hospital and blood culture was performed to 88.8% of them. Based on clinical and laboratory features (a cut off PCT  $\geq 2$  ng/ml) 29.8% of patients were confirmed as sepsis. Mean age of them was 46.5 years old  $\pm 20.7$  s.d.. PCT was very high (4.1-100 ng/ml) in patients with sepsis with average value 23.65 ng/ml. A statistically significant higher PCT level was observed in patients with sepsis compared to those without sepsis (23.65  $\pm 21.3$  vs 0.5  $\pm 1.4$  respectively). In 71.2% of cases (patients without sepsis) PCT was under 2 ng/ml.

#### Conclusion

The serum concentration of PCT is specifically elevated in sepsis patients. Measurement of PCT blood level is a simple and specific test and may be recommended in the diagnosis of systemic infection. Procalcitonin should be included in diagnostic and clinical practice guidelines for sepsis. Both absolute values and variations should be considered and evaluated in further studies.

## P50

### Parathyroid adenoma screening efficacies of different imaging tools and factors affecting the success rates

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#### Introduction

To evaluate the efficacy of 3 different parathyroid adenoma screening tools (high resolution ultrasonography (USG), technetium Tc 99m-methoxyisobutylisonitrile (MIBI) parathyroid scintigraphy and magnetic resonance imaging (MR)) and to determine factors affecting the detection success rates.

#### Methods

Thirty-nine patients (29 female and 10 male) with primary hyperparathyroidism were included in the study. Patient's mean age was 53.9  $\pm$  14.1. Patients were examined with USG, 99mTc-MIBI parathyroid scintigraphy and cervical MR imaging to detect hyperplastic parathyroid tissue and parathyroid adenoma and compare the 3 different imaging tools according to the detection success.

#### Results

Patient's mean age was 53.2  $\pm$  13.7. Mean calcium (Ca), 24 h urine Ca, ALP and parathyroid hormone levels were 11.6  $\pm$  1.3 mg/dl, 624.8  $\pm$  305.7 mg/day, 197.7  $\pm$  84.7 U/l and 254.9  $\pm$  212.7 ng/ml respectively. Among the 39 patients; USG, parathyroid scintigraphy and cervical MR imaging correctly identified 35 adenomas (89.7%), 28 adenomas (71.8%) and 26 adenomas (66.7%) respectively. Positive predictive value for USG (34/35), scintigraphy (27/28) and cervical MR (26/26) imaging were 97.1, 96 and 100%. Parathyroid adenomas were detected with 92.3% (36/39) certainty when both USG and scintigraphy modalities were applied together. Minimally invasive parathyroidectomy under local anesthesia with unilateral incision was successfully performed in 24 (% 61.5) of 39 patients.

#### Conclusions

Minimally invasive surgery for parathyroid adenomas is in development and has equal success with traditional surgery however there is need for accurate localization of adenomas, in this study scintigraphy and ultrasound combination was found to be efficient in localizing adenomas for successful surgery. MR imaging should be reserved for detecting ectopic parathyroid tissue.

## P51

### In vitro testing for goitrogenic potential of glucosinolate extracts

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#### Introduction

Glucosinolates (GLS) are secondary plant metabolites found in members of families within the order Brassicales (e.g. broccoli, pak choi, cauliflower, kale, mustard or rapeseed), but they are also present in plants of Moringaceae (moringa) and Caricaceae (papaya). Some GLS-derived breakdown products, i.e. sulforaphane, were extensively studied for their beneficial effects on colon-cancer incidence. This preventive nutritional approach might require the prophylactic use of highly dosed, enriched GLS-preparations of *Brassica* plant extracts. Goitrogenicity is a known consequence of nutrition containing high *Brassica* content due to the release of thiocyanate and inhibition of thyroperoxidase (TPO). Detailed and recent studies about this topic are rare, but nevertheless needed for a potential preventive or therapeutic use of GLS. Therefore, we used several *in vitro* approaches to assess the goitrogenic potential of various GLS-extracts.

#### Hypothesis

Some GLS exert potential goitrogenic effects via competitive inhibition of the sodium-iodide-symporter (NIS) by thiocyanate ions and/or direct effects of specific GLS breakdown products on type 1 deiodinase or thyroperoxidase (TPO) activity.

#### Methods

NIS-inhibition was tested by their interference with non-isotopic iodide uptake into rat thyrocytes (FRTL5). Interference with Dio1 activity was detected via a non-isotopic iodide-release assay. SCN<sup>-</sup>-release from GLS was tested by a colorimetric reaction. TPO-interference was detected by applying the test compounds to recombinant TPO enzyme.



#### Results/conclusion

To investigate and exclude possible adverse GLS effects, we established a screening platform for the potential goitrogenic action of GLS and their breakdown products. Several of the GLS extracts inhibited Dio1 or TPO activity within a high supra-physiological  $\mu\text{M}$  concentration range. Furthermore, several extracts released significant amounts of SCN<sup>-</sup> after hydrolysis, thus potentially enhancing to the overall SCN<sup>-</sup> load of the organism. It appears mandatory that appropriate *in vivo* studies address these adverse GLS effects. This work is supported by the Bundesministerium für Bildung und Forschung.

#### P52

##### Phytoestrogen genistein causes downregulation of oestrogen receptor $\alpha$ in uterus of female Sprague Dawley rats

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Phytoestrogen genistein is one of the endocrine disrupting chemicals found mostly in soybean. It has become a topic of interest since decades ago in view of its benefits in reducing the risks of breast cancer, cardiovascular disease, obesity and perimenopausal symptoms. However, it has been reported that genistein can cause adverse consequences on female reproductive system and may cause infertility. Its action was said to be associated with its binding affinity to oestrogen receptor (ER) which produce both oestrogenic and antioestrogenic effects depending on dosage, time and duration of exposure. In this study, intact female juvenile Sprague Dawley rats were fed orally with genistein (10 mg/kg per day) from postnatal day 22 (PND 22) till PND 42. At the end of the experiment, animals were sacrificed and selected organs were harvested and processed for light microscopy. We found that ER- $\alpha$  in uterine tissue of rats treated with genistein were downregulated compared to the control group. This is supported by the reduced uterine weight and myometrium thickness of the uteri of genistein treated animals. In conclusion, prepubertal exposure of prepubertal female rats to genistein (10 mg/kg per day) has altered development of their uteri through its antioestrogenic effect.

#### Endocrine tumours and neoplasia

#### P53

##### Insulinoma in crete: a rare tumor?

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Insulinoma, a pancreatic  $\beta$ -cell tumor, comprises 55% of Neuroendocrine tumors with an annual incidence of 3–10/million. Female to male ratio is 3:2 presenting in their 4th decade. The main clinical symptom is severe hypoglycemia irrespective of tumor size.

#### Aim

Presentation of four newly diagnosed insulinoma patients aged 18–60, within an eight month interval.

All patients presented with neuroglycopenic symptoms (irritability, emotional lability, sluggish speech, blurred vision). On admission one patient presented with semicoma, another with acute coronary syndrome, the third had a car accident and hypoglycemia was an incidental finding in the fourth. All patients underwent prolonged fasting test. Whipple's triad, insulin glucose ratio >0.4, c-peptide >2.5 ng/ml during hypoglycemia established the diagnosis. CT and MRI scan of the abdomen revealed hypervascular lesions with intense arterial phase enhancement in the pancreas (three patients). Multiple metastatic hepatic lesions without a primary pancreatic tumor (no pancreatic lesions) were found in the fourth patient (malignant insulinoma). Chromogranin A was marginally increased only in the malignant insulinoma patient. All patients were treated initially with intravenous glucose, Diazoxide and somatostatin analogue. Two patients underwent peripheral pancreatectomy with complete remission of their hypoglycemia. One patient is scheduled for surgery. The fourth patient underwent partial hepatectomy, hepatic chemoembolism and pancreatectomy, with marked improvement of his symptoms. Ninety percent of insulinomas are benign, 10% are malignant, 10% are multiple and 50% of them present in MEN1. Benign or malignant insulinomas cannot be histologically differentiated. Surgery is the treatment of choice for benign, malignant or recurrent disease.

#### Conclusion

High clinical suspicion is a prerequisite for the diagnosis and treatment of these slow growing usually benign tumors. The sudden cluster of four insulinomas over

an 8 month period in our department with complete absence of cases over the past 23 years demands increased vigilance in case this is a non-random but a causal effect in need of further elucidation.

#### P54

##### Frequency, localisation of unusual metastases of medullary thyroid cancer and impact on survival: an observational study in 102 patients

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Medullary thyroid carcinoma (MTC) early spreads to cervical lymph nodes and to distant sites: mostly to the liver, lungs or bones. Unusual sites such as breast, skin or orbit have been also described. The aim of the study was to evaluate the frequency, localisation of unusual metastases of MTC and their impact on survival.

Eligibility criteria for this study were the presence of a pathological confirmed diagnosis of MTC with distant metastases (other than lymph nodes) and a follow-up of at least one visit between January 1st 2000 and May 1st 2010 in 7 University French hospitals.

We retained 102 patients (men/women: 57/45; multiple endocrine neoplasia (MEN) 12.7%; median age at MTC diagnosis: 50.5 yr). At the end of the study, 68.6% patients had bone metastases, 64.7% pulmonary metastases, 59.8% liver metastases. Unusual sites, diagnosed in 21 patients (20.6%), were brain, skin, pancreas, breast, peritoneum, adrenals, prostate, retina, and endometrium. Sex ratio, age at MTC diagnosis, proportion of MEN, tumor staging and calcitoninemia or ECA of metastatic patients with unusual sites were not statistically different from those of other patients. On May 1st 2010, 50 patients (49.0%) were alive, and 45 patients (44.1%) were deceased (data not available for 7 patients). The 10-year overall survival was 55.5% years after MTC diagnosis. Survival of patients with unusual metastases was 54.4% (no significant difference compared with the remaining population).

In a French population of 102 cases of distant metastatic MTC, the frequency of unusual metastases was 20.6%. These metastases were not associated with specific characteristics of patients and had no impact on 10-year survival. These metastases have to be localised in case of elevated calcitoninemia without lymph nodes, lung, bone or liver metastases.

#### P55

##### Diagnosis of post-surgical recurrence in patients with malignant insulinoma

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#### Context

The post-surgical follow-up of malignant insulinomas is often marked by recurrence after initial remission. However, the criteria for the diagnosis of recurrence of these malignant endocrine tumors are not well defined in the literature, unlike diagnostic criteria for hyperinsulinemic hypoglycaemia (Cryer 2009). We retrospectively reviewed the criteria that allowed the diagnosis of recurrence in four patients with malignant insulinoma after initial post-operative remission.

#### Patients and methods

At the initial diagnosis, two patients had a metastatic insulinoma (one in the liver, one in lymph nodes) and two patients had a single tumor whose radiological presentation was benign. After pancreatoduodenectomy ( $n=4$ ), lymph node resection ( $n=3$ ) and left hepatectomy ( $n=1$ ) patients were considered in remission, defined as R0 resection and no clinical or biological post-operative hypoglycaemia.

#### Results

The recurrence occurred after  $14.8 \pm 8.5$  months ( $m \pm s.d.$ ). Three patients had clinical signs of hypoglycaemia, but only one patient had a proved fasting hypoglycaemia (1.26 mmol/l). The remaining three patients did not reach the classical criteria of hyperinsulinemic hypoglycaemia during a 72 h-fast test ( $n=2$ ) or serial plasma glucose, insulin and C-peptide measurements (every 4 h for 24 h) the lowest plasma glucose levels in these three patients were 3.2, 2.5, and 4.3 mmol/l with concomitant C-peptide levels of 0.3, 0.5, and 0.8 ng/ml, respectively. At the same time, radiological (CT scan  $n=3$ , MRI scan  $n=1$ )

evaluation revealed lymph nodes ( $n=2$ ) or liver metastases ( $n=2$ ) attesting recurrence of malignant insulinoma.

#### Conclusion

After initial post-operative remission, the diagnosis of recurrence of malignant insulinoma cannot be based only on clinical and biological criteria, but CT or MRI scan evaluation is mandatory during post surgical follow-up of such patients.

## P56

### The T-box transcription factor TBX1, the candidate gene of 22q11.2 microdeletion/DiGeorge syndrome, is involved in human parathyroid tumorigenesis

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The embryonic transcription factor TBX1 plays a critical role in cell differentiation during organogenesis of the parathyroid glands. Here we demonstrated that TBX1 mRNA and protein were detected in human adult normal parathyroid tissues ( $n=3$ ). Immunostaining showed that TBX1 protein was expressed in endothelial CD31+,  $\alpha$ -SMA+ cells. By contrast, TBX1 mRNA and protein was expressed at higher levels in parathyroid typical adenomas ( $n=23$ ), where TBX1 staining was positive in parathyroid PTH+ cells with a cytoplasmic and nuclear localization. Functional studies were performed in HEK293 cells as they expressed TBX1. The activation of the calcium sensing receptor (CaSR) by stimulating for 24-h HEK293 cells stably transfected with the human CaSR with 1–5 mM calcium as well as with 10–100 nM R-568, the CaSR agonist cinacalcet, induced a significant reduction in TBX1 mRNA levels. Thus, CaSR downregulation in parathyroid tumours might be related with the TBX1 overexpression. Overexpression of TBX1 mRNA and protein were observed also in parafibromin-expressing parathyroid cancers ( $n=3$ ). TBX1 mRNA and protein were downregulated in parafibromin negative parathyroid carcinomas ( $n=14$ ), suggesting that Wnt/ $\beta$ -catenin activation might inhibit TBX1 expression. In HEK293 cells TBX1 mRNA were inhibited by  $\beta$ -catenin accumulation induced by 24-h treatment with 1–20 nM lithium chloride, while  $\beta$ -catenin degradation induced by 40-min pretreatment with 10–100 nM calyculin A determined an increase in TBX1 mRNA levels. Further functional studies on the effect of silencing and transfection of TBX1 are ongoing. In conclusion, the present data demonstrated that: i) TBX1 is expressed in adult parathyroid tissues; ii) TBX1 impaired expression contributes to parathyroid tumorigenesis; iii) TBX1 is a new target of the CaSR and Wnt/ $\beta$ -catenin pathways. TBX1 provides a potential therapeutic target for parathyroid tumors.

## P57

### Clinicopathological characteristics of pancreatic neuroendocrine tumors at diagnosis

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#### Introduction and objective

Neuroendocrine tumors (NETs) are a rare kind of tumor that can have various locations. Pancreatic location is the most common after the gastrointestinal tract.

The aim of this report is to study the characteristics of neuroendocrine tumors located in the pancreatic gland (pNETs) at diagnosis.

#### Material and methods

Descriptive and retrospective study about 22 patients diagnosed of pNET during the period 1993–2010 in our hospital. Data regarding demographic, clinical, analytical, anatomopathological and diagnostic variables were collected. Statistical analysis was performed using SPSS 16.0, results are expressed as mean  $\pm$  s.d.

#### Results

Twenty-two patients (64% men) with a mean age at diagnosis of  $43.0 \pm 14.1$  years were studied. Six (27.3%) patients had type 1 multiple endocrine neoplasia. Fifteen (68.2%) presented typical clinical syndrome of their pNET and pancreatic tail was the most common location (54.5%) followed by head (40.9%). The mean tumor size was of  $3.9 \pm 2.1$  cm. Tumors were diagnosed more frequently by imaging techniques (CT: 54.5%, MR: 31.8% and others: 36.3%), and surgery was required only in one case.

Anatomopathologically, the tumors were classified as insulinomas (40.9%), non-secreting pancreatic tumor (36.4%), gastrinoma (9.1%), and glucagonoma, somatostatinoma and ectopic secretion pancreatic tumor (4.5%, each one). Immunohistochemical markers resulted positive for: insulin (40.9%), glucagon (16.7%), gastrin and progesterone (9.2%) and somatostatin and PTHrP (4.5%). Ki67% was  $<2\%$  in 16.7% of patients, between 2 and 15% in 50.0% and  $>15\%$  in 33.3%.

Fourteen (63.6%) had metastases at diagnosis, all of them hepatic.

#### Conclusions

According to our experience, most pancreatic neuroendocrine tumors produce typical symptoms with a high proliferative index observed in the anatomopathological study. They are usually detected in an advanced metastatic stage, being the liver the most common affected gland. This delay in the diagnosis might be due to a low suspicion of these kinds of tumors.

## P58

### A large adrenal tumour as a phenotypic manifestation of the Birt-Hogg-Dube syndrome

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#### Introduction

The Birt-Hogg-Dube syndrome (BHDS) is a rare autosomal dominant genodermatosis characterised by presence of at least one of the following: benign skin fibrofolliculomas, lung cysts with recurrent pneumothoraces or renal tumours. The phenotypic constellation is due to heterozygous germline mutations of the tumour suppressor gene – FLCN, located on 17p11.2, encoding a 579 aminoacid protein termed folliculin. Case reports have described endocrine tumours of the thyroid, parathyroid, in addition to neuroendocrine tumours, colon cancer, melanomas, meningiomas and squamous cell carcinoma. To our knowledge, only one case of an adrenal tumour has been previously reported in association with BHDS.

#### Case report

A 36-year-old woman, presenting to the urologists with urinary frequency, was found to have a  $110 \times 95 \times 56$  mm left adrenal tumour on CT imaging, following a preliminary ultrasound. She underwent an uncomplicated open adrenalectomy for a presumed non-functioning adrenal carcinoma. Histology revealed a non-functioning adrenocortical tumour with no malignant features. In the view of the family history of BHDS, FLCN mutational analysis was requested. The presence of a frameshift mutation in exon 11 (p.His429ProfsX27) confirmed the diagnosis of BHDS. Post-operative PET scanning did not show any evidence of metastatic disease. A decision was made not to commence adjuvant mitotane chemotherapy and to arrange follow-up imaging. Genetic screening for her two children was discussed.

#### Conclusions

Adrenal tumours are likely to be a rare feature of BHDS, however their presence should be sought in people affected with this condition. The likely mechanism of tumorigenesis is the 'two-hit' process. The relative sparing of the adrenals compared to other endocrine organs merits further investigation.

**P59****Metabolic syndrome related to adrenal incidentaloma: observational study**Eduard Circo, Marian Beciu & Razvan Circo  
'Ovidius' University, Constanta, CT, Romania.**Introduction**

The adrenal incidentaloma is defined as a mass lesion found unexpectedly in an adrenal gland by an imaging procedure performed for another reason than suspected adrenal pathology.

**Objective**

To estimate the incidence of the adrenal mass and to define the algorithm for managing patients with incidental adrenal mass; a comparative analysis to the treatises data. The observational study was set one's face towards by the adrenal incidental features who was diagnose between November 2008 and May 2010 and it was specificate the presence of some diseases proper to metabolic syndrome.

**Methods**

We studied clinical historical of 34 patients (5 men and 29 women) with typical computed tomography features of adrenal mass. The structure of the patients batch with adrenal incidentaloma ( $n=34$ ) was: batch A-nonsecretory tumors ( $n=21$ ) (61.8%-TN) and batch B-secretor tumors ( $n=13$ ) (38.2%-TS) whereby four patients with one-sided pheochromocytoma; five patients with Cushing syndrome; two patients with subclinical Cushing syndrome and two patients with adrenal hypertrophy.

**Results**

Overweight or obesity were found in 33.3% by batch A and 30.8% by batch B; hypertension in 61.9% by batch A and 69.2% by batch B; diabetes mellitus in 19% by batch A and 30.8% by batch B; dislipidemia in 42.9% by batch A and 30.8% by batch B; osteoporosis in 14.3% by batch A and 23.1% by batch B. Diameter was less than 4 cm in 85.7% by batch A and 36.5% by batch B of the tumors, and more than 4 cm in 14.3% by batch A and 63.5% by batch B. 90.5% by batch A and 76.9% by batch B were detected by computed tomography. Surgical adrenalectomy was performed in 69.2% TS and one TN with cyst character. Beside the histopathologic examination wherefore three carcinomas described by CT just two was confirmed, the other remains was adenomas (two pheochromo-cytoma and three cortisol secretor tumors) and one adrenal hypertrophy with cortisol secretion.

**Conclusions**

The tumor assess, the imagistic features and the quality of secretory/nonsecretory tumor is essential. Some features of the metabolic syndrome described to the patients with adrenal incidentalomas are enlarged the medically problem and it can form an argument to supplementary investigations.

vascularization and endocrine potential need to be examined. Nevertheless, these tumor models have the potential to evaluate individualized treatment modalities in the future.

**P61****Liposome based strategies for the treatment of adrenocortical carcinoma**C Hantel<sup>1</sup>, F Lewrick<sup>2</sup>, M Reincke<sup>1</sup>, R Süß<sup>2</sup> & F Beuschlein<sup>1</sup>  
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Medical treatment of adrenocortical carcinoma (ACC) is limited to common cytotoxic agents, which are usually given in combination with mitotane. Recently we have developed a novel therapeutic approach by coupling a monoclonal IGF1 receptor blocking antibody (1H7) to sterically stabilized liposomal doxorubicin (SSLD). 1H7 coupled liposomes showed *in vitro* high and significant cellular association and furthermore internalization in various human tumor cell lines as well as *in vivo* superior anti-tumor efficacy against neuroendocrine tumors of the gastroenteropancreatic system. Flow cytometry and fluorescence microscopy demonstrated high internalization of 1H7 coupled liposomes ( $50.13 \pm 2.2$ ) also for the adrenocortical tumor cell line NCIh295, but surprisingly an even higher uptake of plain liposomes ( $84.57 \pm 0.82$ ;  $P=0.0001$ ). This extraordinary uptake phenomenon was independent on liposomal cholesterol content (cholesterol free preparation,  $82.92 \pm 1.63$ ;  $P=0.11$ ) and was not exclusively dependent on caveolae- ( $66.47 \pm 2.2$ ) and clathrin-mediated endocytosis ( $57.27 \pm 1.9$ ) since specific inhibitors were only partially able to inhibit the investigated effect. We evaluated the therapeutic efficacy of liposomal preparations in an *in vivo* model for ACCs. A significant reduction in tumor size (cm) was detectable 32 days after a single treatment of NCIh295 tumor-bearing mice with SSLD-1H7 ( $0.89 \pm 0.15$ ;  $P=0.006$ ) and diminished for SSLD ( $1.01 \pm 0.19$ ;  $P=0.04$ ) compared with untreated controls ( $1.5 \pm 0$ ) while no significant effects were seen for treatments with free 1H7 ( $1.38 \pm 0.11$ ;  $P=0.36$ ) LD ( $1.2 \pm 0.3$ ;  $P=0.36$ ), unspecific SSLD-IgG ( $1.45 \pm 0.5$ ;  $P=0.36$ ) or SSLD + free 1H7 ( $1.29 \pm 0.17$ ;  $P=0.25$ ). Thus, SSLD-1H7 could represent a promising approach for the treatment of ACCs in the future. Moreover, a combination of mitotane plus encapsulated cytostatic agents instead of the free drugs could also represent an interesting novel treatment option.

**P60****Development and characterisation of patient-individual tumor models for endocrine tumors**C Hantel<sup>1</sup>, F Scheller<sup>1</sup>, A Ozimek<sup>2</sup>, C Chiapponi<sup>2</sup>, T Mussack<sup>2</sup> & F Beuschlein<sup>1</sup><sup>1</sup>Endocrine Research Unit, Medical Clinic Innenstadt, LMU, Munich, Germany; <sup>2</sup>Department of surgery, Medical Clinic Innenstadt, LMU, Munich, Germany.

Only a few cell lines are available for endocrine tumors which furthermore do not reflect heterogenous functional properties and specific therapeutic response rates of individual tumors. To facilitate patient individual treatments and thereby to optimize therapeutic efficacy, we are aiming at the development and characterisation of patient-individual tumor models. Pieces of surgical tumor specimen from four adrenocortical carcinomas, one aldosterone producing adenoma, one pheochromocytoma (pheo), one metastasis of a malignant pheo, one pheo and medullary thyroid carcinoma associated with multiple endocrine neoplasia type 2 and 9 neuroendocrine tumors (NETs, seven NETs of the gastroenteropancreatic system including one metastasis as well as one primary tumor and one metastasis of Merkel cell carcinoma) were implanted subcutaneously in athymic NMRI nude mice at this time. To investigate whether morphological and functional characteristics between tumor samples after mouse engraftment in comparison to the original tumor would be comparable, we started examination of implanted material and original patient tumor by histology and immunohistochemistry. Twelve weeks after engraftment tumors were explanted and investigated for vitality (HE), proliferation (Ki67) and endocrine differentiation marker (SF-1, Chromogranin A). HE stainings revealed successful engraftment with maintained vitality of the implanted tumor tissues. Ki67 stainings exhibited furthermore proliferating tumor cells in the implanted material. In addition, depending on the entity tumors expressed SF-1 or Chromogranin A. First comparisons indicate that the implanted tumors keep the characteristics of the original tumor material in the murine host. These findings need to be further substantiated and additional endpoints such as

**P62****Injection systems for long-acting somatostatin analogs: a comparative assessment of preparation times and nurses' perceptions**D T Adelman<sup>1</sup>, A Burgess<sup>2</sup> & P R Davies<sup>3</sup><sup>1</sup>Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA; <sup>2</sup>The Christie, Neuroendocrine Tumour Department, Manchester, UK; <sup>3</sup>Neuroendocrine Tumour Unit, Royal Free Hospital, London, UK.**Introduction**

The two major long-acting somatostatin analogues (SSA) available on the European market for the treatment of neuroendocrine tumours and acromegaly are Somatuline Autogel (SA) and Sandostatin LAR (LAR). The aim of this study was to gain nurses' insights on the use and ease of administration of SSA devices, including Somatuline Autogel new device (SA-ND), in Europe and the US.

**Methods/design**

Seventy-seven qualified nurses with  $\geq 3$  SSA patients/year were interviewed to gather views regarding SSA injection use. Nurses were timed while preparing and performing test injections with SA-ND and LAR. Attributes of SA-ND and current SSA devices were evaluated and an overall preference score (attributes weighted by importance to nurses) calculated for each.

**Results**

SA-ND scored higher than LAR and SA for nearly all attributes. Mean time for administration was 66 s for SA-ND versus 329 s for LAR. Low clogging risk, prefilled device, safety feature and time to administer were the main perceived advantages of SA-ND. Overall preference score was 63% higher for SA-ND versus LAR (114 vs 70 respectively).

**Conclusion**

Preference for a new SSA device was high among interviewed nurses. Conceivably, the short administration time, confidence that a full dose is delivered and perceived ease-of-use of the new device compared to existing SSA devices could lead to improvement in clinical practice and a benefit to patients/caregivers when administering SSAs at home.

**P63****The influence of different ACTH assays on the ACTH concentrations of patients with small cell lung cancer**J A P Bons<sup>1</sup>, A C Dingemans<sup>2</sup> & P P C A Menheere<sup>1</sup><sup>1</sup>Department of Clinical Chemistry, Maastricht University Medical Centre, Maastricht, The Netherlands; <sup>2</sup>Department of Respiratory Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands.**Introduction**

ACTH is measured to confirm impairments in the hypothalamic–pituitary–adrenal axis. Patients with small cell lung cancer (SCLC) can have ectopic adrenocorticotrophic secretion. In December 2009, a SCLC patient showed an enormous discrepancy between the ACTH results obtained with two different ACTH assays. The aim of this study was to compare different ACTH assays in SCLC patients.

**Methods**

In a pilot study EDTA plasma samples of nine SCLC patients were measured with three different ACTH assays; IRMA, electrochemiluminescent immunometric assay (Elecys), RIA. Twelve EDTA plasma samples of patients who underwent an insulin tolerance test (ITT) were measured with the IRMA and RIA assays. At our laboratory the specific IRMA assay is used as standard method.

**Results**

The average ACTH Elecys/IRMA and RIA/IRMA ratios were respectively 0.84 (range: 0.61–0.94) and 4.66 (range: 1.52–17.0) for the nine SCLC patients. The RIA/IRMA ratio was 1.96 (range: 0.90–5.25) for the ITT group. The average RIA/IRMA ratio in the SCLC group is 2.4 times higher with respect to the ITT group.

**Conclusions**

Ectopic adrenocorticotrophic secretion can lead to discrepancies in the ACTH concentrations measured with different ACTH assays. In the case of ectopic adrenocorticotrophic secretion, not only the concentration of the intact ACTH is of interest, but also in the total ACTH concentration including fragments, degradation products, and precursors. In those cases it happened to be meaningful to use a RIA assay. Mostly, non-competitive ACTH are used in standard laboratory practice. When interpreting ACTH results one should be aware of the specifications of the assay. If there is any doubt that biochemical effects of ACTH-like molecules, not measured with the routine assay, could explain clinical status of a patient, a competitive assay should be used additionally. Competitive assays appear to cross react with more ACTH-like analytes than non-competitive assays.

**P64****Primary hyperparathyroidism in MEN1 patients: preferred surgical procedure and relation with genotype**C R C Pieterman<sup>1</sup>, L T van Hulsteijn<sup>2</sup>, M den Heijer<sup>3</sup>, R B van der Luijt<sup>4</sup>, A R M M Hermus<sup>3</sup>, M R Vriens<sup>3</sup> & G D Valk<sup>1</sup>

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**Background**

Primary hyperparathyroidism (pHPT) is the most prevalent manifestation of multiple endocrine neoplasia type 1 (MEN1). Surgery is the preferred treatment but which type of surgery offers the best chance of cure combined with the lowest risk of postoperative hypoparathyroidism is still uncertain.

**Aims**

Identifying the optimal surgical technique for MEN1 related pHPT. Describing the course of postoperative hypoparathyroidism. To see if a genotype–phenotype correlation exists with regard to recurrent pHPT.

**Methods**

A retrospective longitudinal cohort study performed at the departments of endocrinology of the University Medical Centers (UMCs) of Utrecht and Nijmegen in The Netherlands. Patients were selected from the Dutch MEN1 database which includes all Dutch patients aged  $\geq 16$  years who are treated for MEN1 in one of the Dutch UMCs between 1990 and 2009. Data were collected by medical record review.

**Results**

Seventy-three patients who underwent parathyroidectomy for MEN1-related pHPT were included. The studied surgical procedure was the primary surgery for 42 (57.5%) patients. Persistent or recurrent pHPT was seen in 19 (26%) patients: after less than three parathyroids resected (<SPTX) in 53%, after subtotal resection (SPTX) in 17% and after total resection (TPTX) in 19% of patients. Persistent ( $\geq 6$  months) postoperative hypoparathyroidism was seen in 34 (46.6%) patients: after <SPTX in 23.5%, after SPTX in 39.1% and after TPTX in 65.6% of patients. The median duration of postoperative hypoparathyroidism was 1.5 years. In 65% of the patients successful cessation of substitution therapy (calcium supplements and vitamin D) was possible, even after > 10 years. Patients with nonsense or frameshift mutations in exons 2, 9 and 10 had a significantly lower risk of persistent or recurrent pHPT than patients with other mutations.

**Conclusions**

SPTX is the procedure of choice in MEN1-related pHPT. Patients with nonsense or frameshift mutations in exons 2, 9 or 10 have less risk of persistent or recurrent pHPT. Postoperative hypoparathyroidism lasting  $\geq 6$  months should not be considered permanent and it is advisable to try to taper off the substitution therapy even several years after surgery.

**P65****Significance of BRAF expression in nodular thyroid disease: prognostic and therapeutic implications**Ioana Golu<sup>1</sup>, Adriana Neghina<sup>2</sup>, Marioara Cornianu<sup>3</sup>, Daniela Amzar<sup>1</sup>, Melania Balas<sup>1</sup>, Dana David<sup>2</sup> & Mihaela Vlad<sup>1</sup>

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**Background**

Whereas many papers have reported on clinical and morpho pathological features of thyroid cancer with BRAF<sup>V600E</sup> mutation, a limited number of studies have been focused on the quantification of BRAF mRNA expression in thyroid tumors. The present study aimed to assess the expression of BRAF transcript in fine needle aspiration specimens, prelevated from cases with nodular thyroid disease.

**Material and methods**

The mRNA expression levels of BRAF and GAPDH (housekeeping gene) was evaluated in 23 malignant and 34 benign thyroid tumors by means of semiquantitative RT-PCR, with the assessment of the crossing point values for each sample in triplicates.

**Results**

The mean age of the study group was  $49.1 \pm 19.0$  years, with a female to male ratio (F/M) of 8.6/1. An over expression was documented in about 50% of analyzed carcinomas, with the following distribution in patients with papillary thyroid carcinomas (PTC): four out of seven cases presented the classical variant of the disease, two out of five cases micro PTC and four out of nine cases the follicular variant of PTC. A significant over expression was noticed in the tumor ( $2^{-\Delta\Delta C_T} = 4608.24$ ) and also in the perinodular tissue ( $2^{-\Delta\Delta C_T} = 3929.1$ ) of one case with follicular thyroid carcinoma (FTC) and follicular adenoma, as compared with the samples with benign tumors ( $2^{-\Delta\Delta C_T} = 0.98$ ).

**Conclusions**

The significant over expression of BRAF mRNA could be a feature of FTC, highlighting an over activity of Ras–BRAF–MAP kinase pathway, with subsequent increase of their invasive potential.

**P66****Metabolic endocrine tumor activity is reflected by total <sup>18</sup>F-DOPA PET tumor uptake in patients with a carcinoid tumor**

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**Introduction**

Positron emission tomography (PET) using 6-[F-18]fluoro-L-dihydroxyphenylalanine (<sup>18</sup>F-DOPA) has an excellent sensitivity to detect carcinoid tumor lesions.

<sup>18</sup>F-DOPA-tumor uptake and the levels of biochemical tumor markers are mediated by tumor endocrine metabolic activity. Therefore we evaluated whether total <sup>18</sup>F-DOPA-tumor uptake on PET, defined as the whole-body metabolic burden (WBMTB) reflects tumor load per patient, as measured with tumor markers.

#### Methods

Seventy-seven consecutive carcinoid patients who underwent a <sup>18</sup>F-DOPA PET scan in two previously published studies were analyzed. For all tumor lesions mean standardized uptake values (SUVs) at 40% of the maximal SUV and tumor volume on <sup>18</sup>F-DOPA PET were determined and multiplied to calculate a metabolic burden per lesion. WBMTB was the sum of the metabolic burden of all individual lesions per patient. As tumor markers served 24-h urinary serotonin, urine and plasma 5-hydroxyindole acetic acid (5HIAA); catecholamines (nor) epinephrine, dopamine and their metabolites, measured in urine and plasma, and serum chromogranin A.

#### Results

All but one were evaluable for WBMTB, 74 patients had metastatic disease. <sup>18</sup>F-DOPA PET detected 979 lesions. SUVmax on <sup>18</sup>F-DOPA PET varied up to 29-fold between individual lesions within the same patients. WBMTB correlated with urinary serotonin ( $r=0.51$ ) and urinary and plasma 5HIAA ( $r=0.78$  and  $0.66$ ). WBMTB also correlated with urinary norepinephrine, epinephrine, dopamine and plasma dopamine, but not with serum chromogranin A.

#### Conclusion

Tumor load per patient measured with <sup>18</sup>F-DOPA PET correlates with tumor markers of the serotonin and catecholamine pathway in urine and plasma in carcinoid patients and reflects metabolic tumor activity. Although not yet investigated in neuroendocrine tumor patients, WBMTB on <sup>18</sup>F-DOPA PET might be an interesting measure of treatment response in patients with a carcinoid tumor.

## P67

### Ectopic mediastinal parathyroid adenoma: a case report

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#### Introduction

Incidence of ectopic parathyroid glands in individuals is ~6%, the most common location being the thymic capsule or the superior mediastinum.

#### Case

We present the case of 21-years-old female with a recent history of osteoclastoma affecting the maxillary bone and the mandible, together with increased values of the parathyroid hormone (over 20 times the normal value), total calcium and alkaline phosphatase and decreased serum phosphorus. A cervico-mediastinal MRI was performed with the disclosure of a well delimited 5/5.5/2 cm tumoral mass in the antero-superior part of the mediastinum, as well as multiple cystic bone tumors affecting the maxillary bone, mandible, clavicle, humeral head and scapula - osteitis fibrosa cystica in context of the primary hyperparathyroidism. In September 2010 thymectomy was performed.

#### Material and methods

Tissue fragments from the thymectomy specimen were routinely processed and stained with H&E. Supplementary Ki-67 antigen (MIB 1 clone), En Vision method, DAB visualization was made.

#### Results

Within the capsule of the thymus was found a nodule, embedded in connective tissue, formed mainly by parathyroid epithelial cells, chief cell type, patterned in nests and small follicles; focal pleomorphic nuclei were observed, but no evidence of malignancy such as vascular or soft tissue invasion. Immunohistochemistry for the cell cycle marker Ki67 has been 2%, so the final diagnosis was that of ectopic parathyroid adenoma, with mild atypia.

#### Conclusion

After surgical removal of the mediastinal mass, imagistically suspected and histopathologically confirmed as ectopic parathyroid tissue, the syndrome of primary hyperthyroidism was resolved.

## P68

### Significant prognostic survival factors in patients with pancreatic neuroendocrine tumors

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#### Introduction

Pancreatic neuroendocrine tumors (PNETs) are generally clinically more indolent than adenocarcinomas. But relevant, clinically applicable prognostic survival factors still need to be more precisely defined.

#### Aim

To identify survival factors in patients with PNETs.

#### Methods

We analyzed 66 patients with PNETs (52.62 ± 13.3 years old) treated at our department during 2003–2010. Patients with insulinomas were not included in survival analysis unless manifesting malignant behavior. Kaplan–Meier method was used for univariate survival analysis, and Cox regression model for the estimation of influence of risk factors.

#### Results

Minority of PNETs were hormonally active (13 insulinomas, 7 somatostatinomas, 6 gastrinomas). Majority (53.7%) presented with metastatic disease, in stage IV according to TNM classification. According to WHO criteria, majority were well-differentiated neuroendocrine carcinomas (48.4%). Nine patients (13.6%) had genetically confirmed MEN1 syndrome. Primary tumor was operated in 36 patients (54.5%). Overall median survival was 73 months (2–180 months) with 5-year survival of 66.7%. Significantly shorter survival was noted in patients with metastatic disease at presentation ( $P=0.025$ ), those who did not undergo primary tumor operation ( $P=0.018$ ), tumors with higher mitotic count ( $P=0.001$ ), higher proliferation index Ki-67 ( $P<0.001$ ), and more aggressive behavior as characterized by the WHO group ( $P<0.001$ ). Primary tumor size, hormonal activity, heritability and tumor stage had no significant influence on survival. Significant independent predictor of mortality was group according to WHO classification. (HR 4.722; 95% CI 1.746–12.771),  $P=0.002$ ).

#### Conclusion

Biology of PNETs is best reflected by the criteria of WHO classification. Important favorable survival factor is operation of primary tumor, despite the presence of metastatic disease. Tumor stage, according to TNM classification, did not affect survival of our patients.

## P69

### Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH): is it linked to GH secretion?

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DIPNECH is a rare condition which can present as small airways obstruction physiologically. Currently the pathophysiological basis of the disease is unknown but scarce data on a familiar case suggest a genetic background. Currently, it is unclear whether endocrine intervention may be of help to therapeutically improve patients which may be important as the disease is regarded as a precursor lesion to pulmonary carcinoid tumours. Here we report a family with both twin sisters affected with histologically proven DIPNECH.

A 31-year-old IT technician was referred to endocrine clinic for consideration of GH replacement. She is an identical twin born prematurely. Aged 8 years a problem with growth was discovered and she was diagnosed with GH deficiency (GHD). She was started on GH replacement at age 10 and continued to age 15. After an interval of nearly 10 years she presented to our clinic 5 years ago. She was 145 cm in height. Her MRI pituitary showed small volume of pituitary tissue measuring 6×4 mm consistent with pituitary hypoplasia. A repeated ITT confirmed GH deficiency and she was recommenced on GH replacement with all other pituitary axes intact apart of mild primary hypothyroidism. Our patient further complained of mild but increasing shortness of breath associated with recurrent chest infections. Respiratory investigations showed small airways obstruction and diffuse gas trapping on HRCT. Lung biopsy confirmed DIPNECH.

Her twin sister measuring 165 cm in height suffered of more severe respiratory symptoms, physiology of severe small airways obstruction and a high resolution CT scan showed again diffuse air trapping. A provisional diagnosis of obliterative

bronchiolitis had been suspected prior to lung biopsy. An open lung biopsy was performed which demonstrated changes compatible with DIPNECH. This twin suffers from sensori-neuronal hearing loss. When testing her basal pituitary function she showed decreased IGF1 serum, levels but all other pituitary functions were normal. Currently she is awaiting a dynamic stimulation test (ITT).

Our twin patients with histologically proven DIPNECH raise a number of questions. First, it is unknown whether modern technology of somatostatin receptor scintigraphy using Ga-DOTANOC will help with the diffuse tumour diagnosis as in pulmonary carcinoids. This could lead to early therapeutic intervention in the condition with using octreotide treatment to decrease tumour size and improve local mucus clearance. Secondly, links between DIPNECH and GH secretion have been discussed in a single case of combined DIPNECH and acromegaly but this was attributed to potential MEN1<sup>1</sup>. The familial cause in our patients and the potential relation to GH deficiency suggest a different genetic background. If confirmed it may argue for a closer surveillance of mild respiratory symptoms in patients with GHD.

1. Fessler MB, Cool CD, Miller YE, *et al*. Idiopathic diffuse hyperplasia of pulmonary neuroendocrine cells in a patient with acromegaly. *Respirology* 2004 9 274–277.

## P70

### First prognostic analysis of stage III adrenocortical carcinoma patients after complete resection: a retrospective French Multicentric Study from the INCA-COMETE network

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#### Objective

To assess outcome of stage III-ENSAT adrenocortical carcinoma (ACC) patients after complete macroscopic resection and prognostic factors for disease-free survival (DFS) and overall survival (OS).

#### Methods

A retrospective review of 68 stage III ACC patients treated surgically between 1994 and 2009 in the French COMETE network was performed. Characteristics of patients were reviewed by a single investigator as well as the Weiss score by experienced pathologists. There were 44 females and 24 males. The median age was 47 years (range 21–75). Surgical excision was performed by laparotomy (91%) or laparoscopy. R1 resection and capsular effraction were notified in 18 and 16% of cases, respectively. Stage III-ENSAT definition included: T3 tumors (57%), T4 tumors (43%), N1 (10%) and/or venous tumor thrombi (28%). Adjuvant treatment included mitotane therapy (96%) and radiotherapy of the tumor bed (7%). The median follow-up time was 36 months (5–167). Uni and multivariate analyses were performed.

#### Results

Recurrence was evidenced in 57 (84%) patients within a median time of 9 months (1–71). Anatomic distribution of recurrences was: local only (37%), metastatic only (19%), local and metastatic (15%). The 1-year, 2-year, and 3-year DFS rate were 44, 26, and 18%, respectively. The 1-year, 2-year, and 3-year OS rate were respectively 88, 69, and 50%. Prognostic factors of DFS at multivariate study analysis were positive lymph node status and the size of the primary tumor. Prognostic factors of survival at multivariate analysis were age and positive lymph node status. Early recurrence (before 1 year) and mitotane plasma level < 14 mg/l at the time of recurrence were associated with a poorer survival.

#### Conclusions

Our study demonstrated a high rate of recurrences after complete macroscopic resection of stage III-ENSAT ACC patients supporting a role for neo- and/or adjuvant therapy in the future in this subgroup of patients.

## P71

### Comparison of the efficacy and the tolerance of high-dose calcium and pentagastrin tests in patients with cured or persistent medullary thyroid cancer and in controls

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The recent unavailability of pentagastrin (Pg) in several European Countries, prompted us to evaluate the potency and tolerability of the 'high dose calcium test' (Ca) in the stimulation of serum human calcitonin (hCt). We compared Pg- and Ca-stimulated hCt in 19 patients already treated for medullary thyroid cancer (MTC) in remission ( $n=19$ ) or in persistence ( $n=13$ ), in 18 patients with chronic thyroiditis and multinodular goiter, and in 15 healthy controls. In controls, Ct never peaked above 50 pg/ml. A response of Ct was observed in 4 cases in apparent remission without differences between the 2 tests. The hCt peak in persistent patients ranged 18–3637 for Pg and 60–2932 pg/ml for Ca tests, without significant differences even in the delta increases. The hCt peak in patients with autoimmune or nodular thyroid diseases ranged 21–942 pg/ml for Pg and 5–38 000 pg/ml for Ca tests, without significant differences. Four patients with a Ct response > 100 pg/ml, have been submitted to thyroidectomy. At histology, an intrathyroidal MTC was found in 3 cases while one patient, with a Ct peak after Pg of 162 and after Ca of 599, was negative either for MTC or for C-cells hyperplasia.

The number, intensity and duration of side effects were significantly lower during Ca test, being the feeling of warmth the most frequent discomfort.

In conclusion, this study demonstrated that high dose Ca test has a similar or higher potency than Pg test. In addition, Ca test was well tolerated and highly preferred by patients. These data, associated with the lower cost of Ca and its worldwide availability, indicate that the high-dose calcium is the test of choice for the pre-surgical diagnosis of MTC and for the follow up. Ongoing studies will identify the cut-off point to use for this test in the pre-surgical identification of MTC.

## P72

### Distribution of mitotane and its two metabolites in lipoprotein fractions of patients with adrenocortical carcinoma

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#### Introduction

Adrenocortical carcinoma (ACC) is a rare tumor of the adrenals with poor prognosis (survival rate for metastatic patients < 15% at 5 years). Mitotane (o,p'-DDD) is the main therapeutic option with up to 66% objective response rates in patients with serum levels between 14 and 20 mg/l. Its two main metabolites are o,p'-DDA and o,p'-DDE which plasma levels are not correlated to therapeutic response. Mitotane is a lipophilic drug that accumulates in lipoproteins and induces hypercholesterolemia by activation of HMGCoA reductase. Looking for predictive/prognostic markers of response we assessed the distribution of o,p'-DDD and its metabolites in lipoprotein fractions of ACC patients.

#### Materials and methods

Levels of o,p'-DDD, o,p'-DDA and o,p'-DDE were measured using HPLC-UV method after liquid-liquid extraction with p,p'-DDE as internal standard. Lipoprotein fractions from plasma of ACC patients treated with mitotane were obtained by differential ultracentrifugation process.

#### Results

We developed and validated an HPLC-UV method. The extraction yields of o,p'-DDD, o,p'-DDA and o,p'-DDE are 75, 73 and 38% respectively. This method is linear, precise, accurate and no matrix effect was observed (< 2%). The method of separation of lipoproteins by differential ultracentrifugation allowed us isolating VLDL, LDL and the mixture HDL-proteins. Analyses in a few plasma patients showed a major distribution of o,p'-DDA in HDL (99%) while o,p'-DDD and o,p'-DDE were mainly measured in HDL (> 70%), partially in LDL (12–25%) and barely in VLDL (< 5%).

#### Conclusion

This work allowed us to define conditions for the study of o,p'-DDD, o,p'-DDA and o,p'-DDE distribution in the different lipoprotein fractions. The preliminary

results showed a different distribution profile for o,p'-DDA. The perspectives of this study are multiple: evaluation of lipoprotein distribution over time in a large cohort of patients; dyslipoproteinemia impact; relationship between o,p'-DDD, o,p'-DDA and o,p'-DDE lipoprotein plasma levels and therapeutic response.

### P73

#### **<sup>68</sup>Ga-DOTATATE PET shows a better diagnostic performance than octreoscan in patients with neuroendocrine tumors**

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#### Background and objective

<sup>111</sup>In-DPTA-Octreotide (Octreoscan) has limited spatial resolution and a somewhat lower receptor affinity of the radioligand compared to DOTATATE, a novel somatostatin analogue, which can be radiolabelled with <sup>68</sup>Ga and adapted for PET imaging, resulting in an increased spatial resolution. The aim of this study was to evaluate the diagnostic performance of <sup>68</sup>Ga-DOTATATE PET in patients with NET and to compare this technique with Octreoscan.

#### Patients and methods

Fifty-seven patients with NET were enrolled, located in pancreas (27), duodenum/ileum (4), lung (7), stomach (6), thyroid (12), paraganglia (1). Forty patients had a sporadic and 17 a MEN1-related NET. <sup>68</sup>Ga-DOTATATE PET was performed in all cases by acquiring whole body studies 40–60 min after the radioligand i.v. injection (range 74–111 MBq). Octreoscan was also performed in a subgroup of 27 patients using intravenous injection of Indium-111-DTPA-Phe1-octreotide (Octreoscan, Mallinckrodt Medical, Petten, The Netherlands; 120–200 MBq). At the time of PET/Octreoscan evaluation 40 patients had tumor lesions and 17 were negative as confirmed by CT/MR examination and clinical follow-up.

#### Results

The patient based sensitivity was 77% with a specificity of 100% while the lesion based sensitivity was 64% with a specificity of 85%. Positive and negative predictive values were 100 and 65% respectively. The sensitivity of PET was about 100% in pancreatic and gut NET, about 75% in lung and thyroid NET and lower than 50% in gastric NET. In a subgroup of 27 patients undergone both PET and Octreoscan, the result was concordant in 21 and discordant in six cases. The discordant results included five PET positive and Octreoscan negative NET (three pancreatic NET, two medullary thyroid cancer) and one PET negative and Octreoscan positive NET (an atypical bronchial carcinoid).

#### Conclusions

<sup>68</sup>Ga-DOTATATE PET shows high sensitivity and excellent specificity in patients with NET, revealing a higher diagnostic performance than Octreoscan.

### P74

#### **In vitro effect of the somatostatin analogue BIM 23704 on cell cycle timecourse in an ovarian carcinoma cell line**

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Ovarian carcinoma (OC) is associated with poor prognosis and low survival rate. Eligible treatment for advanced OC is surgery followed by chemotherapy based on a combination of Platinum and Taxol, but unfortunately long-term outcome of such therapy is disappointing. Moreover, systemic administration of cytotoxic agents is usually accompanied by a series of side effects. On the basis of this evidence, more selective and tumour cell-targeted therapeutic approaches are warranted. Somatostatin receptors (SSTRs) are widely distributed in normal and tumour tissues and mediate anti-proliferative, anti-angiogenic and pro-apoptotic effects acting on different molecular pathways. The aim of this study was to analyze SSTRs expression in A2780 human ovarian adenocarcinoma cell line and tested the effect of a SSTR selective agonist on cell line proliferation. PCR analysis showed SSTR1 and SSTR2 expression, suggesting to test the effect of the SSTR agonist BIM 23704, which has high affinity for these two receptors. BIM 23704, administered at the dose of  $10^{-7}$ – $10^{-9}$  M, showed a dose-dependent

inhibitory effect on cell proliferation with a maximum effect after 48 h of continuous exposure (30% at  $10^{-7}$  M and 25% at  $10^{-9}$  M). This effect persist until 96 h of exposure, then treated cells become insensitive. FACS analysis indicated that this inhibitory effect is not due to induction of apoptosis nor to block of cell cycle but to a slowdown of cell cycle timecourse (reduction of 11%). Western blot analysis showed a reduction of pERK1/2(Tyr 204) kinases in treated cells protein extracts compared with untreated control cells suggesting an involvement of these proteins in A2780 cell cycle. These preliminary data, if confirmed, might suggest the use of this compound as adjuvant of chemotherapeutic agents in the effort to reduce their dose and consequent side effects and to bypass the onset of chemoresistance due to high dosage and continuous exposure in the management of OC.

### P75

#### **T-type calcium channel blockers inhibit hormone secretion and induce apoptotic cell death in a medullary thyroid cancer cell line**

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Medullary thyroid carcinoma (MTC) accounts for ~5–10% of thyroid cancers. Intense efforts are currently directed toward the identification of new druggable targets for the treatment of MTC. The aim of this study was to investigate whether drugs acting at voltage-gated T-type calcium channels could affect hormone release and/or cell proliferation in a MTC cell line, the TT cells. The expression of the three isoforms of T-type calcium channels (CaV3.1, CaV3.2 and CaV3.3) was evaluated by RT-PCR whereas T-type currents were recorded by whole cell patch clamp electrophysiology. The effect of T-type calcium channel blockers (Ni<sup>2+</sup>, mibefradil and NNC 55-0396) on calcitonin secretion and cell proliferation and viability was evaluated respectively by measuring calcitonin levels, and the percentage of cell cycle arrest and/or apoptosis by WST assay, flow cytometry and FLICA assay. At RT-PCR analysis, all three isoforms of T-type channels were found. Whole cell patch clamp experiments showed that T-type currents represent the only form of Ca<sup>2+</sup> current elicited by depolarization in these cells. The amplitude of T-type currents was reduced to <30% of their basal values when TT cells were perfused with an extracellular solution containing either the transition metal Ni<sup>2+</sup> (150 M) or mibefradil (10 M) or NNC 55-0396 (10 M). Basal calcitonin release from cultured TT cells was dose-dependently inhibited in the presence of T-type blockers (IC50: ~15 M). The viability of TT cells was inhibited by Ni<sup>2+</sup> (32%), NNC 55-0396 (76%) and Mibefradil (70%) at the concentration 1 mM, 10–30 μM and 30 μM respectively. Moreover, NNC 55-0396 dramatically increased the percentage of cells undergoing apoptosis whereas only limited effects were observed on cell cycle. At FLICA assay a significant apoptotic effect was observed by binding of red fluorochrome to caspases in cells treated with NNC 55-0396, confirming the evidence that upon NNC 55-0396 exposure TT cells underwent apoptosis. In conclusion, T-type channels seem to play a relevant role in controlling both hormone secretion and cell viability in MTC cells. These data prompt further studies aimed to investigate the potential of T-type channel blockers in the pharmacological treatment of MTC.

### P76

#### **Management of type III neuroendocrine tumors of the stomach**

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#### Background

Type 3 neuroendocrine tumors of the stomach (type 3 NETV) are very rare subtypes of NETV with poor prognosis, not fully understood biology and not well investigated diagnostic and therapeutic procedures. The aim of the study was to analyze tumor biology and the outcome of differentiated treatment in patients with type 3 neuroendocrine tumors of the stomach.

#### Methods

A prospective 10 years follow up of five patients with type 3 NETV treated in our institution was undertaken.

#### Results

We investigated and followed up for 11 years five patients with histopathologically confirmed type 3 NETV. The diagnosis and gastric resection (total 3/5,

partial 2/5, palliative 1/5) was performed 3–12 months after first tumor related symptoms presentation 5/5. The solitary tumors 5/5, were from 20 to 90 mm in diameter, located in fundus 4/5 and in cardia 1/5, infiltration beyond submucosa occurred in all cases. Metastases to regional lymph nodes were present in 5/5 cases during surgery, liver and distant (peritoneum, retroperitoneal space) metastases occurred in 4/5 in 0–11 months from diagnosis/gastrectomy. Chemotherapy was given in 3/5 in 2–6 months from diagnosis/gastrectomy. One patient was treated with 90Y in 6 months after diagnosis. 3/5 patients died in 8–18 months from diagnosis/gastrectomy. 2/5 patients are alive in 20–38 months from diagnosis/gastrectomy, 1/5 patient is free of disease in 38 months from diagnosis/gastrectomy.

#### Conclusions

The increasing number of type 3 NETV related probably to improved diagnostic methods and their poor prognosis require more profound investigation of the tumor biology and improvement of the treatment procedures.

## P77

### Could <sup>99m</sup>Tc labeled long-acting agonist of GLP-1 (Exendin-4) scintigraphy be a new tool in the diagnostics of unknown focus of insulinoma? preliminary results

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#### Introduction

GLP-1 receptors have been found on different types of neoplasm cells and density of these receptors appears to be more frequent in some of them. GLP-1 receptor scanning offers a new approach for successful localization of small or not detectable by other methods insulinomas and other cancers such as MTC, gastrinoma, pheochromocytoma, gastrinoma, etc.

#### Aim of the study

The aim of study was to present first successful experiences with the use of <sup>99m</sup>Tc-labeled [Lys<sup>40</sup>-(Ahx-HYNIC)NH<sub>2</sub>]-Exendin-4 (Exendin-4 is a long-acting GLP-1 agonist) scintigraphy in localization of unknown insulinoma focus.

#### Material and method

[Lys<sup>40</sup>(Ahx-HYNIC-<sup>99m</sup>Tc/EDDA)NH<sub>2</sub>]-Exendin-4 receptor scintigraphy was performed in three patients. The insulinoma was suspected in two of them: a 57-year-old woman with severe clinical hypoglycaemias, no lesion in CT and no uptake of the tracer in somatostatin receptor scintigraphy (SRS), and in 16-year-old man with hyperinsulinemia, small unclear lesion in CT and no uptake of the tracer in SRS. A 62-year-old woman with malignant insulinoma after surgery with metastases to the liver in CT was qualified to scintigraphy with suspicion of the disease recurrence and no pathological uptake in SRS.

#### Results

In both suspected insulinoma cases, the uptake of [Lys<sup>40</sup>(Ahx-HYNIC-<sup>99m</sup>Tc/EDDA)NH<sub>2</sub>]-Exendin-4 in pancreas was found. The first patient had successful surgery, revealing small lesions of insulinoma. For the second patient, fusion of images confirmed that pathological accumulation of tracer localized in pancreas; patient has been referred to surgery. In patient with malignant insulinoma the uptake of the tracer was in the place suspected as recurrence.

#### Conclusion

Promising pharmacokinetic data and the preliminary results of our study showed that [Lys<sup>40</sup>NH<sub>2</sub>-(Ahx)-HYNIC]-Exendin-4 - <sup>99m</sup>Tc could be suitable candidate for clinical GLP-1 receptor imaging in clinical practice.

## P78

### A randomized, double-blind, placebo-controlled, multicenter phase III trial of everolimus + octreotide LAR versus placebo + octreotide LAR in patients with advanced neuroendocrine tumors (NET) (RADIANT-2): updated safety results

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#### Background

Patients with advanced NET have limited treatment options. In the phase III RADIANT-2 study of patients with advanced low- or intermediate-grade NET

and a history of carcinoid syndrome, everolimus, an oral mTOR inhibitor, + octreotide LAR provided a clinically meaningful 5.1-month increase in median progression-free survival (PFS) compared to placebo + octreotide LAR (ESMO 2010 Abstract #LBA8). An updated safety analysis from this trial is presented.

#### Methods

Patients were randomized to everolimus 10 mg/day orally + octreotide LAR 30 mg IM q28d (n=216), or placebo + octreotide LAR (n=213). The primary endpoint was PFS. Adverse events (AEs) were collected and coded to a preferred term using the medical dictionary for regulatory activities. AEs were graded using the National Cancer Institute's Common Terminology Criteria (v 3.0). Safety population included 407 patients (215 for everolimus; 211 for placebo).

#### Results

Median safety follow-up was 31.1 months. Everolimus safety was consistent with the primary analysis (median follow-up 28 months). Most frequent drug-related AEs (everolimus + octreotide LAR versus placebo + octreotide LAR, %) were stomatitis (47.4 vs 10.9), rash (37.2 vs 12.3), fatigue (31.6 vs 24.2) and diarrhea (27.4 vs 15.6). Most frequent drug-related grade 3/4 events (everolimus + octreotide LAR versus placebo + octreotide LAR, %) were fatigue (6.5 vs 2.8), diarrhea (6.0 vs 2.4), hyperglycemia (5.1 vs 0.5), thrombocytopenia (4.7 vs 0) and stomatitis (3.7 vs 0). Drug-related AEs rates leading to study drug discontinuation were 19.5% for everolimus + octreotide LAR vs 3.8% for placebo + octreotide LAR. Disease progression was the primary reason for treatment discontinuation in both arms.

#### Conclusions

Safety of everolimus observed in this updated analysis is consistent with previous experience and provides further evidence of the favorable risk-benefit profile of everolimus in patients with advanced NET and a history of carcinoid syndrome.

#### Acknowledgment

Study sponsored by Novartis.

## P79

### A randomized, double-blind, placebo-controlled, multicenter phase III trial of everolimus in patients with advanced pancreatic neuroendocrine tumors (PNET) (RADIANT-3): updated safety results

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#### Background

In the phase III RADIANT-3 trial, everolimus, an oral mTOR inhibitor, demonstrated superiority in progression-free survival with a median of 11.0 vs 4.6 months for placebo (hazard ratio, 0.35; 95% confidence interval, 0.27 to 0.45; *P*<0.0001) in patients with advanced pNET (ESMO 2010, Abstract #LBA9). Here we present an update of the safety analysis from this trial.

#### Methods

Patients with progressive advanced low- or intermediate-grade pNET were randomized to everolimus 10 mg/day orally (n=207) or placebo (n=203); both arms received best supportive care. Adverse events (AEs) were collected and coded to a preferred term using the medical dictionary for regulatory activities. AEs were graded using the National Cancer Institute's Common Terminology Criteria for AEs (v 3.0). Safety population included 407 patients (204 for everolimus; 203 for placebo).

#### Results

Median study follow-up was 20.1 months. Safety of everolimus was consistent with the primary analysis (median follow-up 17 months). Median exposure to everolimus was 2.3-fold longer than placebo (37 vs 16 weeks), corresponding to exposures of 175.2 vs 104.9 patient-years, respectively. Most common drug-related AEs with everolimus versus placebo were stomatitis (52.9 vs 12.3%), rash (48.5 vs 10.3%), diarrhea (34.3 vs 10.3%), and fatigue (32.4 vs 14.3%). Anemia (5.9 vs 0%), hyperglycemia (5.9 vs 2.5%), stomatitis (4.9% vs 0%), and thrombocytopenia (3.9 vs 0%) were the most common drug-related grade 3/4 events for everolimus and placebo, respectively. Most AEs resolved with dose modification/interruptions or concomitant medication. Rate of drug-related AEs leading to discontinuation was relatively low (13.7% everolimus versus 2.0% placebo). Disease progression remained the primary reason for discontinuation from both treatment arms.

#### Conclusions

Safety of everolimus in pNET patients observed in this updated analysis is consistent with previous experience and provides further evidence of the favorable risk-benefit profile of everolimus in advanced pNET patients.

#### Acknowledgment

Study is sponsored by Novartis.



**P80****Mitotane reduces the chemoresistance phenotype in an adrenocortical carcinoma cell line**

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Adrenocortical carcinoma (ACC), a rare tumor, with incidence of 1–2 per million population annually, has a bimodal distribution by age, with cases clustering in children under 6, and in adults 30–40 years old. ACC has a dismal prognosis. The only curative treatment is complete surgical excision of the tumor, but late diagnosis prevents surgical cure, since ACC frequently recurs and metastasize. Chemotherapy is frequently ineffective, due to the overexpression of the MDR-1 gene, encoding for detoxifying pump P-gp, which confers chemoresistance to ACC. Mitotane, an adrenolytic drug, is being used as adjuvant therapy to prevent recurrence, despite several and important side effects appearing at therapeutic concentrations (14–20 mg/l; 40–60 µM).

We aimed at identifying therapeutic strategies to overcome adrenocortical carcinoma chemoresistance by using mitotane.

Human adrenocortical cells, NCI-H295R, expressing high P-gp levels, were treated with mitotane (2.5–80 µM) and doxorubicin (1–100 nM), identifying minimal cytotoxic concentrations. Cell viability and caspase 3/7 activity under 25 and 50 nM doxorubicin without or with 5 µM mitotane were tested. These drug concentrations are much lower than those reached *in vivo*. Combination treatments significantly and more potently reduced cell viability (40–60%) by inducing caspase-3/7 activity. In addition, we found that mitotane significantly reduced P-gp activity, promoting the intracellular accumulation of fluorescent compounds.

Our results indicate that mitotane enhances the cytotoxic effects of doxorubicin in a chemoresistant adrenocortical carcinoma cell line, likely inhibiting P-gp activity.

**P81****Treatment of patients with metastatic well-differentiated gastroenteropancreatic tumors with RAD001 (EVEROLIMUS)**

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**Purpose**

The efficacy and safety of RAD, an mTOR inhibitor, in patients with well differentiated metastatic gastroenteropancreatic neuroendocrine tumors (GEP-NET).

**Material and methods**

We studied seven male patients (mean age 55 years, range 36–75) with well differentiated non functioning stage IV GEP-NETs (five pancreatic, one hindgut carcinoid, and one NET of unknown primary). All received RAD 10 mg/day in combination with long acting octreotide 30 mg/month. Five patients had progressive disease despite previous treatment (three chemotherapy, two radiolabelled somatostatin analogues), while RAD was the initial treatment for two patients. The efficacy of treatment was evaluated with biochemical (chromogranin A, CgA) and radiological (RECIST) criteria.

**Results**

The mean time of follow up was 7.8 months (range 2.7–19). Using RECIST criteria, five patients showed stable disease (three with previous progressive disease) and two patients showed progression (both with previously documented progressive disease). The mean value of CgA before RAD treatment was 149.14 ng/ml (range 63–242), and 181 ng/ml (range 66–345) after last assessment; four patients had biochemically stable disease. Four patients developed grade I aphthodous stomatitis, two grade I rash, and two grade I diarrhea. Hyperglycemia was observed in all of them (four grade I, three grade II), and hyperlipidemia in six. Anemia developed in four patients (three grade I, one grade II), grade I thrombocytopenia in one, and leucocytopenia in three (two grade I, one grade II). No patient discontinued RAD treatment because of a serious adverse event.

**Conclusion**

Although the time of follow up is relatively short, the administration of 10 mg everolimus orally in combination with octreotide is a well tolerated and efficient treatment, in patients with well differentiated metastatic GEP-NETs with documented progression before treatment.

**P82****The role and extend of surgical resection in patients with gastric carcinoid tumours type 1**

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**Background**

The exact indications and/or the type of operation requiring for the treatment of gastric carcinoids (GC) type 1, are not clearly stated. The present study was designed to evaluate the value of the oncological gastrectomy in the treatment of GC type 1.

**Materials and method**

Between January 2004 and December 2010, we advocated gastric resection in cases of GC type 1 when at least one of the following criteria was present: i) multiple recurrent lesions, ii) lesions with positive margins following endoscopic resection, iii) lesions with malignant potential (deep gastric parietal wall invasion, lymph node enlargement, Ki67 proliferative index >2%) and/or iv) presence of metastatic disease. Preoperatively, all patients had undergone laboratory tests (serum gastrin and chromogranin A measurements) and imaging investigations for exclusion of the Zollinger-Ellison and the MEN1 syndromes. In addition, all patients underwent either computerized tomography (CT) and/or magnetic resonance imaging (MRI) of the abdomen, as well as scintigraphy with <sup>111</sup>In-pentetreotide (Octreoscan). Endoscopic ultrasound (EUS) was performed in four patients. A modified subtotal D2 lymphadenectomy was routinely performed.

**Results**

Seven patients (five females) were surgically treated. Four patients fulfilled one and three patients fulfilled two criteria. Fifteen to thirty-six perigastric lymph nodes were histologically retrieved per patient, but metastatic infiltration was noticed only in a patient with hepatic metastases. Within a median follow-up of 34 months, all patients are alive without any evidence of recurrence.

**Conclusion**

Extended peri- and extra-gastric lymphadenectomy did not prove to be necessary for non-malignant localized GC type 1, either for staging or therapeutically.

**P83****Effectiveness of zoledronic acid yearly for preventing bone loss in patients with neuroendocrine tumors on somatostatin analogue therapy**

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**Background**

Neuroendocrine tumors awareness is developing. Carcinoid syndrome and hormonal overproduction may be controlled with somatostatin analogues in majority of cases. However, due to unspecific symptomatology in early stages of disease, majority of cases are diagnosed when process is disseminated. Co-morbidities and coexisting diseases are present in most of patients. Bone loss may be accelerated by metastasizing tumor, peptides secreted or decreased absorption of vitamin D and calcium.

Aim was to investigate zoledronic acid effectiveness in preventing of bone loss not related to metastases.

**Group**

Eight patients with carcinoid syndrome due to GEP-NET, with no metastases to bones at octreoscan and strontium scintigraphy, on stable therapy with octreotide LAR (*n*=6) or lanreotide AutoGel (*n*=2), with excluded multiple endocrine neoplasia and deficit of vitamin D.

**Intervention**

Zoledronic acid 5 mg i.v. given every 12 months, DXA of lumbar spine and femoral neck and history of fractures to assess intervention effectiveness.

**Results**

Overall L2–L4 BMD did not changed significantly (0.876 SD 0.113 vs 0.911 SD 0.134; *T*<sub>score</sub> –1.6 SD 0.8 –1.4 SD 0.9) during first year and second year of observation. Femoral neck BMD increased during first year in 5 out of 8 and trend was significant during 2nd year (*n*=6; 0.814 SD 0.134 vs 0.947 SD 0.098 *P*=0.03; *T*<sub>score</sub> –1.7 SD 0.7 vs –1.1 SD 0.5 *P*=0.05). Two patients were excluded from further observation due to disease progression and bone metastases detection. No fractures were recorded in whole group.

**Conclusions**

Zoledronic acid 5 mg given yearly may be useful to treat concomitant osteoporosis in patients with neuroendocrine tumors during disease stabilization on somatostatin analogue therapy.

**P84****The demethylating agent 5-aza-2-deoxycytidine upregulates somatostatin type 2 receptor expression and enhances internalization of radiolabeled somatostatin analogue in human carcinoid tumour cells**M J Veenstra<sup>1</sup>, P M van Koetsveld<sup>1</sup>, W E Farrel<sup>2</sup>, F Dogan<sup>1</sup>, A M Waaijers<sup>1</sup>, D M Spruij-Mooij<sup>1</sup>, S W J Lamberts<sup>1</sup>, G Vitale<sup>3,4</sup> & L Hofland<sup>1</sup><sup>1</sup>Division of Endocrinology, Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; <sup>2</sup>Department Human Disease and Genomics Group, Institute of Science and Technology in Medicine, School of Medicine, Keele University, Stoke on Trent, Staffordshire ST4 7QB, UK; <sup>3</sup>Department of Medical Sciences, University of Milan, Milan, Italy; <sup>4</sup>IRCCS Istituto Auxologico Italiano, Milan, Italy.**Introduction**

Neuroendocrine tumors (NET) are rare tumors originating from various types of neuroendocrine cells. The somatostatin receptor type 2 (sst<sub>2</sub>) is expressed in NET cells and is a target for therapy with somatostatin analogues.

Epigenetic changes including methylation of DNA at CpG dinucleotides, and particularly in the promoter region of genes can inhibit transcription. Methylation can be inhibited by cytidine analogues such as 5-aza-2-deoxycytidine (AZA). Since hypermethylation of the sst<sub>2</sub> gene has been shown to influence sst<sub>2</sub> expression we hypothesize that upregulation of sst<sub>2</sub> expression by AZA would lead to increased uptake of [<sup>125</sup>I-Tyr<sup>3</sup>]octreotide.

**Materials and methods**

Cells of the human pancreatic carcinoid cell line BON were cultured 7 days with increasing doses of AZA to determine the effect on cell proliferation. To determine the effect of AZA on uptake of [<sup>125</sup>I-Tyr<sup>3</sup>]octreotide, cells were cultured 6 days at 3 conditions: untreated control and AZA 50 and 100 nM. Thereafter, the cells were plated and cultured without or with AZA for 2 days. Subsequently, an internalization experiment was performed using [<sup>125</sup>I-Tyr<sup>3</sup>]octreotide. Cells were subsequently collected for RT-qPCR of sst<sub>2</sub>. To determine the methylation status of the CpG island, methylation specific PCR and subsequent sequencing was performed on bisulfite treated DNA isolated from untreated cells.

**Results**

AZA induced a dose-dependent inhibition of cell proliferation with an IC<sub>50</sub> value of 80 nM. AZA, at 50 and 100 nM, induced a 2- and 2.5-fold increased uptake of [<sup>125</sup>I-Tyr<sup>3</sup>]octreotide. RT-qPCR results showed a dose-dependent increase of sst<sub>2</sub> mRNA expression. Partial CpG methylation of the sst<sub>2</sub> gene was found in the CpG island, downstream of the transcription start site.

**Discussion and conclusion**

Measurement of internalized [<sup>125</sup>I-Tyr<sup>3</sup>]octreotide revealed increased dose dependent uptake after treatment with AZA, likely due to upregulation of sst<sub>2</sub> expression. This indicates that either the region of the CpG island we determined to be methylated influences expression or there is an indirect mechanism causing upregulation of sst<sub>2</sub>.

**P85****Comments on a series of patients affected by MEN1**

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by variable combination of more than 20 types of endocrine and non-endocrine tumors: typically parathyroid glands, pancreatic islet cells and anterior pituitary gland are involved. We studied 20 patients (10M and 10F), affected to our Pituitary Unit in a period of 10 years. All patients had clinical diagnostic criteria suspected for MEN1 (presence of two endocrine tumors at least) and underwent genetic test (sequence analysis) for MEN1 mutations that were confirmed in five patients. Kindred of patients with MEN1 mutations were also tested and four people (in one family) were found to have MEN1 mutations. 18 patients had parathyroid tumors, 18 had pituitary tumors (9 GH-secreting adenomas, 5 prolactinomas and 6 non-functioning adenomas). One patient had colorectal carcinoid tumor and in three patients we found also gastric neuroendocrine cells hyperplasia. Five patients had pancreatic gastrinomas, four of them were MEN1 mutations carriers. One of them underwent total pancreatectomy while the others underwent excision of pancreatic gastrinomas or pancreaticoduodenectomy. After <10 years two of them (both carriers of MEN1 mutations) showed extensive disease recurrence (despite medical therapy and radiotherapy) and so underwent total pancreatectomy, maybe suggesting that

these kinds of MEN1 mutations lead to a more aggressive type of pancreatic NET. Moreover, despite the data present in literature, we report a higher prevalence of GH-secreting pituitary adenomas, which appear to be more frequent than prolactinomas. Finally high proportion of MEN1 patients without detected MEN1 mutations suggests that unknown mutations of MEN1 gene may be involved in the occurrence of MEN1.

**P86****Role of peptide receptor radionuclide therapy (PRRT) in well and moderately differentiated neuroendocrine tumours**

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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 SSA. We investigated the role of medical therapy with SSA and PRRT in patients with well and moderately differentiated neuroendocrine tumours. We evaluated 55 patients with well or moderately differentiated neuroendocrine tumours; 31 patients with either metastatic, unresectable or functioning tumours were treated with SSAs. Ten of them with metastatic disease or unresectable tumours, all with increased uptake on Octreoscan, underwent PRRT with <sup>90</sup>Y or <sup>177</sup>Lu. SSA allowed biochemical control of hormone secretion in all patients but one refractory carcinoid syndrome; six patients treated with PRRT showed minor or partial response, three had stable disease, one had progression. Three of the six patients with response to PRRT had further progression with a mean PFS of 12.7 months. 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.

**P87****Anatomical distribution of primary adrenal tumors: 10-year single center experience**

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The incidental discovery of adrenal tumors in the course of computer tomography scanning for various reasons has led to the more detailed study of this type of tumors.

The aim was to study the anatomical distribution of primary adrenal tumors at diagnosis.

The archives of all patients with primary adrenal tumors having been hospitalized in the Department of Endocrinology and Metabolism of Metaxa Hospital, Pireaus, Greece from 01.01.2000 up to 30.06.2010 were studied. Patients with secreting and non secreting adrenal adenomas, pheochromocytomas, carcinomas of the adrenal cortex and malignant pheochromocytomas were included.

During the above-mentioned period 223 patients with primary adrenal tumors were hospitalized. Amongst this group of patients 170 (76%) were female and 53 male (24%). In their majority adrenal tumors presented in the left adrenal. In 58% of the male patients (31/53) the tumors were found in the left adrenal, in 30% (16/53) in the right, while in 12% (6/53) of male patients bilateral adrenal tumors were observed. In 80/170 (47%) of female patients tumors were found in the left adrenal, in 58/170 (34%) in the right adrenal and in 32/170 (19%) bilateral adrenal tumors were observed.

The aetiology of adrenal oncogenesis has not been elucidated. In the cohort of patients studied a tendency for the tumors to arise within the left adrenal was observed. However, although the left from the right adrenal differ as far as the blood supply and outflow is concerned, further studies are needed to fully elucidate the anatomical distribution of primary adrenal tumors.

**P88****cAMP induces PATZ1 and estrogen receptor  $\beta$  nuclear re-localization in the human seminoma TCam-2 cell line**F Boscia<sup>1</sup>, F Esposito<sup>1</sup>, S Kitazawa<sup>2</sup>, L Looijenga<sup>3</sup> & P Chieffi<sup>4</sup><sup>1</sup>Università di Napoli Federico II, Naples, Italy; <sup>2</sup>Kobe University, Kobe, Japan; <sup>3</sup>ErasmusMC-University, Rotterdam, The Netherlands; <sup>4</sup>Seconda Università di Napoli, Naples, Italy.

Estrogen exposure has been linked to a risk for the development of testicular germ cell cancers. The effects of estrogen are now known to be mediated by estrogen receptor  $\alpha$  (ER $\alpha$ ) and ER $\beta$  subtypes, but only ER $\beta$  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. PATZ1 is a recently discovered zinc finger protein that, due to the presence of the POZ domain, acts as a transcriptional repressor affecting the basal activity of different promoters. We have previously described that PATZ1 plays a crucial role in normal male gametogenesis and that its up-regulation and mis-localization could be associated to the development of testicular germ cell tumours. Here, we show that ER $\beta$  interacts with PATZ1, and PKA (regulatory subunit) in normal germ cells, while down regulation of ER $\beta$  associates with transcriptional coregulator PATZ1 delocalization in human testicular seminomas. Interestingly, we found that the translocation of PATZ1 from the cytoplasm into the nucleus is regulated by cAMP that induces also an ER $\beta$  increased expression and nuclear localization, while this effect is counteracted by using the anti-estrogen ICI 182-780.

Taken together, our results demonstrate for the first time the interaction between PATZ1 and ER $\beta$  in normal germ cells; consistently, the PATZ1 delocalization associated with ER $\beta$  down regulation in human testicular seminomas, could be ascribed to an impaired cAMP mediated signalling to generate the testicular neoplasia.

**P89****The management of thyroid nodule in a patient with a non-thyroid primary cancer**Rene Baloescu<sup>1</sup>, Mara Carsote<sup>2</sup>, Raluca Trifanescu<sup>1,2</sup>, Madalina Musat<sup>1,2</sup>, Ruxandra Danculescu-Miulescu<sup>2</sup>, Dumitru Ioachim<sup>1</sup>, Dana Terzea<sup>3</sup> & Catalina Poiana<sup>1,2</sup><sup>1</sup>CI Parhon National Institute of Endocrinology, Bucharest, Romania; <sup>2</sup>Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; <sup>3</sup>Babes Institute of Development and Research, Bucharest, Romania.**Introduction**

The management of a thyroid nodule in a patient who is newly diagnosed with an active cancer depends not only of the thyroid disease itself but also of the prognosis of the second neoplasia.

**Aim**

We present a case of a male who was admitted to an endocrinologist for a thyroid nodule evaluation.

**Case presentation**

B.C., 66-year-old male patient, smoker from the last 17 years, was treated with thyamazol for hyperthyroidism. A CT exam showed a right thyroid nodule of 2.5 cm, but also a left inferior lobe lung tumor of 3.8 cm, with unhomogenous and spiculiform aspects, several micronodules of maximum 6 mm under pleura (bilateral), and left hill lymph nodes were found. The investigations lead to bilateral lobectomy (atypical resection) of a lung tumor. The pathological exam showed pulmonary tissue with neoplastic infiltration of differentiated mixt adenocarcinoma with pleural invasion (T2N3M1). The IHC report showed positive reaction for TTF1 and CEA and negative for tireoglobulin. Later, he was admitted for a recent rapid growth of the nodule. The FNAB revealed epithelial proliferation with compact, trabecular, and papillary aspects, and also with squamous areas, multiple microcalcifications, suggestive for a papillary thyroid cancer but a pulmonary lung metastasis cannot be excluded. The serum calcitonin was normal, but serum thyroglobulin was twice normal – 141.4 ng/ml (normal: 1.4 and 78 ng/ml). Total thyroidectomy was performed and the diagnosis of papillary thyroid cancer was established. Radioiodine therapy was started and also levo-thyroxin suppressive therapy, associated with chemotherapy.

**Conclusion**

The practitioners should be aware that hyperthyroidism does not exclude the presence of a thyroid cancer. The management of a thyroid nodule in a patient already diagnosed with a malign non thyroid tumor is more difficult.

**Female reproduction****P90****Ovulation induction by metformin in lean and obese women with polycystic ovary syndrome**P V Popova<sup>1</sup>, L I Ivanova<sup>3</sup>, T L Karonova<sup>1</sup> & E N Grineva<sup>1,2</sup><sup>1</sup>Saint Petersburg Pavlov State Medical University, Saint Petersburg, Russian Federation; <sup>2</sup>Almazov Federal Heart, Blood and Endocrinology Centre, Saint Petersburg, Russian Federation; <sup>3</sup>Kirov Military Medical Academy, Saint Petersburg, Russian Federation.**Introduction**

Metformin is now one of the most common treatments for PCOS. However controversy exists about the usefulness of metformin in PCOS women with normal insulin sensitivity and normal weight. The aim of this study was to compare efficacy of metformin treatment for the purpose of restoration of ovulation in lean and obese women with polycystic ovary syndrome.

**Design**

Thirty-six unovulatory women with PCOS (21 lean with mean body mass index (BMI)  $21.4 \pm 2.3$  kg/m<sup>2</sup> and 15 obese with BMI  $32.3 \pm 3.3$  kg/m<sup>2</sup>) received metformin 1700 mg/day during 6 months. We estimated ovulation induction (by ultrasound scanning), change in menstrual cycle, anthropometric measurements, endocrine parameters and insulin sensitivity.

**Results**

A total of 20 lean and 14 obese women completed the trial. The groups were comparable by age, androgen levels and severity of menstrual dysfunction at baseline. Ten obese patients (67%) and one lean patient (5%) had resistance to insulin (homeostasis model assessment (HOMA) index  $> 2.5$ ),  $P < 0.001$ . Lean women with PCOS treated with metformin had restoration of menstrual function (55%) and ovulation (45%) more often than obese women with PCOS (only one patient – 7% – responded to the treatment),  $P = 0.018$ . Significant decrease of testosterone level (from  $3.1 \pm 1.0$  to  $2.7 \pm 0.8$  nmol/l;  $P = 0.049$ ), fasting glucose (from  $5.2 \pm 0.4$  to  $4.9 \pm 0.4$  mmol/l,  $P = 0.013$ ) and HOMA (from  $1.6 \pm 0.7$  to  $1.2 \pm 0.7$ ,  $P = 0.045$ ) during metformin treatment was shown only in lean women with PCOS. There was no change in BMI and waist circumference in both groups.

**Conclusions**

Treatment with metformin 1700 mg daily was more effective in lean than in obese women with PCOS. Its efficacy was independent of initial surrogate markers of resistance to insulin.

**P91****Detection and exploring the left shift kurtosis of the fertile window in ovulatory cycles by biophysical biomarkers of the cervical secretion: case report**Jose María Murcia Lora & María Luisa Esparza Encina  
Clinical Consulting G&E, Logroño La Rioja, Spain.**Introduction**

The main purpose of this case is to present a case in which the left kurtosis interval of the fertile phase curve, may be evaluated by checking the biophysical changing pattern of reproducible methods of viscoelasticity and transparency of the cervical secretion.

**Case report**

This case report included a 24 years old woman who plots a curve in which integrates two biomarkers in relation to the fertile window at the follicular phase. It was recording the evolution of biophysical parameters of the cervical secretion when approaching to the fertile window. Filancia is quantified by measuring the distance in cm of cervical secretion and transparency in tree degrees of density. Biomarkers are located within the window of fertility in relation to the maximum peak day of filancia plotted on the chart. The fertile window was detected with the threshold level of urinary oestrone-3-glucuronide (E3G). It was identify the ovulation day (EDO) by test of LH post peak.

**Results**

The fertile window was 10 days estimated by cervical mucus, 4 days longer than the monitor E3G, in which the length of the fertile period was 6 days. It was possible to detect the cervical mucus evolution of 4 days at the left kurtosis of the 6-day fertile period of the fertile window when it used biophysical parameters of the cervical secretion.

**Conclusion**

The objective evaluation help to check better the changing pattern of the left kurtosis of the fertile window by cervical secretion, and detect better the time shift of this left kurtosis of the fertile window. Integrating the different biomarkers parameters may identify better the left changing patterns of the fertile window when it used cervical secretion.

**P92****Prevalence of thyroid pathologies in patients with polycystic ovary syndrome**

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**Introduction**

Polycystic ovary syndrome (PCOS) is a common disorder characterized by chronic anovulatory oligo-amenorrhea and signs of hyperandrogenism. In this study, we aimed to determine prevalence of thyroid nodules and autoimmune thyroid diseases in patients with PCOS.

**Methods**

Patients diagnosed with PCOS in our endocrinology clinic were included in the study. In patients with irregular menses and/or hirsutism, the diagnosis of PCOS was made by high serum LH/FSH ratio, high serum free testosterone levels and/or polycystic ovaries in pelvic ultrasonography. Serum thyrotropin, free thyroid hormones, antithyroid peroxidase (AntiTPO) and antithyroglobulin (AntiTg) antibody levels were evaluated. Thyroid ultrasonography was performed in all subjects by the same examiner. Thyroid fine needle aspiration biopsy (FNAB) was carried out when indicated.

**Results**

There were 107 women with PCOS with a mean age of  $23.96 \pm 5.70$ . Seventeen (15.9%) patients had hypothyroidism and 2 (1.8%) had hyperthyroidism. 30.5% patients had positive AntiTPO, 30.5% had positive AntiTg and 37.8% had positive AntiTPO or AntiTg antibodies. Thyroid nodules were detected in 29 (27.1%) patients, 10 had solitary and 19 had multiple nodules. FNAB was performed in 11 patients with nodules; 10 were cytologically benign and 1 was malignant (papillary thyroid carcinoma was confirmed histopathologically). Totally, in 54 (50.5%) patients, there was a thyroid related disease such as positive thyroid antibodies, thyroid dysfunctions or nodules. Insulin resistance calculated with HOMA-IR was  $\geq 2$  in 47 (43.9%) and  $< 2$  in 60 (56.1%) patients. Nodule prevalence, thyroid dysfunctions, thyroid autoantibody positivity and thyroid volume did not differ between PCOS patients with and without insulin resistance.

**Conclusion**

Thyroid pathologies are observed in half of the patients with PCOS. Measurement of thyroid hormones are usually a part of investigation in PCOS patients, but we think serum thyroid autoantibodies and presence of thyroid nodules should also be searched in these patients.

**P93****Metformin treatment with or without life style changes in the polycystic ovary syndrome**

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Metformin 500 mg three times daily had been advised to our patients suffering from the polycystic ovary syndrome by the Rotterdam criteria who did not want to take contraceptive pills. More recently, we have included life style changes to metformin taking: increased physical activity, low glycaemic index diet (with calorie restriction for the obese). The efficacy of the two treatment forms was compared. The metformin only group consisted of 27 patients (age 18–39 years, mean, 26); the metformin + life style changes group of 28 age-matched patients. The following parameters were registered at the beginning and the end of a 6-month treatment period: global acne score, Ferriman-Gallwey hirsutism score (FG), body mass index (BMI), and the waist-hip circumference ratio (W/H). By the end of the treatment period, both acne and FG improved significantly in both treatment groups, without significant difference between treatment forms: acne  $8.6 \pm 5.7$  vs  $9.3 \pm 6.0$  ( $P=0.67$ ); FG  $2.5 \pm 2.0$ , vs.  $2.6 \pm 1.6$  ( $P=0.83$ ). BMI and WH remained practically unchanged in the metformin only group:  $0.26 \pm 1.0$  (NS) and  $0.001 \pm 0.02$  (NS). BMI improved in the metformin + life style group by  $0.9 \pm 1.1$  ( $P<0.001$ ); and W/H by  $0.015 \pm 0.03$  ( $P<0.05$ ). Life style changes did not show in additional improvement of hyperandrogenic symptoms compared to metformin only but diminished BMI and W/H. Based on these results we advise the continuation of the triple basal treatment consisting of metformin with low glycaemic index diet and increased physical activity (as triple basal therapy) to all patients with PCOS for long-term assessment.

**P94****Development of a mouse model with polycystic ovary syndrome**

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women in their reproductive age. Based on the Rotterdam criteria PCOS is defined by two out of the following three criteria: hyperandrogenism, oligo/anovulation, and polycystic ovaries. In addition, PCOS patients are often abdominal obese, which can lead to insulin resistance. The subsequent increased insulin levels stimulate the ovary to further increased androgen production. Thus, the elevated androgen and insulin levels may result in a detrimental vicious circle between ovary and adipose tissue. Our aim was to develop a mouse PCOS-like model, allowing the future use of transgenic mouse models to study the interaction between ovarian and adipose function in PCOS.

Prepubertal female mice received a 60 or 90 days continuous release pellet containing the non-aromatizable androgen dihydrotestosterone (DHT) or vehicle. At the end of the treatment period the estrous cycle was analyzed and ovaries were collected. Furthermore, body weight was measured, fat depots were morphologically analyzed and an intraperitoneal glucose tolerance test (IPGTT) was performed.

DHT treatment for 60 days did not result in differences in reproductive and metabolic characteristics compared to vehicle-treated mice. In contrast, 90 days DHT-treated mice were in continuous an-estrous and their ovaries lacked corpora lutea, consistent with anovulation. Antral follicles of 90 days DHT-treated mice had a cyst-like structure and there was an increase in the number of atretic follicles. These mice also showed a significantly higher body weight. In addition, fat depots of DHT-treated mice displayed an increased number of adipocytes of increased size. Furthermore, blood glucose levels during IPGTT were higher, suggesting that these mice are glucose intolerant.

We conclude that DHT treatment for 90 days in mice results in a metabolic and reproductive phenotype resembling the phenotype found in PCOS women.

**P95****Kisspeptin: new marker of placental dysfunction?**

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Kisspeptin produced by placenta is involved in the regulation of early placental development. Aberrant placental development and placental dysfunction are common in pregnancies with diabetes and hypertension. Parameters of fetal growth, fetoplacental circulation, pregnancy outcome, placental weight and morphology were recorded and correlated with kisspeptin levels in pregnant women with diabetes (chronic insulin dependent-IDDM and gestational-GD) and hypertension (chronic-H, pregnancy induced-PIH and pre-eclampsia-PE). Kisspeptin was evaluated in 129 singleton pregnancies by an in-house RIA assay. Samples were collected in the 1st, 2nd and 3rd trimester (T) in patients with IDDM ( $n=16$ ), H ( $n=22$ ) and healthy pregnant women ( $n=25$ ) and in the 2nd and 3rd trimester in patients with GD ( $n=20$ ), PIH ( $n=18$ ) and PE ( $n=28$ ). In pregnancies with IDDM, GD, H and PE kisspeptin levels were significantly lower compared to control group at all time points. Kisspeptin levels in patients with PIH were not significantly different from control group. In PIH and controls no adverse events and no abnormalities in placental morphology and function were recorded. In the 1st T severely decreased kisspeptin levels were associated with an increased risk of spontaneous abortion. In the 2nd T low kisspeptin levels were associated with higher incidence of abnormal placental morphology. In the 3rd T low kisspeptin levels correlated with higher incidence of adverse outcomes ( $P<0.01$ ) and disturbances in fetoplacental circulation ( $P<0.01$ ). Gestational age, neonatal and placental weight were significantly lower in PE group compared to others ( $P<0.01$ ). No correlation was found between placental weight and kisspeptin ( $P>0.05$ ). Significant associations between reduced kisspeptin levels and parameters of placental dysfunction and pregnancy outcome were found in pregnancies with pre-eclampsia, diabetes and chronic hypertension. Larger studies are needed to investigate the role of kisspeptin as a potential new marker of placental dysfunction.

**P96****Tissue specific regulation of CGB gene expression in the common marmoset (*Callithrix jacchus*) by SP-1 and AP-2**

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**Objective**

In the marmoset (*Callithrix jacchus*) the LH is functionally replaced by the chorionic gonadotropin (CG). However, disparate to the human LH/hCG system, marmoset CG is expressed in both, the pituitary and in the placenta. Previously, we could show the presence of a tissue-specific promoter system in the marmoset. Alternative promoters, as well as different first exons, either a pituitary or a novel placenta specific one, are being employed to direct tissue specific expression of CGB. This study aims to elucidate the regulation of marmoset CG in the placenta and the general mechanism for tissue-specific expression.

**Results**

Sequence analysis and reporter gene assays revealed the presence of two SP-1 and AP-2 binding sites in the placental CGB promoter. Mutation of any of these binding sites led to a significant decrease in promoter activity by 90%. Further sequence analysis by MethPrimer revealed the presence of a prominent CpG-island within the placental promoter. A tissue specific DNA-methylation pattern was identified for the placental promoter region, with being hypermethylated in the pituitary and hypomethylated in the placenta. *In vitro* methylation of the placental promoter sequence led to a drastic decrease in CGB promoter activity.

**Conclusion**

We propose a concurring model where AP-2 and SP-1 work synergistically in a cassette modus on placental CGB expression. Our results further indicate an epigenetic regulation of the CG promoter in the placenta. This links CGB expression to a combined epigenetic and transcription factor regulatory mechanism.

**P97****Sexual dysfunction in polycystic ovary syndrome**Selvinaz Velet, Mustafa Velet, Pinar Kadioglu & Sezai Sahmay  
Cerrahpasa Faculty of Medicine, Istanbul, Turkey.**Objective**

We aimed to examine sexual dysfunction frequency and relationship between sexual dysfunction and androgen level in polycystic ovary syndrome.

**Patients and methods**

Twenty-five PCOS patients were recruited from the outpatient clinics of the Division of Endocrinology in the Department of Internal Medicine and outpatient clinics of the Department Obstetrics and Gynecology at the Cerrahpasa Medical Faculty. Twenty-five healthy volunteer age and sex matched are included in control group. Patients and control subjects were evaluated with female sexual function index (FSFI) and the beck depression inventory (BDI). Serum free testosterone, total testosterone, DHEA-S, androstenedione and prolactin were measured. Free androgen index (FAI) was calculated.

**Results**

Total FSFI score  $26.17 \pm 4$  was in the PCOS group whereas, healthy women had a total FSFI score of  $28.61 \pm 4.68$ . FSD was diagnosed in 4 of 25 patients (16%) while 5 of 25 (20%) healthy women had FSD ( $P=0.57$ ). There is no statistically significant difference between sexual desire ( $P=0.89$ ), arousal ( $P=0.10$ ), lubrication ( $P=0.057$ ), orgasm ( $P=0.18$ ) and satisfaction ( $P=0.78$ ) domain scores. Pain domain score was significantly lower in PCOS women ( $P=0.002$ ). BDI scores was significantly higher in PCOS women ( $P=0.002$ ). No correlation was seen between androgen level, FAI and total FSFI score and domain score.

**Conclusion**

Change in external appearance in women with PCOS do not make them feeling less attractive and do not lead to sexual dysfunction. Sexual desire, arousal, lubrication, satisfaction and orgasm disorder in women with PCOS is similar to healthy women but depression is more frequent in women with PCOS than healthy women.

**P98****Oligo-amenorrhea and hyperandrogenaemia in postmenarcheal adolescence**P Pinola<sup>1</sup>, H Lashen<sup>2</sup>, A Bloigu<sup>3</sup>, K Puukka<sup>4</sup>, M Ulmanen<sup>4</sup>, M R Järvelin<sup>3,5</sup>,A L Hartikainen<sup>1</sup> & L Morin-Papunen<sup>1</sup>

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**Introduction**

Hyperandrogenaemia in adolescence is associated with hirsutism, acne and menstrual irregularity and is considered as an important risk for polycystic ovary syndrome and subsequent risk of cardiovascular disease and type 2 diabetes. The aim of this study was to establish if self-reported oligo/amenorrhea were associated with hyperandrogenemia and metabolic disturbances in adolescence.

**Subjects and methods**

A cross sectional study using postal questionnaire targeting 16 years old girls in the Northern Finland Birth Cohort 1986 ( $n=4567$ ). Of them, 3237 girls (71%) responded to the questionnaire and attended a clinical examination. After excluding pregnant girls, girls taking oral contraceptives or other hormonal treatment, and the subjects with missing data, 2448 girls were included in the analyses.

**Results**

Seven hundred and fifteen girls reported oligo-/amenorrhea (symptomatic girls) and 1753 had regular periods (symptomless girls). Girls with menstrual disorders exhibited significantly higher serum concentrations of testosterone ( $P=0.010$ ), lower serum levels of sex hormone binding globulin (SHBG,  $P=0.042$ ) and higher levels of free androgen index (FAI, mean: 3.37 (95% confidence interval, CI: 2.32, 4.42) versus 3.07 (95% CI: 2.04, 4.10),  $P=0.002$ ). The two groups did not differ as regards body mass index (BMI), waist-hip ratio, serum levels of glucose, insulin and lipids, or insulin sensitivity. There was a significant linear trend towards higher FAI levels in the higher BMI quartiles both in symptomatic and asymptomatic girls. In the whole population there was a statistically significant linear decreasing in high-density lipoprotein concentrations ( $P<0.001$ ) and higher triglyceride concentrations ( $P=0.004$ ) in the upper FAI quartile.

**Conclusion**

Oligo/amenorrhea at the age 16 is a good marker of hyperandrogenaemia. Girls in the highest BMI quartiles exhibited the greatest degree of hyperandrogenaemia, and those in the highest FAI quartile had a more unfavorable lipid profile, thus confirming earlier data on an association between obesity, hyperandrogenism and metabolic risks.

**P99****Cardiovascular disease risk markers in women with polycystic ovary syndrome: systematic review and meta-analysis**

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**Introduction**

The relation between polycystic ovary syndrome (PCOS) and cardiovascular disease (CVD) remains unclear. The aim of the study was to systematically review the relevant trials that have studied CVD risk factors in woman with PCOS (CRP, Hcy, TNF- $\alpha$ , PAI-1, Lp(a), AGEs, VEGF, IL6, ADMA and fibrinogen) and to meta-analyze the best evidence available.

**Patients and methods**

Search was conducted in the MEDLINE, EMBASE and CENTRAL d (last update June 2010). Eligible for the systematic reviews were studies, which reported on CVD risk markers levels in women with PCOS compared to controls. Weighted mean differences (WMD) and 95% confidence intervals (CI) were calculated in each of the CVD risk markers for all eligible studies and combined using random effects model. To ensure synthesis of the best available evidence, sensitivity analyses were performed.

**Results**

One hundred and thirty studies were including in 11 different meta-analyses, involving in total 6260 women with PCOS and 4546 controls. Women with PCOS demonstrated significantly elevated CRP (WMD (95% CI)=0.96 (0.74 to 1.19)), Hcy (2.25 (1.46 to 3.03)), PAI-1 antigen (16.96 (7.65 to 26.28)), PAI-1 activity

(0.70 (0.17 to 1.23)), VEGF (1.72 (0.96 to 2.48)), ADMA (0.19 (0.08 to 0.3)) and AGEs (3.91 (2.36 to 5.45)) levels as compared – controls, yet with significant between-study heterogeneity. Borderline significance was detected for TNF- $\alpha$  (0.75 (0.07 to 1.44)) and fibrinogen (0.20 (0.01 to 0.39)) whereas no significance was detected for IL6 (0.71 (-0.16 to 1.59)).

#### Conclusions

Women with PCOS have increased serum concentrations of CVD risk factors as compared to controls. If this apparent risk is translated into increased incidence of CVD in later life remains to be elucidated.

## P100

### The aldosterone/renin ratio during administration of oral contraceptives with different types of gestagens

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Aldosterone/renin ratio (ARR) is obtained by measurements of plasma aldosterone (ALD) and either plasma renin activity (PRA) or direct renin concentration (DRC). The various gestogen components (desogestrel, DSG; gestoden, GD; drospirenon, DRSP) of the frequently used oral contraceptives (OC) can influence the ARR differently. Aim of the study was to analyze the effect of OCs with different gestagens and low-dose estradiol component on ARRs (ALD/PRA versus ALD/DRC).

#### Methods

PRA, DRC (DiaSorin) and ALD (Immunotech) were simultaneously measured with RIA in 61 plasma samples of healthy, normotensive volunteers: 22 women taking no OC were controls; 19 women received DSG, 15 women GD and 5 women DRSP.

#### Results

There was a moderate correlation ( $r=0.78$ ;  $P<0.01$ ) between PRA ( $1.72 \pm 1.31$  ng/ml/h) and DRC ( $12.3 \pm 10.1$  ng/l). ALD was significantly ( $P<0.01$ ) higher ( $43.5 \pm 29.5$  ng/dl) in DRSP group. The DRC ( $7.96 \pm 6.56$  ng/l) was significantly ( $P<0.05$ ) lower in women receiving DSG than in control and in those taking DRSP ( $14.5 \pm 9.5$  and  $20.1 \pm 17.6$  ng/l), respectively. The ALD/DRC, but not the ALD/PRA was significantly ( $P<0.05$ ) higher in women receiving DSG and GD ( $2.85 \pm 3.19$  and  $3.07 \pm 3.3$  ng/dl/ng/l) than in controls ( $1.32 \pm 0.78$  ng/dl/ng/l). The number of cases with ALD/PRA out of the cut-off value ( $> 37.6$  ng/dl/ng/ml/h) were rare (2 in DSG and 1 GD groups), but much frequent (9 women) are occurred by determining ALD/REN (cut-off  $> 3.9$  ng/dl/ng/l; 5 in DSG, 3 in GD and 1 in DRSP groups).

#### Conclusions

There is a weak analytical and clinical correlation between PRA and DRC. In patients taking OC the ALD/PRA is usually more reliable. Our preliminary results indicate that in women receiving DRSP-containing OC not only higher ALD (caused by *in vitro* interactions) but also high PRA and DRC are present because of the antimineralocorticoid effect of these gestagens. Consequently, the ARR rarely exceeds the cut-off level.

## P101

### Inhibitory effect of DHEAS on leiomyoma growth

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#### Objective

This study aims to investigate whether DHEAS (Dehydroepiandrosterone sulfate) inhibit the growth of leiomyoma.

#### Method

Total 131 patients were recruited according to the leiomyoma size by transvaginal ultrasonography. Excluding twenties and over fifties, 88 patients were analyzed. The leiomyoma group was classified into three groups, such as mild, moderate and severe. The mild group was defined as the presence of at least one or two leiomyomas smaller than 3 cm in diameter. The moderate group was defined as having of at least one 3 to 5 cm leiomyoma. The severe group was defined as having leiomyomas greater than 5 cm. The control group was defined as no presence of leiomyoma. Serum DHEAS level was measured by radioimmunoassay.

#### Results

DHEAS mean value of four groups are control ( $n=19$ ,  $125.7 \pm 77.6$ ), mild ( $n=24$ ,  $122.4 \pm 72.3$ ), moderate ( $n=24$ ,  $90.6 \pm 40.2$ ) and severe ( $n=21$ ,  $87.7 \pm 43.8$ ) ( $P=0.069$ ). Comparing after recombination four group into two group, control plus mild (group 1) and moderate plus severe (group 2  $n=45$ ), their mean values are group 1 ( $n=43$ ,  $123.9 \pm 73.8$ ) and group 2 ( $n=45$ ,  $89.2 \pm 41.5$ ) ( $P=0.0076$ ).

#### Conclusion

This study shows that DHEAS may inhibit the growth of leiomyoma.

Keywords: Leiomyoma, DHEAS (dehydroepiandrosterone sulfate), RIA (radioimmunoassay).

## P102

### The importance of PIK3R1 gene in polycystic ovary syndrome

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#### Introduction

Insulin resistance is a main characteristic of polycystic ovary syndrome (PCOS) and contributes to the increased risk of type 2 diabetes and cardiovascular disease. It is thought that in PCOS there is an alteration in the insulin signaling pathway. An important kinase in this pathway is the phosphatidylinositol 3 kinase (PI3K), the activity of which has been found to be decreased in PCOS. Based on this observation we investigated whether a single nucleotide polymorphism (rs1043526, A>G) of *PIK3R1* gene that encodes the phosphatidylinositol 3 kinase regulatory subunit 1 (p85a) is involved in PCOS. This polymorphism is located in the 3'-UTR of *PIK3R1* gene, where miRNAs can bind and thus regulate gene expression.

#### Subjects and methods

We studied 68 women with PCOS and 136 healthy women of reproductive age. Both patients and controls had normal weight. Hormonal profile was determined on 3–5th day of menstrual cycle. Insulin resistance was assessed by fasting glucose to insulin ratio and HOMA index. DNA was extracted from peripheral blood leucocytes and the rs1043526 polymorphism of *PIK3R1* gene was genotyped. All subjects gave their consent and the local Ethical Committee approval was obtained.

#### Results

The *PIK3R1* genotypes were found to be in Hardy-Weinberg equilibrium in both study groups ( $P>0.05$ ). A significant difference was found in the distribution of rs1043526 genotypes between patients and controls. Women with PCOS had a greater frequency of AG and GG genotypes than normal women (35.3% vs 19.9%,  $P=0.016$ ). Furthermore, among patients, those with AG and GG genotypes had higher fasting glucose levels compared to those with the AA genotype ( $90.1 \pm 6.1$  mg/dl vs  $86.2 \pm 8.8$  mg/dl,  $P=0.03$ ). Also, a non significant increase was found in fasting insulin levels and insulin resistance indexes in women with AG and GG genotypes.

#### Conclusion

The rs1043526 A>G polymorphism of *PIK3R1* gene may be involved in the pathogenesis of PCOS and affects glucose metabolism.

## P103

### Effect of paraoxonase genes on metabolic profile of women with polycystic ovary syndrome

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#### Introduction

Increased oxidative stress is considered to be implicated in the pathophysiology of polycystic ovary syndrome (PCOS). While the regulation of oxidative stress is multifactorial, many studies have been focused on the antioxidant role of paraoxonase (PON). The aim of this study was to investigate the possible association of the Q192R and the L55M polymorphisms of *PON1* and the Ser311Cys polymorphism of *PON2* with PCOS.

#### Subjects and methods

We studied 165 women with PCOS and 168 healthy women. The body mass index (BMI) was recorded. Insulin resistance was assessed by fasting glucose to insulin ratio and HOMA index. Furthermore, 100 overweight-obese patients underwent a 75gr OGTT and areas under the curve (AUC) for insulin and glucose were estimated. DNA was extracted from peripheral blood leucocytes and the Q192R and the L55M polymorphisms of *PON1* and the Ser311Cys polymorphism of *PON2* were genotyped.

## Results

PON1 and PON2 gene polymorphisms were in Hardy–Weinberg equilibrium ( $P > 0.05$ ) in both PCOS and control group. There was no significant difference in the distribution of all polymorphisms between PCOS women and controls. However, the L55M polymorphism was found to be associated with insulin levels after glucose load. Patients with the LL genotype compared with those with LM or MM genotype, had higher AUC<sub>insulin</sub> values whereas the basal insulin concentrations, fasting glucose to insulin ratio, HOMA index and AUC<sub>glucose</sub> were similar. This association remained significant after adjustment for the confounding factors BMI, age, and AUC<sub>glucose</sub> ( $P = 0.012$ ,  $\beta = -0.243$ ). Regarding the Q192R polymorphism of PON1 and the Ser311 polymorphism of PON2 no association with biochemical parameters was found.

## Conclusion

PON1 and PON2 gene polymorphisms are not associated with PCOS. However, the association found between the L55M polymorphism and insulin levels after glucose load shows that this polymorphism may be implicated in the insulin resistant phenotype of PCOS.

## P104

### Glucocorticoid-induced leucine zipper (GILZ), a factor which controls tumor cell proliferation, is estradiol responsive in epithelial ovarian cancer

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Little is known about the molecules that contribute to tumor growth in epithelial ovarian cancer (EOC) that remain the most lethal gynecological neoplasm. Glucocorticoid-Induced Leucine Zipper (GILZ), a leucine zipper protein of 17 kDa, is frequently detected in epithelial tissues with a hormonal background. We detected GILZ in EOC<sup>1</sup> and we further demonstrated that this factor which expression is enhanced by estradiol (E<sub>2</sub>), activates tumor cell proliferation and up-regulates CXCR4, a chemokine receptor highly involved in tumor expansion. GILZ was detected by immunohistochemistry in tumors from 50 patients surgically treated for diagnosis of EOC at Antoine Bécélère hospital from 1998 to 2007. Its expression scored from 1 to 7 was positively correlated to the proliferation marker Ki-67 ( $P < 0.00001$ ). It was also higher in tumors containing high amount of phosphorylated protein kinase B (p-AKT) ( $P < 0.01$ ). Cell proliferation increased in BG1 cells overexpressing GILZ by stable transfection of a GILZ-encoding vector whereas it decreased after silencing GILZ by the use of small interfering RNA. Modulation of GILZ expression changed the level of p21, cyclin D1 and phospho-retinoblastoma, three proteins that regulate cell cycle and are AKT targeted. Further GILZ increased phospho-AKT cellular content and AKT kinase activity. In mice xenografted with BG1 cells, tumor growth was faster in females. BG-1 cells treated with  $10^{-9}$  M estradiol had a higher cellular content of GILZ. In parallel growth rate was faster and the expression of CXCR4 was higher, a chemokine which expression was also enhanced by GILZ as revealed by DNA microarray analysis of BG1 cells overexpressing GILZ. In conclusion, GILZ, a protein produced by ovarian cancer cells and which controls their proliferation and CXCR4 expression, is up regulated in estradiol-treated cells like proliferation and CXCR4. This reveals GILZ as a key molecule for estradiol action in EOC.

## P105

### rs7566605 SNP of INSIG2 gene is not associated with the polycystic ovary syndrome diagnosis and its phenotypic traits in Romanian subjects

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## Introduction

The polycystic ovary syndrome (PCOS) and obesity are related, yet different complex diseases, with the same genes possibly playing different roles in each

disease. INSIG2, an obesity-associated gene (by rs7566605 C/C genotype) is a good candidate for PCOS. Its genotypes were not associated with obesity in PCOS patients. To date, there are no published association studies of rs7566605 with PCOS itself.

## Aim

To perform a case-control association study between rs7566605 and PCOS in the Romanian population, and assess genotype-phenotype correlations within the PCOS cases group.

## Patients and methods

We recorded anthropometrical, biochemical and hormonal parameters for 141 PCOS patients (Rotterdam-criteria) and 89 healthy female controls. Insulin-resistance was evaluated by HOMA-IR. Genotyping of rs7566605 was done by an original protocol of allele-specific PCR (MS-PCR) coupled with detection by high-resolution melting (HRM) analysis.

## Results

36.03% PCOS patients and 7.14% controls were obese ( $P < 0.0001$ ). rs7566605 allelic frequencies and genotypes were not significantly different between cases and controls, suggesting no association with PCOS. Stratification by obesity status showed no significant differences in rs7566605 allelic frequencies between non-obese controls (0.388), non-obese PCOS (0.383) and obese PCOS (0.408). The C/C genotype frequency was also not significantly different between these groups. A genotype-phenotype correlation analysis within the PCOS cases group showed no association with BMI, waist circumference, basal glycemia, insulinemia, HOMA-IR, blood lipids, Ferriman–Gallwey score and total testosterone.

## Conclusions

This is a first association study of rs7566605 with PCOS and our results suggest that INSIG2 is not a significant PCOS gene in the Romanian population. Still, results should be regarded as preliminary, given that the statistic power of our study was limited.

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## P106

### C-peptide serum concentration in obese women with polycystic ovary syndrome

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## Introduction

It is believed that key pathogenic mechanism of PCOS is hyperinsulinemia. Insulin synthesis is linked to C-peptide release, but the role of C-peptide in PCOS remains unknown.

## Methods

The aim of this study was to evaluate of C-peptide serum levels in obese women with PCOS with concomitant assessment of possible link between C-peptide concentrations and metabolic disturbances and androgens levels in those individuals.

Sixty obese women (aged  $26.2 \pm 6.3$  years; body mass index  $35.0 \pm 4.45$  kg/m<sup>2</sup>) diagnosed with PCOS were included to the study. The control group consisted of 10 healthy, ovulatory women with normal weight (aged  $28.8 \pm 4.8$  years; BMI  $21.2 \pm 2.1$  kg/m<sup>2</sup>).

Folliculotrophin (FSH), lutrophin (LH), 17 $\beta$ -estradiol (E<sub>2</sub>), testosterone (T), dehydroepiandrosterone sulfate (DHEAS) and insulin serum concentrations were measured. Serum fasting glucose levels and lipid profile was assessed: total cholesterol (TCH), triglycerides (TGA), low and high density lipoproteins (LDL, HDL).

## Results

C-peptide serum concentrations in studied group were  $1.31 \pm 1.05$  nmol/l, whereas among healthy women  $1.62 \pm 1.56$  nmol/l. Obtained differences were not statistically significant ( $P > 0.05$ ). C-peptide serum level were not correlated significantly with the FSH, LH, E<sub>2</sub>, T, DHEAS levels. Negative correlation between glucose and C-peptide serum levels was found (Spearman index  $R = -0.71$ ,  $P < 0.05$ ) in control group, whereas in PCOS and obesity group there was no correlation between these values.

## Conclusion

There were no differences in C-peptide serum level between study and control group. C-peptide serum concentrations were not correlated with androgens. The relation between C-peptide and hyperinsulinemia and metabolic disturbances is complicated and requires further studies.

**P107****Higher metabolic risk with nih diagnostic criteria versus rotterdam criteria for polycystic ovarian syndrome**

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**Introduction**

Polycystic ovary syndrome (PCOS) is a heterogenous disease with well established metabolic abnormalities among women of reproductive age. There are various diagnostic criteria to define and establish PCOS. However, there are some conflicting data regarding the the optimal diagnostic criteria for PCOS and the metabolic consequences. We evaluated the clinical, endocrine and metabolic features between main PCOS phenotypes according to different diagnostic criteria.

**Methods**

One hundred seventy-five consecutive women with PCOS, 41 ovulatory women with idiopathic hirsutism and 109 healthy, non-hirsute, ovulatory controls were enrolled. Hirsutism was defined by a value of  $\geq 8$  using the modified Ferriman-Gallwey. Ovulatory function, ovarian sonography, gonadotropins, testosterone, DHEAS, 17 OH progesterone, oral glucose tolerance test, fasting insulin, homeostasis model assessment for insulin resistance, lipids, body mass index, waist circumference, frequency of metabolic syndrome were detected.

**Results**

In total 175 women who fulfilled the Rotterdam 2003 criteria were diagnosed with PCOS. Of these, 121 (69%) had both androgen excess and ovulatory dysfunction and met the NIH criteria. The remaining 54 (31%) had PCO confirmed by ultrasonography with either androgen excess or ovulatory dysfunction. There were 41 ovulatory women with only hirsutism, and 109 healthy, non-hirsute, ovulatory women. Among groups, body mass index, waist circumference, levels of testosterone, DHEAS, triglyceride, insulin, homeostasis model assessment (HOMA-IR) index were higher in NIH group. Also prevalence of insulin resistance (HOMA-IR $>3$ ) and metabolic syndrome were higher as well.

**Conclusions**

NIH criteria has detected patients with higher risk for insulin resistance and metabolic syndrome.

**Conclusion**

Our results indicate that insulin resistance could be responsible for increased level of oxidative stress in young, non-obese PCOS women. Presence of insulin resistance, hyperinsulinemia, and oxidative damage are likely to accelerate development of cardiovascular disease in PCOS women.

**P109****PCOS, coronary heart disease, stroke and the influence of obesity: a systematic review and meta-analysis**

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**Background**

Patients suffering from polycystic ovarian syndrome (PCOS) are warned for arterial disease. We examined the risk of (non)fatal coronary heart disease (CHD) or stroke between PCOS patients and ovulatory, non-PCOS women. Further we assessed the effect of obesity on this risk

**Methods**

We performed a systematic review and meta-analysis of controlled observational studies. Four definitions of PCOS were considered: WHO type II anovulation (1976), NIH criteria (1990), Rotterdam consensus (2003) and Androgen-excess criteria in 2006. Obesity was defined as a BMI  $>30$  kg/m<sup>2</sup> and/or a waist circumference  $>88$  cm. Study quality was assessed using the Newcastle-Ottawa Scale. Fatal and nonfatal CHD or stroke was the primary outcome. The definition of CHD and stroke was based on the criteria used by the various authors. The effect measure was the pooled relative risk in a random effects model. Risk ratios and rate ratios were combined in the present analysis.

**Results**

Five follow-up studies published between 2000 and 2008 were included. The studies showed heterogeneity in design, definitions and quality. In a random effects model the relative risk for CHD or stroke was 2.02 comparing PCOS women to non-PCOS women (95% ci 1.47, 2.76). Pooling the two studies with BMI adjusted risk estimates, showed a relative risk of 1.55 (1.27, 1.89).

**Conclusions**

This meta-analysis showed a two-fold risk of arterial disease for PCOS patients relative to non-PCOS patients. The risk remained increased after adjustment for BMI.

**P108****Relation of insulin resistance and lipid peroxidation in non-obese women with polycystic ovary syndrome**

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**Introduction**

Polycystic ovary syndrome (PCOS) is a frequent metabolic disorder in women of the reproductive age, and is characterized by multi-factorial cardiovascular risk factors. Insulin resistance is proposed as a central factor linking PCOS to increasing risk of cardiovascular disease. The aim of our study was to explore a link between insulin resistance and oxidative stress, as a predictor of cardiovascular risk, in young, non-obese PCOS women.

**Methods**

A cross-sectional controlled study in 29 young PCOS women (age:  $23.8 \pm 3.5$  years; body mass index, BMI:  $22.5 \pm 4.6$  kg/m<sup>2</sup>) and 13 matched controls (age:  $25.3 \pm 2.8$  years; BMI:  $20.6 \pm 3.3$  kg/m<sup>2</sup>) was performed. Fasting blood samples were collected for the determination of malondialdehyde (MDA), glucose, insulin, testosterone, and sex hormone binding globulin. Insulin resistance was calculated using homeostasis assessment model (HOMA-IR).

**Results**

MDA concentrations showed no difference between PCOS and controls ( $4.0 \pm 1.6$  vs  $4.3 \pm 2.3$  uM,  $P=0.685$ ). Indices of insulin resistance were significantly higher in PCOS group in comparison to controls (insulin:  $16.6 \pm 7.5$  vs  $10.7 \pm 2.6$  mU/l,  $P=0.009$ ; HOMA-IR:  $3.2 \pm 1.3$  vs  $2.2 \pm 0.6$ ,  $P=0.015$ ). MDA had significant positive correlation with insulin ( $P=0.046$ ) and HOMA-IR ( $P=0.016$ ) in PCOS group only.

**P110****MTHFR mutations in female patients with autoimmune thyroiditis**

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**Introduction**

Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme involved in folate and homocysteine metabolism. The enzyme is encoded by a gene located on chromosome 1p36.3. MTHFR mutations frequently met in the population are C667T and A1298C. The impairment of homocysteine metabolism due to MTHFR gene polymorphism influences the risk for diseases such as CVS diseases, certain types of cancer and is associated with certain complications of pregnancy including chromosomal abnormalities, congenital malformations, neural tube defects: spina bifida, anencephaly, recurrent abortions, diseases of the placenta and preeclampsia. Autoimmune thyroiditis is a relatively common disorder found in the female population, which can influence fertility in a negative sense.

**Purpose**

The study aims to assess the risk of thrombophilia in patients with autoimmune thyroiditis.

**Subjects and methods**

There were assessed 50 patients aged 22 years to 39 years diagnosed with autoimmune thyroid disease, diagnosis established by means of thyroid ultrasound and by detection of ATPO and antithyroglobulin antibodies titre. The reasons for presentation for examination were primary and secondary infertility. Because the patients presented in their personal history spontaneous abortions there were also performed further evaluation of coagulation profile: V Leiden factor, C protein, S protein, detection of MTHFR mutations.



## Results

In this group of patients with autoimmune thyroiditis 15 patients had MTHFR mutations: 3 patients with C667T homozygous mutation, 5 patients with heterozygous C667T mutation, 2 patients with A1298C homozygous mutation, 4 patients with heterozygous A1298C mutation and 1 patient with compound heterozygous C667T/A1298C.

## Conclusions

MTHFR mutations are relatively frequent in the population (11%). Additional studies are needed to explain the association of MTHFR mutations with autoimmune thyroiditis, an increased prevalence of these mutations among the patients with autoimmune thyroid disease being observed.

## P111

### Plasma testosterone fractions in women with hyperandrogenism

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Plasma total testosterone (TT) does not provide reliable information about the biological active testosterone, since a large proportion of this hormone is bound to either specific (sex hormone binding globulin, SHBG) or non-specific binding proteins (albumin). Free testosterone (fT) represents not more than 0.7–2.2% of TT while bioavailable testosterone (BA-T) in females is about 15–48% of the TT. The aim was to investigate the analytical and clinical correlations among measured TT and calculated free testosterone index (fTi), BA-T and fT levels.

## Methods

Sera from 61 women (age: 26 ± 10 years) including 12 healthy controls and 49 patients who had either high (*n* = 26, HRP) or low risk (*n* = 23, LRP) for true hyperandrogenism on the basis of clinical and ultrasound findings were analysed. Samples were examined for TT and SHBG using electrochemiluminescence immunoassays (Roche) and for albumin using colorimetric method, then the fT, fTi and BA-T concentrations were calculated.

## Results

All sera had normal albumin levels (mean ± s.e.m., 46 ± 3 g/l). There were significant (*P* < 0.01) but weak correlations between TT and fTi (*r* = 0.68), between TT and BA-T (*r* = 0.79) and between TT and fT (*r* = 0.79), while correlations between fTi and BA-T (*r* = 0.94) and between fTi and fT (*r* = 0.89) were stronger. All T fractions were the highest in HRP (*P* < 0.001) compared to the control and LRP groups. For detecting hormonal hyperandrogenism, the sensitivities and specificities of testosterone fractions were as follows: fTi, 54 and 85%; BA-T, 54 and 88%; TT, 50 and 76%; fT, 46 and 74%, respectively.

## Conclusions

In patients with normal albumin levels there are only marginal differences among sensitivities of fTi, BA-T, TT and fT. Our data confirm that when TT is out of the reference range the SHBG and albumin concentration should be measured to calculate the fTi or BA-T because of their highest specificity.

## P112

### Polycystic ovary syndrome and autoimmunity

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Polycystic ovary syndrome (PCOS) is characterized by laboratory and/or clinical features consisting of hyperandrogenism with chronic anovulation, and is currently one of the most common endocrinopathies in women of fertile age. PCOS is associated with a variety of endocrine and metabolic disturbances. It is well known that women are more prone to develop autoimmune diseases compared to men and levels of sexual hormones are thought to be responsible for such a difference. Our aim is to prove that women with PCOS are more prone for autoimmune diseases even though their protective levels of androgens. It was already demonstrated that the prevalence of autoimmune thyroiditis is 3fold higher among these patients. Recent studies reveal a higher incidence of autoantibodies such as antihistone, anti dsDNA presented in systemic autoimmune disease, however their clinical significance is still unknown.

## Materials and method

We analyzed sera of 180 patients with PCOS using commercially available kits for presence of various autoantibodies (such as ANA, ENA, aTPO, aTG, ANCA, ACLA and their typization) known in systemic and organ specific autoimmune diseases and compared it with 43 healthy controls. Moreover we tried to prove presence of anti-ovarian autoantibodies in patients' sera what could mean that disease is autoimmune itself.

## Results

We found no significant differences between the presence of systemic autoantibodies in patients sera compared to controls. Significant difference was seen in presence of antibodies typical for Hashimoto tyreoiditis. Only in some PCOS patients we could observe a tendency to bind ovarian cancer cell-lines meaning there could be a subgroup of PCOS. Clinical significance is still unknown and whether these patients will develop autoimmune disease in future remains unclear. It raises a question of hormonal influence on immune system.

## P113

### 17-hydroxysteroid dehydrogenase type 5 gene polymorphism (71A/G HSD17B5 SNP) and effects of oral contraceptive pill on hirsutism, androgens and metabolic profile in non-obese PCOS women: a pilot study

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An association of the single nucleotide polymorphism (SNP) in the promoter gene that codify for 17b-HSD5 enzyme (71A > G) with polycystic ovary syndrome (PCOS) has been suggested in previous studies. Given the role of 17b-HSD5 in androgenic metabolism producing testosterone from precursors, the aim of this study was to assess whether non-obese PCOS women with the variant allele G have different response on hirsutism and on hormonal and metabolic variables to oral contraceptive pill (OCP) treatment. Twenty-eight PCOS outpatients, defined by the Rotterdam criteria, were included in the study. None had received any drugs known to interfere with hormonal levels for at least 3 months before the study. Patients with diabetes, BMI ≥ 30 or insulin resistance (HOMA-IR > 3.8) were excluded. Women received OCP (ethinyl oestradiol 20 µg plus gestodene 75 µg) during 6 cycles. Hirsutism, hormone and metabolic variables were evaluated before and after the treatment. DNA was extracted from peripheral blood by a standard salting out procedure, and the samples were genotyped by allelic discrimination assay with Real Time PCR. Data were analyzed by Anova for repeated measures. Participants were stratified by the presence of the allele G (AA or AG + GG). The genotypic distribution (AA:46.4%, AG:42.9%, GG:10.7%) was similar to previous studies. Hirsutism regressed equally with OCP in both groups (33.5 and 45.5% for AA and AG + GG, respectively). SHBG levels were increased and androgens and LH declined from baseline (*P* < 0.05) independently of the presence of the allele G. Triglycerides, total, and HDL-cholesterol increased in the two groups during treatment, but the levels remained normal. Data from this pilot study suggest that, for selected PCOS patients without major metabolic comorbidities, OCP may be a safe and effective treatment and the presence of allele G appears not to contribute with the improvements in the studied variables.

## P114

### Does uric acid predict insulin resistance in polycystic ovary syndrome? O Oz Gul<sup>1</sup>, C Ersoy<sup>1</sup>, B Gul<sup>2</sup>, S Cander<sup>1</sup>, O K Unal<sup>1</sup>, E Erturk<sup>1</sup>, E Tuncel<sup>1</sup> & S Imamoglu<sup>1</sup>

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## Introduction

Women with polycystic ovary syndrome (PCOS) have an increased prevalence of insulin resistance and related disorders. Endothelial dysfunction and chronic inflammation are early findings in the atherosclerotic process. Elevated serum levels of uric acid may reflect low-grade chronic inflammation. But there are controversial data whether increased the uric acid level in PCOS patients. This study was aimed to examine the relationship between uric acid, lipoprotein levels and insulin resistance in obese and non-obese patients with PCOS.

## Methods

Forty-two young women with PCOS and 42 controls of similar age were included this study. Plasma levels of glucose, insulin and uric acid were

measured. Anthropometric variables, hormonal and metabolic profiles were evaluated in both groups. Insulin resistance was evaluated by homeostasis model assessment (HOMA-IR).

#### Results

Plasma uric acid levels and HOMA-IR were significantly higher in women with PCOS than in healthy women. Serum fasting total cholesterol, triglycerides (TG) and hemoglobin A1c levels were similar between PCOS and control groups. Compared with non-obese PCOS subjects, obese PCOS subjects had high HOMA-IR, insulin, TG and uric acid levels. Plasma glucose levels, serum low-density lipoprotein cholesterol and androgen levels were similar between obese and non-obese women with PCOS. No correlation was observed between plasma uric acid, HOMA-IR and serum androgen levels.

#### Conclusion

In this study we demonstrate increased levels of uric acid in PCOS. Obesity is the main determinant of serum uric acid concentrations in PCOS patients. Our results suggest that measurement of serum uric acid does not provide new means for identification of insulin resistance and related disorders in patients with PCOS.

## P115

### Injected FSH overriding ageing related elevated FSH through high activity of the hypothalomo-pituitary axis, enforces wanted multiple follicle growth in women undergoing IUI

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#### Introduction

The hypothalomo-pituitary response to the loss of ovarian function due to ageing is an increase in GnRH induced gonadotrophic activity: FSH. It is thought that the pituitary gives maximum endogenous stimulation leading to FSH levels above the ovarian threshold to develop follicular growth and it is a matter of debate whether exogenous FSH (r-FSH) will or will not improve ovarian response. In intrauterine insemination (IUI) treatment r-FSH is used to induce multifollicular growth to improve treatment efficacy. This study evaluated whether addition of r-FSH in women with endogenous elevated basal FSH levels, results in a higher percentage of cycles with multifollicular growth.

#### Methods

A randomized controlled trial was performed in women undergoing IUI treatment with elevated basal FSH levels ( $\geq 10$  IU/l on cycle day 3). Patients were assigned to the control (3 natural cycles followed by 3 stimulated cycles) or the intervention group (6 stimulated cycles). The primary outcome was the occurrence of multifollicular growth. Statistical analysis was done using Cox regression and multivariate analysis.

#### Results

Forty-eight women were included; 23 in the control versus 25 in the intervention group. There were no significant differences in baseline characteristics. The intervention group was 2.7 times more likely to develop multiple follicular growth (95% CI, 1.16–6.11,  $P=0.02$ ). Lower FSH levels at the beginning of a treatment cycle compared to basal FSH levels predicted multifollicular growth with an odds ratio of 0.81 (95% CI, 0.72–0.92,  $P=0.0003$ ).

#### Conclusion

Administration of r-FSH in women with elevated basal FSH levels undergoing IUI treatment leads to a higher percentage of cycles showing multifollicular growth. A lower FSH level in the beginning of the treatment cycle compared to basal FSH levels predicts multifollicular growth. Therefore, women with elevated basal FSH levels should be offered controlled ovarian stimulation in order to improve treatment outcome.

## P116

### The influence of LH substitution for GnRH antagonist blocked endogenous LH in older IVF patients

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#### Introduction

With IVF stimulation a premature pituitary LH surge can ruin the whole procedure. Traditionally prevention of this event is done by administering

a GnRH antagonist against endogenous hypothalamic GnRH. The obvious consequence is low LH levels, one of the two gonadotrophins usually not given for ovarian stimulation since for adequate action FSH suffices. However there are indications that LH levels may render too low, in particular in older women. The aim of this study was to investigate whether the addition of LH to an IVF stimulation protocol with FSH and GnRH antagonist will improve the ovarian response and consequently, implantation and pregnancy rates in women of 35 years and older.

#### Methods

A prospective randomized multicentre study was performed in couples who were undergoing IVF/ICSI. Women were 35 years or older and received ovarian stimulation with FSH and GnRH antagonist from day 6 of stimulation. Randomization took place on day 6 of stimulation to receive both FSH and LH or continue with FSH alone. Primary endpoint of the study was implantation rate (the chance of an embryo to implant) and clinical pregnancy rate (defined as a pregnancy diagnosed by ultrasonographic visualisation of at least one gestational sac).

#### Results

Of 249 subjects randomized, 128 received both FSH and LH and 121 received only FSH. There were no demographic or clinical differences between the groups. Intention to treat analysis revealed that of those receiving FSH and LH, 33 (27.5%) had a clinical pregnancy, compared with 38 (30.6%) receiving only FSH ( $P=0.6$ ). Implantation rates were similar: 17.2 vs 21.1% ( $P=0.4$ ) in the 'FSH and LH' and 'FSH only' groups respectively.

#### Conclusions

Our study shows that replenishment of low hypothalomo-pituitary regulation of LH is not crucial for ovarian response in IVF in older women.

## P117

### Adiponectin and metabolic syndrome in PCOS patients – beyond obesity

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#### Introduction

Polycystic ovary syndrome (PCOS) have an increased rate of metabolic syndrome (MS). Many studies have proved that adiponectin is closely associated with MS and participate in the disturbances of gonadal axis. The aim of the study was to evaluate the association of adiponectin levels with MS in PCOS.

#### Patients and methods

Study group included 38 patients with PCOS (Rotterdam criteria) compared to 30 healthy volunteers age and sex matched, all with BMI  $> 25$  kg/m<sup>2</sup>. Blood pressure (BP), waist circumference, serum adiponectin, fasting glucose, HOMA, lipids levels were performed in all subjects.

#### Results

In the PCOS group, adiponectin was lower than in the control group, but the difference was not significant ( $P=0.2$ ). There were no differences between adiponectin values in overweight versus obese women with PCOS ( $P=0.15$ ), but the difference was significant in the non-PCOS group ( $P=0.018$ ). Metabolic syndrome was more frequent in PCOS patients, but the difference was not significant (58 vs 46%); in women with MS adiponectin levels were lower, the difference being significant ( $P=0.012$  in control group;  $P=0.014$  in PCOS group). Adiponectin levels were negatively correlated with waist circumference, fasting glucose, triglycerides, and diastolic BP, and positively correlated with HDL. In both groups, adiponectin levels were significantly lower in women with abdominal obesity ( $P<0.05$ ) and in older women ( $> 35$  years old;  $P<0.03$ ). Insulin resistance had a significant negative correlation with adiponectin in PCOS subjects ( $r = -0.446$ ) but not in the normal group ( $r = -0.08$ ).

#### Discussion

Our results are similar to those of the literature. We presume that adiponectin was not significantly lower in PCOS because both groups had BMI  $> 25$  kg/m<sup>2</sup>. Despite the similar values, MS was more frequent in PCOS. Our results suggest that adiponectin is an independent risk factor for MS and probably involved in PCOS pathogenesis.

**P118****Ovarian ageing and twinning**

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Dizygotic twinning is the result of fertilization of multiple follicles that develop during one ovulatory cycle. Significant factors contributing to natural DZ multiples are heredity, increased maternal age and higher parity. Older women are at higher risk for DZ twinning because they have a strongly increased risk of multiple follicle growth as a result of diminished ovarian feedback from the due to a smaller available cohort of follicles. FSH increases allowing all follicles present to develop and potentially ovulate. With familial twinning elevations of FSH are also found. Another hormone may be a good estimate for ovarian aging; anti mullerian hormone (AMH). Lower levels are associated with aging ovaries. AMH has been determined in the residual serum of the 16 mothers of DZ twins and 14 controls. This may reveal that the twin mothers of our study may already have shown some subtle features of limited ovarian reserve indicating protracted organ ageing. The average AMH levels were not different:  $2.13 \pm 0.50 \mu\text{l}$  the twin mothers and  $1.85 \pm 0.60$  in the controls. The average calendar age was 35 years in both groups. Among the 16 familial twin mothers, 7 had elevated FSH values over 10 U/l in the early follicular phase compared to 1/14 in the control ( $P < 0.04$ ). Their AMH was very low compared to the 9 twin mothers with normal FSH values:  $0.57 \pm 0.15$  and  $3.37 \pm 0.88 \mu\text{l}$  ( $P = 0.016$ ). None of the twin mothers (currently >45 years), reported early menopause (<45 years). Our data indicates that among mothers with familial DZ twins, elevated FSH occurs often and seems to be associated with signs of limited ovarian reserve. These findings support the hypothesis that with familial DZ twinning potentially inheritance of processes that advance ovarian ageing can be responsible.

**P119****The role of metabolic hormones ghrelin, leptin and obestatin in control of ovarian functions**

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The recent original data concerning interrelationships between metabolic hormones (ghrelin, leptin, obestatin) and feeding, action of metabolic hormones on basic ovarian functions (proliferation, apoptosis, secretory activity, response to gonadotropins, oocyte maturation) and intracellular mechanisms of its action in different species (rabbit, pig, human, chicken) are reviewed. It was shown, that metabolic hormones ghrelin, leptin and obestatin can control proliferation, apoptosis, release of ovarian hormones and alter response of ovarian cells to gonadotropin and other hormones, oocyte maturation and fecundity. Action of pharmacological blockers of protein kinases and of cell transfection with gene constructs for transcription factors demonstrated, that effects of metabolic hormones on ovarian cells are mediated by protein kinases A, MAP kinase, CDC2 kinase and transcription factors CREB, p53 and STAT-1. Malnutrition affects release of metabolic hormones and their action on ovarian cells, whilst administration of metabolic hormones can mimic, promote or prevent effect of food restriction on ovarian cells both *in vivo* and *in vitro*. The species-specific differences in ghrelin, leptin and obestatin action are discussed.

The present data suggest that metabolic hormones play 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 metabolic hormones output, which in turn, through protein kinases and transcription factors, can affect ovarian cell proliferation, apoptosis, secretory activity, response to gonadotropins and other hormonal stimulators and fecundity.

This study was performed during realization of the project CEGEZ 26220120042 supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

**P120****Smoking is associated with increased adrenal responsiveness, decreased prolactin levels and a more adverse lipid profile in 650 Caucasian patients with polycystic ovary syndrome**

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**Objective**

Smoking may be associated with changes in metabolic risk factors and sex hormones in PCOS.

**Design**

Retrospective trans-sectional study.

**Patients**

Six hundred and fifty caucasian premenopausal women with the diagnoses hirsutism or PCOS were divided according to smoking status: Non-smokers (NS-PCOS=390) and smokers (S-PCOS=260). 119 healthy women were studied as controls (NS-Control=105, S-Control=14).

**Interventions**

Clinical evaluation, hormone analyses, transvaginal ultrasound. Oral glucose tolerance tests (OGTT) and ACTH tests.

**Main outcome measures**

Clinical, metabolic, and endocrine parameters. ACTH stimulated cortisol and 17-hydroxyprogesterone (17OHP) levels.

**Results**

S-PCOS has significantly higher fasting lipid profile and 17OHP levels (basal and ACTH stimulated) than NS-PCOS patients, whereas prolactin levels were decreased. No significant differences were found in body composition and measures of insulin resistance between NS-PCOS and S-PCOS. PCO was more prevalent in NS-PCOS patients. During multiple regression analyses, smoking was positively associated with 17OHP and cholesterol, triglycerides, and low density lipoprotein and inversely associated with prolactin and high density lipoprotein.

**Conclusion**

Smoking was associated with increased adrenal responsiveness, decreased prolactin levels and a more adverse lipid profile in PCOS patients, whereas smoking was unassociated with body composition and insulin resistance. Smoking may be associated with the prevalence of individual Rotterdam criteria.

**P121****Normalization of blood loss in women with heavy menstrual bleeding treated with an oral contraceptive containing estradiol valerate/dienogest**

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**Introduction**

Two identically designed randomized, placebo-controlled double-blind studies (one conducted in North America and the other one in Europe and Australia) showed that an oral contraceptive containing estradiol valerate (E<sub>2</sub>V)/dienogest (DNG) in an estrogen step-down, progestogen step-up 28-day regimen was effective in women with heavy and/or prolonged menstrual bleeding without organic pathology. Both studies applied a strict definition of treatment success, based on a composite of eight individual criteria. To make these results comparable with published data, data were pooled and re-analyzed using a definition of successful treatment more commonly referred to in literature, applying more clinically meaningful criteria.

**Methods**

Women  $\geq 18$  years with objectively-confirmed heavy menstrual bleeding (HMB, defined as menstrual blood loss (MBL)  $\geq 80$  ml/cycle in two cycles during a 90-day run-in period) without organic cause were randomized to E<sub>2</sub>V/DNG ( $n = 220$ ) or placebo ( $n = 135$ ) for 7 cycles of 28 days. MBL was measured objectively using the alkaline-hematim method. The primary variables were

absolute change in MBL volume from baseline and proportion of women who were successfully treated at cycle 7. Treatment success was defined as MBL volume  $\leq 80$  ml and  $\geq 50\%$  reduction in MBL volume from baseline to cycle 7. Results

At cycle 7, the proportion of patients with successful treatment was 63.6% with E<sub>2</sub>V/DNG and 11.9% with placebo ( $P < 0.001$ ). A MBL volume  $\leq 80$  ml was observed in 68.2 and 15.6% of women, respectively, and a MBL volume reduction  $\geq 50\%$  was observed in 70.0 and 17.0% of women, respectively (both  $P < 0.001$ ). From baseline to cycle 7, median MBL volume reduced from 179.8 to 35.7 ml with E<sub>2</sub>V/DNG, and from 174.9 to 163.7 ml with placebo ( $P < 0.001$  for E<sub>2</sub>V/DNG versus placebo).

#### Conclusions

E<sub>2</sub>V/DNG was highly effective for treating HMB and was associated with a high overall response rate.

## P122

Abstract withdrawn.

## P123

### Self-reported health related quality of life and depression in women with polycystic ovary syndrome: associations with selected metabolic and hormonal PCOS features.

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#### Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age causing symptoms that can lead to depression and reduced health-related quality of life (HRQoL). Associations of depression and HRQoL indices with hormonal and metabolic PCOS features have not been adequately studied.

#### Methods/design

Thirty-seven premenopausal women (age:  $28 \pm 6.4$  years; BMI:  $28.3 \pm 7.2$  kg/m<sup>2</sup>) with PCOS according to ESHRE criteria were recruited. HRQoL was assessed using the PCOS Questionnaire (PCOQ6). Degree of depression was assessed by Beck Inventory. Hormonal (testosterone, SHBG, androstenedione, estradiol, LH, FSH) and metabolic (fasting glucose, insulin, lipids) parameters were measured. Trunk fat was determined by bioimpedance (Viscan, Tanita). Data were analyzed using multivariate regression with reduction of dimensionality.

#### Results

The weight dimension PCOQ6 score significantly correlated to i) insulin resistance parameters: HOMA-IR ( $R = -0.84$ ,  $P < 0.01$ ); fasting insulin ( $R = -0.82$ ,  $P < 0.01$ ); SHBG ( $R = -0.70$ ,  $P < 0.01$ ) and ii) obesity parameters: BMI ( $R = -0.92$ ,  $P < 0.01$ ); percent of trunk fat ( $R = -0.95$ ;  $P < 0.01$ ). Additionally, the infertility dimension PCOQ6 score exhibited significant correlations with HOMA-IR ( $R = -0.86$ ,  $P < 0.01$ ); fasting glucose ( $R = -0.80$ ,  $P < 0.01$ ); fasting insulin ( $R = -0.82$ ,  $P < 0.01$ ) and waist-to-hip ratio ( $R = -0.74$ ;  $P < 0.01$ ). Prevalence of mild depression was 21% and of moderate to severe depression 13%. Beck score significantly correlated with the weight dimension ( $R = -0.91$ ;  $P < 0.01$ ) and emotion dimension ( $R = -0.75$ ;  $P < 0.01$ ) PCOQ6 scores, but not with hormonal or metabolic parameters.

#### Conclusion

These results suggest that PCOS features relating to obesity and insulin resistance are associated with decreased HRQoL in the weight and infertility PCOQ6 domains, but not with depression. Supported by grant IGA MH NS 9839/4.

## P124

### Two hormone for one receptors: dissecting out LH and hCG activity with an in vitro approach.

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#### Introduction

LH and hCG act on the same receptor (LHCGR), have different half-lives and *in vivo* biopotency. It is not known whether they elicit the same cellular and molecular response. The aim of this study was to compare the kinetics of cAMP response to recombinant LH and hCG.

#### Design

In COS-7 cells permanently expressing the human LHCGR (COS-7/LHCGR) we evaluated LH and hCG dose-response curves, by measuring total cAMP after 3 h of incubation. We then evaluated the time-course of intracellular cAMP production in the presence of ED<sub>50</sub> doses of LH and hCG over 3 h. Finally we evaluated the long-term response to LH and hCG by exposing human primary granulosa lutein cells (hGLC) to ED<sub>50</sub> doses over 12 h. All incubations were performed in the presence of IBMX.

#### Results

In COS-7/LHCGR cells, we observed significantly different ED<sub>50</sub> for LH ( $475.75 \pm 137.33$  pM, mean  $\pm$  s.d.) and hCG ( $101.75 \pm 44.63$  pM) (Mann-Whitney's *U*-test,  $P = 0.029$ ;  $n = 4$ ). Maximal LH stimulation of intracellular cAMP, about 50 fold over control, reached a plateau in 10 min, while maximal hCG stimulation at similar levels was attained only after 1 h (Anova;  $P < 0.05$ ;  $n = 3$ ). In hGLC continuous exposure to LH and hCG resulted in a repetitive, pulsatile increase of intracellular cAMP with peaks every 3–4 h and significantly higher levels of stimulation in the presence of hCG vs LH (Anova;  $P < 0.05$ ;  $n = 3$ ).

#### Conclusions

Equimolar concentrations of human recombinant LH and hCG result in significantly higher *in vitro* biopotency of hCG (about 5-fold). Equipotent concentration (ED<sub>50</sub>) of LH and hCG stimulate a faster response to LH within the first 3 h, but a quantitatively higher response to hCG over 12 h. hGLC respond to constant LH/hCG stimulation in a pulsatile fashion, suggesting a novel control of gonadotropins action at the receptor level.

## P125

### Anti-müllerian hormone as a predictor of ovarian response to metformin in lean women with polycystic ovary syndrome

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#### Introduction

Anti-Müllerian hormone (AMH) has an inhibitory role during folliculogenesis, which may contribute to anovulation in polycystic ovary syndrome (PCOS). AMH level has been shown to be a predictor of reproductive response to weight loss and clomiphene citrate in women with PCOS. The aim of this study was to evaluate the role of AMH as a predictor of improvements in menstrual cyclicity and ovulation induction by metformin in lean women with PCOS.

#### Design

Twenty-one lean unovulatory women with PCOS (age  $24.2 \pm 3.2$  year, body mass index (BMI)  $21.4 \pm 2.3$  kg/m<sup>2</sup>) received metformin 1700 mg/day during 6 months. We estimated ovulation induction (by ultrasound scanning), change in menstrual cycle, anthropometric measurements, endocrine parameters and insulin sensitivity.

#### Results

Twenty women completed the trial. 11 of them (55%) responded to the intervention by improvement in menstrual cyclicity (responders, 9 of which recommenced ovulation). There was no difference between responders and nonresponders in age, BMI, waist circumference, basal levels of gonadotropins, androgens, fasting insulin and homeostasis model assessment. Compared to

nonresponders, responders had lower AMH levels at baseline ( $7.2 \pm 3.1$  vs  $12.7 \pm 2.6$  ng/ml;  $P=0.021$ ). Using ROC-curves we calculated that the value of AMH  $<8$  ng/ml can predict improvement of menses (sensitivity 72.7%, specificity 99%) and ovulation induction (sensitivity 66.7%, specificity 81.8%) by metformin in lean women with PCOS. Likewise, the mean follicle number per ovary was lower in responders than in nonresponders ( $11.0 \pm 1.5$  vs  $15.7 \pm 5.9$ ,  $P=0.011$ ). It was significantly related to the serum AMH levels ( $r=0.579$ ,  $P=0.006$ ).

#### Conclusions

AMH measurement can be useful in the prediction of ovarian response to metformin therapy in lean patients with PCOS.

## P126

### Cigarette smoking and progesterone and androgen metabolites in premenopausal women

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#### Objective

Chronic smoking alters the circulating levels of sex hormones and possibly also the neuroactive steroids, however, the data available is limited.

#### Patients and methods

Therefore, a broad spectrum of free and conjugated steroids and related substances was quantified by GC-MS and RIA in premenopausal smokers and in age-matched ( $38.9 \pm 7.3$  years of age) non-smokers in the follicular (FP) and luteal phases (LP) of menstrual cycle (10 non-smokers and 10 smokers, in the FP, and 10 non-smokers and 8 smokers in the LP). The local Ethics Committee approved the study.

#### Results

Smokers in both phases of the menstrual cycle showed higher levels of conjugated 17-hydroxypregnenolone, 5 $\alpha$ -dihydroprogesterone, conjugated isopregnanolone, conjugated 5 $\alpha$ -pregnane-3 $\beta$ ,20 $\alpha$ -diol, conjugated androstenediol, androstenedione, testosterone, free testosterone, conjugated 5 $\alpha$ -androstane-3 $\alpha$ / $\beta$ ,17 $\beta$ -diols, and higher free testosterone index. In the FP, the smokers exhibited higher levels of conjugated pregnenolone, progesterone, conjugated pregnanolone, lutropin and higher lutropin/follitropin ratio, but lower levels of cortisol, allopregnanolone, and pregnanolone. In the LP, the smokers exhibited higher levels of free and conjugated 20 $\alpha$ -dihydroprogrenolone, free and conjugated dehydroepiandrosterone, free androstenediol, 5 $\alpha$ -dihydrotestosterone, free and conjugated androsterone, free and conjugated epiandrosterone, free and conjugated etiocholanolone, 7 $\alpha$ / $\beta$ -hydroxy-dehydroepiandrosterone isomers, and follitropin but lower levels of estradiol and sex hormone binding globulin (SHBG) and lower values of lutropin/follitropin ratio.

#### Conclusion

Chronic cigarette smoking augments serum androgens and their 5 $\alpha$ / $\beta$ -reduced metabolites (including GABAergic substances) but suppresses levels of estradiol in the LP and SHBG and may induce hyperandrogenism in female smokers. The female smokers had pronouncedly increased serum progestogens but paradoxically suppressed levels of their GABA-ergic metabolites. Further investigation is needed concerning the machinery of these effects. The study was supported by grant 10215-3 IGA MZCR.

## P127

### Influence of smoking cessation on steroid spectrum in premenopausal women

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#### Background

Smoking represents the most widespread substance dependence in the world. Several studies show the nicotine's ability to alter women hormonal homeostasis. Women smokers have higher testosterone and lower estradiol levels throughout life compared to women non-smokers. This negatively affects women

reproductive function. Furthermore, alteration of neuroactive and neuroprotective steroids occurs in women smokers, which plays an important role in the activity of the central nervous system, cognition, mental condition and substance dependence seriousness.

#### Methods

We monitored the effect of smoking discontinuation on steroid spectrum in 40 premenopausal women smokers. These women were examined before they began to discontinue smoking: after 6, 12, 24 and 48 weeks of abstinence. In each examination, blood was collected to determine steroid spectrum, LH, FSH and SHBG, also basic anthropometric data were measured using GC-MS or immunoanalysis. Repeated measures ANOVA model was used for evaluation of the data. The local Ethics Committee approved the study.

#### Results

Given the small number of women who endured not to smoke, the data could be analysed only after 6 weeks. No changes in C21 steroids were found. A slight increase in androgens after the smoking discontinuation occurred.

#### Conclusion

Chronic smoking causes hyperandrogenism in fertile women; after smoking discontinuation its further increase occurs. Longer-term monitoring is necessary for illustrating the effect of smoking discontinuation on steroid spectrum.

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## P128

### Metabolic and cardiovascular outcomes in adult patients with turner's syndrome under hormonal replacement therapy

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Turner's syndrome (TS) is one of the more common genetic disorder, occurring in about 50 per 100.000 live-born girls. TS is caused by complete or partial X chromosome monosomy in a phenotypic female, and it is associated with increased morbidity and mortality for cardiovascular diseases, impaired glucose tolerance and dyslipidemia. In order to examine the metabolic and cardiovascular profile, in 30 adult TS patients under hormonal replacement therapy (HRT), 17 $\beta$ -estradiol (E<sub>2</sub>), BMI, waist circumference, fasting glucose and insulin, HOMA index, serum lipids, OGTT, blood pressure by 24-h ambulatory monitoring (ABPM) and intima-media thickness (IMT) were evaluated and compared with those in 30 age- and sex-matched controls (CS). No difference was found between TS and CS in E<sub>2</sub> and BMI, while waist circumference was higher ( $P < 0.05$ ) in TS ( $77.7 \pm 2.5$  cm) than in CS ( $69.8 \pm 1.0$  cm). Fasting glucose was similar in TS and in CS, while fasting insulin, HOMA index and 2 h-glucose after OGTT were higher ( $P < 0.0005$ ) in TS ( $13.2 \pm 0.8$  mUI/l,  $2.5 \pm 0.2$  and  $108.9 \pm 5.5$  mg/dl, respectively) than in CS ( $9.1 \pm 0.5$  mUI/l,  $1.8 \pm 0.1$  and  $94.5 \pm 3.8$  mg/dl, respectively). Total cholesterol was higher ( $P < 0.05$ ) in TS ( $199.4 \pm 6.6$  mg/dl) than in CS ( $173.9 \pm 4.6$  mg/dl), while no significant differences in HDL, LDL and triglycerides were found between the two groups. In 13% of TS ABPM showed arterial hypertension, while IMT was  $<0.9$  mm in all TS and CS. A negative correlation between insulin levels, HOMA index or 2 h-glucose after OGTT and E<sub>2</sub> was present in TS. In conclusion, our results indicate that adult patients with Turner's syndrome are connoted by higher frequency of central obesity, insulin resistance, hypercholesterolemia and hypertension, suggesting an increased cardiovascular risk, even under HRT.

## Growth factors

### P129

#### Extreme ketogenic, but not moderate, low-carbohydrate/high fat diets lead to loss of lean body mass in rats due to impairments of the GH/IGF system

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In rats, we have previously demonstrated that consumption of low-carbohydrate/high-fat diets (LC-HFD) led to significant reductions in circulating IGF1.

We further observed that rats fed an extreme, ketogenic, but comparably low protein LC-HFD (LC-HF-2) showed loss of bodyweight, which was mainly due to loss of lean body mass (LBM). Consumption of a moderate protein LC-HF diet (LC-HF-1) did not affect LBM acquisition compared to controls. We now investigated whether the effects on LBM could be explained by specific differences in muscle growth regulation through the local GH/IGF system.

#### Methods

We pair-fed 12-week old Wistar rats either a control chow (CH), LC-HF-1 or LC-HF-2 diets ( $n=8$ /diet group). After 3 weeks, GH profiles were determined by immunoassay using samples from serial blood samplings ( $n=80$ /diet group). After 4 weeks rats were sacrificed to analyze muscle samples (M. quadriceps femoris) by quantitative real-time PCR and Western blot analyses.

#### Results

Circulating IGF1 was lower in both LC-HF groups compared to CH. GH medians were significantly increased with LC-HF-1 ( $P<0.05$ ), but unchanged in the LC-HF-2 group. Muscle GH-receptor mRNA expression was unaffected in both LC-HFD groups. Muscle IGF1 mRNA expression was increased in rats fed LC-HF-1 ( $+110\%$ ,  $P<0.05$ ). High expression of IGF-1 in muscle resulted in a specific activation of IGF1 receptor signalling pathways. Phosphorylation of AKT ( $+110\%$ ,  $P<0.001$ ) and GSK3 $\beta$  ( $+54\%$ ,  $P<0.05$ ) were higher in muscles of rats fed the LC-HF-1 diet.

In conclusion, our data suggest that loss of LBM with LC-HF-1 was prevented by the rise in pituitary GH secretion, which might be triggered by low liver-derived IGF1 concentrations. With LC-HF-1, higher GH secretion resulted in increased muscle IGF1 activation of associated signalling pathways. In contrast, rats fed the ketogenic LC-HF-2 diet showed unchanged muscle IGF1 expression and loss of LBM, probably due to lower systemic IGF1 levels and normal pituitary GH output.

## P130

### Zilpaterol hydrochloride effects on carcass composition, growth performance and blood metabolites of Lory Bakhtyary lambs

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To determine the effects of zilpaterol hydrochloride (Zh) on carcass composition and growth performance of Lory growing lambs, 21 lambs with age 7–8 month and body weight  $37.5 \pm 1.2$  kg was assigned in three groups with 7 replications: group one as control, group 2 received Zh every two days, and group 3 received Zh every day. Lambs orally received 0.2 mg/kg of live body weight mixed with their daily diets during finishing period (as zilpaterol hydrochloride, Zilmax, Intervet South Africa). Dry matter intake compared to control group was significantly decreased by treatments ( $P<0.01$ ). Zilpaterol supplementation had no significant effect on gain and feed efficiency, daily and total gain in treatment groups compared to control group. Also, Zilpaterol supplementation did not affect carcass weight, longissimus muscle area (LM) and carcass dressing percentage. The results showed that the tail-fat weight in treated groups was significantly reduced ( $P<0.05$ ) compared to control group. The back fat (in group 3), kidney-pelvic fat (in group 2), and liver fat compared to control group was significantly reduced ( $P<0.05$ ). In treatment groups, lean protein in fillet muscle was higher than control group. Zilpaterol supplementation did not affect the level of insulin, T4 and T3, triglycerides, cholesterol and glucose in plasma. We conclude that Zh decrease the fat of tail and carcass and enhance growth performance but had no effect on carcass protein in Lory growing lambs.

## P131

### Comparative characterization of influence of replacement therapy with growth hormone on the level of secretion of growth hormone, insulin-like growth factor-1 and binding protein-3 in girls with endocrine and hereditary types of stunting

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#### Purpose

To study comparative influence of replacement GH therapy on a level of secretion of GH, IGF1 and IGFBP-3 in girls with endocrine and hereditary stunting.

#### Materials and methods

Forty-six girls aged 5.5 to 17 were examined. Of them somatotrophic insufficiency (STI) was found in 13 girls, multiple deficiency of adenohipophyseal hormones (MDAH) in 11, idiopathic nanism (IN) in 12, Turner syndrome (TS) in 10 girls. Patients received subcutaneous injections of rGH NordiLet in the dose of 0.07–0.1 IU/kg of the body weight daily. GH treatment was continued during 6 months. Levels of GH, IGF1 and IGFBP-3 were measured before and after GH treatment.

#### Results and discussion

On the background of GH treatment all patients had a positive growth dynamics (growth rate before treatment was  $2.5 \pm 0.05$  cm/year and  $10.1 \pm 2.45$  cm/year following the GH treatment, ( $P<0.0001$ ). The growth dynamics was less expressed in 4 adolescent girls with TS at 3 ones with IN. SDS made  $-3.3 \pm 0.92$  ( $P<0.0001$ ) on the background of GH treatment. The level of GH, IGF1 and IGFBP-3 was reliably low in patients with MDAH, STI-insufficiency before GH treatment in comparison with girls with IN and TS who had GH levels at the bottom normal range. On the background of GH treatment all patients had a reliable rise in IGF1 and IGFBP-3 levels. Correlation between IGF1 and IGFBP-3 ( $P<0.001$ ) was established.

#### Conclusion

A comparative analysis of the results obtained has shown that GH treatment accelerates reliably growth processes and increases IGF1 and IGFBP-3 levels in various types of stunting of endocrine and non-endocrine origin.

## P132

### Bone turnover and mineral density in thalassemia: relationships with gh secretory status and circulating somatomedins

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#### Introduction

Previous data from our group suggested a role for the GH/IGF1 axis in the pathophysiology of osteoporosis in thalassemia (Clin Endocrinol 69:202, 2008). The present study was aimed at evaluating the relationships between circulating IGFs and bone metabolism and density in a very large series of adult thalassemic patients.

#### Study design

One hundred and thirty-nine patients affected by thalassemia major (mean age  $32.3 \pm 7.87$  years) underwent the following evaluations: assessment of lumbar and femoral BMD by DEXA; measurement of serum osteocalcin and CTx, and urinary NTx; measurement of serum IGF1 and IGF2; assessment of the GH response to GHRH + arginine.

#### Results

Lumbar osteoporosis and femoral osteoporosis were detected in 54.3 and 58.1% of patients, respectively. IGF1 was low in 86.7% of subjects, whereas none of them displayed low IGF2. Severe GH deficiency was documented in 27.9% of subjects. In the whole group of patients, IGF1 was positively correlated with GH peak ( $P<0.01$ ), osteocalcin ( $P<0.0001$ ), CTx ( $P<0.0001$ ) and NTx ( $P<0.0001$ ), and negatively correlated with age ( $P<0.0001$ ), but was not correlated with either lumbar or femoral T-scores. No correlations were found between IGF2 and any of the above parameters, with the exception of age (negative correlation,  $P<0.05$ ). The same picture of correlations held true when considering the subset of osteoporotic patients.

#### Conclusions

Our study confirms the high prevalence of both osteoporosis and GH/IGF1 deficiency in thalassemic adults. GH and IGF1 secretory status, but not circulating IGF2, appears to positively affect bone turnover in this clinical condition. Further, GH secretory status, but not circulating IGFs, is likely to influence also bone mineralization.

### P133

#### Effects of the d3 GHR allele on disease activity in acromegalic patients

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#### Background

The exon-3 deletion in the growth hormone receptor gene (d3 GHR) leads to an enhanced GH signal transduction. Acromegalic patients with the d3 GHR genotype could be expected to have more marked clinical or biochemical manifestations for a given GH level than those with the wild (fl) type GHR. We studied acromegalic patients for clinical and/or biochemical disease recurrence after surgery in relation to GHR genotype status to assess whether d3 GHR influences the relationship between GH and disease activity.

#### Methods

Adults with initial diagnosis between 2000 and 2009 and pituitary surgery for acromegaly at our centre, with histologically proven GH-secreting adenoma and consenting to GHR genotyping were included. GH (random or during oGTT) and IGF-1 levels were analysed during follow-up.

#### Results

Forty-eight patients were included. 24 were homozygous for the fl-GHR, 24 had at least one d3 GHR allele. Transsphenoidal surgery markedly decreased disease activity in all patients. 19 of them were not cured as judged clinically and by failure to reach postoperative random low (<0.4 ug/l) GH levels or GH levels suppressible to a nadir of <1 ug/l during oGTT 2–3 months postoperatively. 29 patients had no biochemical evidence for remaining activity and could be classified as in remission. Two of these patients subsequently developed recurrent symptoms of acromegaly during follow-up, along with an increase in IGF1. Both carried the d3 GHR genotype.

#### Conclusions

Despite biochemical 'cure' as inferred from low postoperative GH levels, carriers of the d3 GHR seem to be at increased risk of recurrence. Longer follow-up periods should be monitored in a larger number of patients to find out whether a 'safe' cut-off for postoperative nadir GH levels ought to be lower for carriers of a d3-GHR than for those with the wild (fl) type GHR.

### P134

#### Circulating insulin-like growth factor 1 (IGF1) and mortality: meta-analysis and dose-response meta-regression

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#### Background

Insulin-like growth factor 1 (IGF1) plays a central role in metabolism and growth regulation. High IGF1 levels are associated with an increased risk of cancer, low IGF1 levels with increased risk for cardiovascular disease. We hypothesized that the relationship between circulating total IGF1 levels and mortality is U-shaped.

#### Methods

We performed a systematic review searching PubMed, EMBASE, Web of Science, Cochrane Library (1985 to September 2010) and undertook a random-effects meta-analysis of population-based studies assessing the association between IGF1 and mortality. Mortality in subjects with low or high IGF1 levels was compared with mid-centile reference categories. A dose-response meta-regression model was constructed to quantify the effect of IGF1 on mortality across the full IGF1 range.

#### Results

Twelve studies with 14 906 participants were included in the analysis. All-cause mortality was increased in subjects with low as well as high IGF1 categories, hazard ratio (HR) 1.27 (95% CI 1.08–1.49) and HR 1.18 (95% CI 1.04–1.34), respectively. Dose-response meta-regression confirmed a U-shaped relation of IGF1 and all-cause mortality ( $P=0.003$ ). The predicted HR for the increase in mortality comparing the 10th IGF1 to the 50th percentile was 1.56 (95% CI 1.31–1.86); the predicted HR for the increase in mortality comparing the 90th to the 50th percentile was 1.29 (95% CI 1.06–1.58). A U-shaped relationship was present for both cancer mortality and cardiovascular mortality.

#### Conclusions

This meta-analysis showed that both low and high IGF1 concentrations are associated with increased mortality in the general population.

### P135

#### Long acting analogs of glycoprotein hormones developed by using gene fusion and gene transfer are safe for use in human

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Glycoprotein hormones are used clinically in the treatment of many diseases. One major issue regarding the clinical use of many peptides is their short half-life due to the rapid clearance from the circulation. The major strategies for overcoming this problem by pharmaceutical companies are based on chemical techniques. To overcome this problem, we used genetic engineering techniques that have been found successful for designing long acting hormones. Overlapping PCR techniques, 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  $\beta$  (hCG $\beta$ ) subunit. The CTP contains 28 amino acids with 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), thyrotropin (TSH), erythropoietin (EPO), GH 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. Moreover, FSH long acting was approved by the European Commission (EC) for treatment of fertility. In addition, our results indicated that long acting GH is not toxic in monkeys and the results from clinical trials phase I seem to be promising. Designing long acting peptides will diminish the cost of these drugs and perhaps reduce the number of injections in the clinical protocols.

### Male reproduction

#### P136

#### Experience with a 1% testosterone gel in treatment of aging males with hypogonadism and type 2 diabetes mellitus

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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 (Total testosterone at diagnosis <12 nmol/l) between the age 50–65 years (55.00 (50.00;58.00)) were treated with a 1% testosterone gel (AndroGel) (5 g/day) within 6 months. Patients were assessed before the first using of AndroGel, in one week interval and in 6 months. At each consultation laboratory results, quality of life, mood, sexual function and skin reactions were monitored. Data are presented as mean  $\pm$  s.d. or median (25th–75th percentile).

#### 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 24 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)  $\mu$ g/l to 5.25 (4.03;8.23)  $\mu$ g/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 quality of life.

#### Conclusions

Treatment with the 1% testosterone gel (AndroGel) was associated with significant improvements in the testosterone, triglyceride, HbA1C, leptin, HOMA-IR levels in men with late-onset hypogonadism and T2DM.

**P137****Hypogonadism, insulin resistance and visceral adiposity in myotonic dystrophy**E Passeri<sup>1,2</sup>, E Bugiardini<sup>2,3</sup>, V Sansone<sup>2,3</sup>, C Fulceri<sup>4</sup>, E Costa<sup>4</sup>, B Ambrosi<sup>1,2</sup>, G Meola<sup>2,3</sup> & S Corbetta<sup>1,2</sup><sup>1</sup>Endocrinology and Diabetology Unit, Department Medical-Surgical Sciences, IRCCS Policlinico San Donato, San donato Milanese, Italy; <sup>2</sup>University of Milan, Milan, Italy; <sup>3</sup>Neurology Unit, Department Medical-Surgical Sciences, IRCCS Policlinico San Donato, San donato Milanese, Italy; <sup>4</sup>Clinical Chemistry Laboratory, IRCCS Policlinico San Donato, San donato Milanese, Italy.

Hypogonadism occurs in myotonic dystrophy (DM) type 1 and 2, a multisystemic autosomal dominant disorder. DM patients have genetically induced insulin-resistance (IR) and visceral obesity, providing a model to study the impact of metabolic disruptions on hypogonadism. We assessed hypothalamic-pituitary-gonadal function and metabolic features in 22 DM1 and 8 DM2 males (mean  $\pm$  s.d.,  $46 \pm 1$  years). Hypogonadism, defined as total testosterone (T)  $< 320$  ng/dl, freeT  $< 6.4$  ng/dl and sexual dysfunction), occurred in 1/3 of patients. Response to hCG was impaired in 33% of patients. Serum Insulin-Like factor 3 (INSL3), a marker of Leydig cell function, was lower in hypogonadic than eugonadic patients as well as 31 age-BMI-matched controls ( $0.33 \pm 0.07$  vs  $0.47 \pm 0.09$  and  $0.41 \pm 0.07$  ng/ml,  $P=0.004$ ) and correlated with age and freeT. LH and FSH levels were significantly higher in DM patients and 38% of eugonadic patients showed LH and FSH  $> 95^{\circ}$  centile of controls. Nonetheless, GnRH failed to induce a normal response in a subset of patients. Interestingly, muscle test, using MRC score, negatively correlated with LH and FSH levels ( $r = -0.412$ ,  $P=0.032$ ;  $r=0.479$ ,  $P=0.011$ ). DM patients showed an increased visceral body fat versus controls: waist/hip and body fat mass were significantly higher, hepatosteatosis, abnormal liver tests and increased epicardial fat occur in 60 and 46% of patients, respectively. IR was diagnosed in 36%, 4 had overt diabetes. FreeT levels were predicted by HOMA-IR after adjustment for age and fat mass. In conclusion, nearly 30% of DM patients showed symptomatic hypogonadism and subclinical hypogonadism is even more frequent. Leydig cells impairment is associated with INSL3 reduction and is mainly related to IR rather than visceral adiposity. Interestingly, the relationship between muscle strength and gonadotropin rise suggests that LH and FSH might be a precocious and sensitive marker of muscle degeneration in male DM patients.

**P138****Perceived ejaculate volume reduction in subjects with erectile dysfunction: psycho-biological correlates**Giovanni Corona<sup>1,4</sup>, Valentina Boddi<sup>1</sup>, Mauro Gacci<sup>3</sup>, Alessandra Sforza<sup>4</sup>, Gianni Forti<sup>1,2</sup>, Edoardo Mannucci<sup>5</sup> & Mario Maggi<sup>1</sup><sup>1</sup>Andrology and Sexual Medicine Unit, University of Florence, Florence, Italy; <sup>2</sup>Endocrinology Unit, University of Florence, Florence, Italy; <sup>3</sup>Urology Unit, University of Florence, Florence, Italy; <sup>4</sup>Endocrinology Unit, Azienda Usl di Bologna, Ospedale Maggiore, Bologna, Italy; <sup>5</sup>Diabetes Section Geriatric Unit, Department of Critical Care, University of Florence, Florence, Italy.**Introduction**

The disorders of orgasm/ejaculation include premature ejaculation, delayed ejaculation and perceived ejaculate volume reduction (PEVR). To explore the specific associations of PEVR in subjects with erectile dysfunction (ED).

**Methods**

A consecutive series of 3141 subjects (mean age  $51.6 \pm 13.1$  years old) was studied. Among these, 71 have been excluded because they underwent prostate surgery. Different clinical and biochemical factors were evaluated along with parameters derived from SIEDY structured interview evaluating the contribution of organic, relational and intrapsychic factors to ED.

**Results**

After adjustment for confounders, PEVR was specifically associated with the use of androgen deprivation therapy as well as with different other medications including alpha-blockers, serotonergic reuptake inhibitor antidepressants and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers. A higher prevalence of diabetes mellitus and hypogonadism was observed in subjects with PEVR when compared to the rest of the sample (23.0 vs 14.1% and 9.1 vs 5.3% respectively; both  $P < 0.05$ ). In addition, different sexual parameters such as severe ED (HR = 1.25(1.11–1.41);  $P < 0.0001$ ) patient's (HR = 1.53(1.38–1.70);  $P < 0.0001$ ) and partner's (HR = 1.21(1.07–1.36);  $P < 0.005$ ) hypoactive sexual desire (HSD) were also significantly related to PEVR. Furthermore, PEVR was associated with an impairment of both the relational and intrapsychic components of ED. In a multivariate model, adjusting for age, BMI,

smoking habit and medications, both hypogonadism, diabetes mellitus, and severe ED as well as patient's and partner's HSD were all independently associated with PEVR.

**Conclusions**

Our results indicate that PEVR is important not only for couple reproductive purposes, but it also has a distinct role in the couple's sexual performance.

**P139****Perceived reduced sleep-related erections in subjects with erectile dysfunction: psycho-biological correlates**Giovanni Corona<sup>1,3</sup>, Giulia Rastrelli<sup>1</sup>, Alessandra Sforza<sup>3</sup>, Gianni Forti<sup>4</sup>, Edoardo Mannucci<sup>2</sup> & Mario Maggi<sup>1</sup><sup>1</sup>Andrology and Sexual Medicine Unit, University of Florence, Florence, Italy; <sup>2</sup>Diabetes Section Geriatric Unit, Department of Critical Care, University of Florence, Florence, Italy; <sup>3</sup>Endocrinology Unit, Azienda Usl di Bologna, Maggiore-Bellaria Hospital, Florence, Italy; <sup>4</sup>Endocrinology Unit, University of Florence, Florence, Italy.**Introduction**

Perceived reduced sleep-related erections (PR-SREs), along with erectile dysfunction (ED) and hypoactive sexual desire, have been recently recognized as the most important symptoms characterizing late onset hypogonadism in community-dwelling European men. However, the clinical correlates of PR-SREs have not been thoroughly investigated. The aim of this study is to evaluate the psychological correlates of PR-SREs in a large series of subjects consulting for ED.

**Methods**

A consecutive series of 3888 (mean age  $51.6 \pm 13.0$  years) ED patients attending an Outpatient ED clinic was retrospectively analyzed. PR-SREs were investigated using validated question #13 of SIEDY structured interview, which showed an accuracy of about 70% in predicting RigiscanTM parameters in a consecutive subset of 199 subjects. Clinical, biochemical, hormonal, instrumental (penile color doppler ultrasound; PCDU) and intrapsychic (MHQ-questionnaire) correlates were also evaluated.

**Results**

63.6% of patients reported PR-SREs. After adjustment for age, total, analog free, calculated free and calculated bioavailable testosterone (T) were significantly lower in subjects reporting more severe PR-SREs. After adjusting for T levels and other confounders, PR-SREs were still associated with higher BMI, glucose and triglyceride levels, as well as with an increased 10 year-cardiovascular risk score. Accordingly, PR-SREs were more prevalent in subjects showing a reduced dynamic peak systolic velocity at PCDU or reporting severe ED. Among intrapsychic parameters, depressive and histrionic traits were significantly higher and lower, respectively, in subjects with any degree of PR-SREs.

**Conclusions**

Our study indicates that investigating PR-SREs represents an important step during the andrological consultation. In fact, reduced SREs might indicate an endocrine, organic and/or psychiatric ED background that might help in directing further investigation.

**P140****Sexual interests and hypogonadism in Prader-Willi syndrome (PWS)**H J Hirsch<sup>1</sup>, T Eldar-Geva<sup>1</sup>, F Benarroch<sup>2</sup>, O Rubinstein<sup>1</sup> & V Gross-Tsur<sup>1</sup>  
<sup>1</sup>Shaare Zedek Medical Center, Jerusalem, Israel; <sup>2</sup>Hadassah-Hebrew University Medical Center, Jerusalem, Israel.**Background**

Hypogonadism is a major feature of Prader-Willi syndrome, but clinical manifestations are variable. Sexual interests and behavior in this population have not been previously described.

**Objectives**

We studied PWS adolescents and young adults to assess: i) satisfaction with physical and sexual development ii) frequency of romantic and sexual experiences, iii) aspirations and expectations regarding marriage, iv) investigate the relation between sexual interests and hormone levels, and v) assess the desire for hormonal replacement therapy.

**Methods**

The study population consisted of 27 individuals (13 males) ages 17 to 32 (mean 23.5) years with genetically confirmed PWS. Mean IQ was 75 (range 50–100). We conducted structured interviews using questionnaires specifically designed for this study.



## Results

There was a significant negative correlation between IQ and body image in both males and females. IQ showed a positive correlation with interest in dating and romantic activities. Approximately half of PWS males and females reported having gone on a date and kissing romantically. All males and 64% of the females wished to be married. Seventy-seven percent of PWS males wanted hormonal treatment to increase phallic size. We found no correlation between hormone levels and sexual interests. Only 43% of PWS females wanted hormonal medication to achieve regular menstruation

## Conclusions

Despite documented hypogonadism, PWS young adults are interested in sexual and romantic issues. The range of sexual activities and expectations is variable. Understanding specific sexual characteristics of each individual is important in order to offer proper anticipatory sexual guidance counseling and for appropriate recommendations for hormone replacement.

## P141

### Erectile dysfunction does not mirror endothelial dysfunction in hiv-infected patients

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## Background

The penis has been compared to a barometer of endothelial health, being erectile dysfunction (ED) an early sign of endothelial dysfunction. The aim of the study was to investigate the association between ED and endothelial dysfunction in patients with HIV infection on ART.

## Methods

In this observational cross-sectional study we evaluated the prevalence and factors associated with ED in a cohort of 133 HIV-infected men. Evaluation tools included: the International Index of Erectile Function, ultrasound assessment of brachial artery flow mediated dilatation (FMD) and multi-slice computed tomography for coronary artery calcifications (CAC) as surrogates of endothelial dysfunction, the ATP III criteria to diagnose metabolic syndrome, plasma total testosterone (hypogonadism), and a visual analogue scale (VAS) of aesthetic satisfaction of the face and of the body (psychological distress associated with lipodystrophy).

## Results

Thirty-nine (29.32%) patients had mild, 14 (10.52%) moderate and 26 (19.55%) severe ED. Prevalence of ED ranged from 45% to 65% respectively in patients less than 40 and more than 60 years old. Metabolic syndrome (MS) was present in 20 (25%) patients with ED and 13 (24%) without ( $P$  value=0.87). Prevalence of ED did not appear to be associated with MS as a single clinical pathological entity, nor with the numbers of its diagnostic components. FMD <7% was present in 25 (32%) patients with and 18 (33%) without ED ( $P$  value=0.83) and CAC >100 was present in 8 (10%) patients with and 5(9%) without ED ( $P$  value=0.87). A stepwise multivariable logistic regression analysis was used to find predictors of ED. Independent predictor were VAS face (OR=0.85, 95% CI 0.73-0.99,  $P$ =0.049) and age, per 10 years of increase (OR=1.73, 95% CI 1.02-2.94,  $P$ =0.04).

## Conclusions

Age constituted the most important risk factor for ED, which was related with aesthetic dissatisfaction of the face leading to negative body image perception.

## P142

### P450 aromatase over-expressing mice (AROM+) as a model for infertility in men

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AROM+ mice express universally the human P450 aromatase enzyme, leading to increased serum and intratesticular E2/T ratio. AROM+ mice are infertile and

present with testicular inflammation characterized by a high amount of macrophages, mast cells and fibrosis. Interestingly, testis macrophage and mast cell infiltration together with fibrosis has also been connected to infertility in men. The aim of the study was to find out whether there are similar changes at the molecular level in the testes of AROM+ mice and infertile men having testicular fibrosis. Illumina gene expression array was carried out in AROM+ testes and testes of infertile men having fibrosis, and wild type mice or fertile men were used as controls, respectively. Immunohistochemical and qRT-PCR analyses were further performed to confirm findings in the array, and human peritubular cell culture was used for functional studies. The most interesting finding was that decorin, an extracellular proteoglycan, was up-regulated in AROM+ testis and in the testis of infertile men as compared with the respective controls. Decorin expression was localized to the extracellular matrix of the interstitial tissue both in AROM+ and infertile human testis. Functional studies with human peritubular cells further showed that decorin blocks growth factor-induced proliferation and differentiation of peritubular cells. Increased decorin is, thus, disturbing paracrine signaling pathways in the human testis. AROM+ mouse serves as a model for studying infertility associated with testicular fibrosis in men. Further studies are needed to define the mechanism by which the hormonal imbalance, especially the increased testicular E2/T ratio, brings about chronic inflammation and fibrosis in the human testis.

## P143

### The obstructive azoospermia and TESA-ICSI

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## Introduction

We evaluated the efficiency of TESA-ICSI method in infertile men with obstructive azoospermia. Testicular spermatozoa are obtained by fine-needle aspiration of testis (23 G) and then injected into cytoplasm of the oocyte.

## Methods

In the study, there were 173 married couples with 253 attempts of artificial fertilization (1.45 attempts per couple). Average age of women were 31 years and of men 36 years. Obstructive azoospermia was detected with clinical examination, semen analyses, hormone tests, fine-needle aspiration of testes and genetic tests (CFTR-DNA analysis). Stimulation of folliculogenesis was performed in accordance with the short/long protocol (GnRH analogues-rec. FSH-HCG) with usual oocyte aspiration time. Fertilization is performed by one single testicular spermatozoon which is injected into the oocyte's cytoplasm (ISCI).

## Results

The laboratory results are expressed as mean value (and range). Volume of the semen 2.6 ml (0.2-5.1); acid phosphatase 712 U/l (125-1770); fructosid 12.5 mmol/l (0.0-33.6); LDH-C4 unmeasurable; LH, FSH, testosterone, oestradiol and prolactin were normal; sperm/serum index: left 0.27 (0.09-1.40), right 0.29 (0.11-1.51). CFTR-DNA analysis found 9 heterozygotic mutation carriers. There were no female mutation carriers enabling fertilizations. On average 8.8 (range 2-21) aspirated oocytes, 5.3 (range 1-8) embryos were obtained. There were 88 pregnancies.

## Conclusion

TESA-ICSI seems to be very safe, simple and successful technique of assisted reproduction (34.5% per embryo transfer and cumulative 50.6% of pregnancies).

## P144

### Does local testosterone therapy in adult men measure up to the 2006 Endocrine Society's Guidelines?

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## Introduction

The 2006 Endocrine Society - testosterone therapy in adult men guidelines were audited against the clinical practice at a UK teaching hospital endocrine department. The aim of the audit was to determine whether patients were undergoing appropriate screening tests for prostate cancer and polycythaemia as described in these guidelines.

## Method

Patients were initially identified by searching the endocrine clinic letter database for the names of all testosterone replacement preparations. Patients were randomised and the following data was collected: indication for testosterone use;

date testosterone replacement commenced; baseline and post treatment measurements of PSA, digital rectal examination & haematocrit.

#### Results

The initial search revealed a possible 499 patients that could be receiving testosterone replacement therapy. We audited 180 patients selected at random. 159 of the 180 patients were receiving testosterone replacement therapy at the time of the audit. Of these 14% of patients were commenced on testosterone therapy within the previous twelve months. The most common indication for commencing therapy was due to pituitary pathology (59%). PSA was measured in 48% of patients receiving testosterone therapy within a one year period. Digital rectal examination was never performed or recorded in any patient receiving testosterone replacement therapy. Haematocrit was measured in 47% of patients receiving testosterone therapy within a one year period.

#### Conclusion

This audit highlights the difference between clinical practice and the 2006 Endocrine Societies guidance. It is important that local practice is regularly audited either against national or local guidelines to ensure high standards. We were not surprised to discover digital rectal examinations were not regularly undertaken, but expected a higher rate of PSA testing. In the poster we also discuss recent US, European and Japanese recommendations on screening for prostate cancer.

### P145

#### Cryptorchidism, varicocele, azoospermia and Leydig cell tumor in a couple of identical twins with a unique de novo balanced reciprocal translocation.

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Chromosomal translocations or inversions, may cause spermatogenic failure. We present a case of a 35-years-old couple of identical twins: GD and GS. Both had cryptorchidism and bilateral varicocele and underwent medical treatment first, and orchipexy and varicocelectomy subsequently at 7 years and 28 years respectively. GD at 31 years had left orchiectomy because of Leydig cell tumor. They were referred to our clinic for azoospermia and fatherhood desire. The patients were a nonsmokers and nonalcoholics, with no history of infections or traumas in the genital area and were taking no regular medication. Physical examination showed only the right testicle in GD and both testicles in GS, all of elastic consistency and a volume of approximately 15 ml; deferent ducts, epididymides and prostate were normal at palpation. A semen analysis after 5 days of sexual abstinence (performed twice within 2 months according to the WHO guidelines), demonstrated azoospermia in both twins with a normal ejaculate volume (around 2.5 ml). Bacterial cultures excluded genital infection with pathologic bacteria. The levels of FSH and LH were elevated, testosterone levels within normal limits. A cytogenetic analysis demonstrated a balanced translocation (karyotype 46, XY – 3p25; 14q22). Both GD and GS underwent multiple testicular biopsies that revealed almost identical alterations, namely 'seminiferous tubules with slightly diminished diameters, thickened fibrous membranes, and sparse germinative epithelial tissues. Rare spermatogonia and spermatocytes were observed within the tubules, but no spermatids or spermatozoa, with 80–90% tubules with Sertoli cells'.

To our knowledge, this is the first report about a reciprocal translocation in a couple of identical twins with cryptorchidism, varicoceles, Leydig cell tumor and azoospermia.

### P146

#### Transcriptional regulation of germ cell differentiation.

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#### Introduction

Cellular differentiation and development of germ cells critically depend on a coordinated activation and repression of specific genes. The underlying regulation mechanisms, however, are largely unknown. Here, we describe the interplay between two transcription factors to be critical for cell type-specific expression in testis.

#### Methods

We investigated the binding of CREM $\tau$  (cAMP response element modulator tau) and GCNF (germ cell nuclear factor) to DNA by Band Shift and ChIP assays.

Cell-based assays of wild type and mutated versions were performed by reporter gene experiments. Protein-protein interactions were measured by GST-pull down and immunoprecipitation assays. Finally, *in vivo* relevance of the critical binding sites was monitored using a transgenic mouse model.

#### Results

*In vivo*, expression of the CREM/GCNF-driven transgene is detectable in haploid spermatids, but not in any somatic tissue or at any other stages during germ cell differentiation. CREM $\tau$  acts as an activator of gene transcription whereas GCNF suppresses this activity. Both factors compete for binding to the same DNA response element. Effective binding of CREM and GCNF highly depends on composition and epigenetic modification of the binding site. CREM and GCNF bind to each other via their DNA binding domains, indicating a complex interaction between the two factors. There are several testis-specific target genes that are regulated by CREM and GCNF in a reciprocal manner, showing a similar activation pattern as during spermatogenesis.

#### Conclusion

Our data indicate that a single common binding site for CREM and GCNF is sufficient to specifically direct gene transcription in a tissue-, cell type- and differentiation-specific manner.

### P147

#### Efficacy of recombinant human follicle-stimulating hormone (rhFSH) in the treatment of male-factor infertility.

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To study the efficacy of rhFSH treatment on male infertility we evaluated the response of 79 hypo-normogonadotropic patients referring to our clinic for couple infertility after a period of not <12–18 months of finalized unprotected intercourse without conception. rhFSH 150 IU were given subcutaneously every other day for 12 months (mean 15  $\pm$  3 months). All men, between 23 and 56 years (mean 34  $\pm$  8 years), were divided into three subgroups: i) hypogonadotropic hypogonadism with hypopituitarism ( $n=24$ ), ii) functional hypogonadotropic hypogonadism ( $n=40$ ), iii) hypogonadism associated with other conditions ( $n=16$ : betathalassemia, testicular dysgenesis syndrome, adrenogenital syndrome). FSH levels were in the low-normal range (5.9  $\pm$  2.8 mU/ml) in all patients. Testosterone and LH serum levels and testicular volume were compared before and after treatment in all groups. According to WHO standards the total number of sperms (NTS), the sperm morphology and motility were assessed. After rhFSH treatment, there was no significant change in either values of testosterone and LH (respectively 4.3  $\pm$  1.2 ng/ml and 3.5  $\pm$  1.8 mU/ml), or testicular volume (Prader 18  $\pm$  5). rhFSH treatment induces in group B a significant improvement in sperm number (NTS from 4.1  $\pm$  3.0 to 15.0  $\pm$  12.0 mln,  $P<0.002$ ) and motility (23 vs 35%,  $P>0.03$ ), while there was no effect on sperm morphology. Males in the group A, completely azoospermic before treatment, had a weak response of NTS (up to 4.2  $\pm$  2 mln, in 25% of patients) after treatment. In group C there was no significant results. In conclusion rhFSH treatment in males with functional hypogonadotropic hypogonadism induces a marked increase in sperm count, a slight increase in sperm motility, no change in sperm morphology. Our preliminary data shows that rhFSH prolonged treatment can cause a noticeable improvement especially in those males with functional forms of hypogonadotropic hypogonadism.

### P148

#### Testosterone changes over time in men with human immunodeficiency virus infection: preliminary results

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#### Introduction

Male hypogonadism is very frequent in men treated with highly active anti-retroviral therapy (HAART) for Human Immunodeficiency Virus-1 (HIV-1) infection, reaching a prevalence of about 20%. Literature data regarding the time course of serum total testosterone (T) levels in these patients are lacking.

#### Aim of the study

To evaluate changes of T levels over time in HIV-positive men with an initial finding of low T (<300 ng/dl).

#### Methods

Measurement of T and serum LH in 111 hypogonadic HIV positive outpatients aged 31–68 years (mean 46.3 years) at baseline and after 12 months.

#### Results

Mean T value at baseline was 235.8 ng/dl. After 12 months, 36 subjects (32.4%) had no change in T (a variation of <50 ng/ml), 3 patients (2.7%) had a decrease (more than 50 ng/ml) and 72 patients (64.9%) had an increase in T (more than 50 ng/ml). 63 patients (56.7%) normalized T (>300 ng/ml). Mean T value at baseline of the 63 patients who restored T was 236.3 ± 66.9 ng/ml, while mean T value at baseline of the 9 patients who didn't normalize was 151.4 ± 86.5 ng/ml.

#### Conclusions

Most of the HIV patients under HAART with an initial finding of low T present normalized T in the following months. Baseline T values seem to be predictive of the future evolution of the disease, higher T level being associated with subsequent T normalization. In HIV patients a single finding of hypotestosteronemia needs further confirmation before starting androgen therapy and often a wait-and-see approach is mandatory.

### P149

#### Intramuscular testosterone undecanoate for substitution in male hypogonadism – the experience of 13.5 years

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#### Background

Testosterone substitution with favourable kinetics and tolerance is important for hypogonadal men. Intramuscular injections of long-acting testosterone undecanoate (TU) offer a convenient modality.

#### Methods

We report data from 281 patients (134 with primary, 88 with secondary hypogonadism and 59 with late-onset ('mixed' or 'metabolic') hypogonadism aged 15 to 72 years (mean 40 ± 13 years) receiving altogether 4913 intramuscular injections of 1000 mg of TU during a maximal treatment time of 13.5 years, overall corresponding to 1069 treatment years.

Components of the metabolic syndrome were assessed in 216 men receiving 2864 injections.

#### Results

Trough levels of testosterone were generally within the low normal range, indicating sufficient substitution. Individual dosing intervals ranged from 10 to 14 weeks. The proportion of men fulfilling the new joint consensus criteria of the International Diabetes Federation and the National Cholesterol Education Program for definition of the Metabolic Syndrome decreased from initially 87 to 52% within 2 years ( $\chi^2$  for trend:  $P < 0.001$ ). Especially waist circumference decreased from 112.0 ± 10.3 to 96.4 ± 9.1 cm ( $P < 0.001$ ) within a year (body mass index from 30.4 ± 5.1 to 28.6 ± 3.8 cm ( $P = 0.001$ )). Lipoprotein subfractions, blood pressure and fasting glucose levels improved in a similarly meaningful manner. Hematocrit was significantly elevated under treatment but remained within the normal range, except for occasional measurements (maximal value 56.3%).

#### Conclusion

Injections of testosterone undecanoate represent a feasible, safe and well tolerated modality of androgen substitution in hypogonadal men of a wide age-range, substantiated by more than one decade of experience, facilitating a decrement of metabolic/cardiovascular risk factors.

### P150

#### Testosterone administration to hypogonadal men for a period of up to 42 months improves features of the metabolic syndrome

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#### Objectives

Elderly men often show a concurrence of a decline of testosterone with features of the metabolic syndrome.

#### Materials and methods

One hundred and twenty-two men of a mean age of 59.5 ± 6.0 years, with baseline testosterone 5.9–12.1 nmol/l were treated with parenteral testosterone undecanoate for 42 months as the sole intervention.

#### Results

Plasma testosterone rose from 9.3 ± 1.7 to 18.7 ± 2.1 nmol/l reaching their maximum at 9 months and remaining stable over the next 33 months. Body weight and waist circumference declined progressively over the full study period. Plasma glucose, cholesterol, triglyceride, and LDL-cholesterol decreased significantly over the 24 month study period and then stabilized. Plasma HDL increased significantly over the first 24 months and then declined. There was a significant decrease of levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) over the first 24 months, then values leveled off. At baseline 47/122 met the criteria of the metabolic syndrome (ATPIII criteris) after two years of testosterone treatment this number had declined to 11/122.

#### Conclusion

With testosterone treatment over 42 months, the most significant improvement of the metabolic syndrome was noted over the first 12 months with further improvement over the following 12 months. Body weight and waist circumference declined further but glucose and lipids and liver functions did not further improve but were stable with continued testosterone treatment.

### P151

#### Safety of 42 months of treatment with of long-acting parenteral testosterone undecanoate in men with late-onset hypogonadism

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#### Objectives

To investigate the safety of the administration of long-acting parenteral testosterone undecanoate (TU) to hypogonadal, mainly elderly men.

#### Design and methods

One hundred and twenty-two men of a mean age of 59.5 ± 6.0 years, with baseline testosterone 5.9–12.1 nmol/l were treated with parenteral TU. These 122 patients were followed for 42 months.

#### Results

Plasma levels of testosterone rose from 9.3 ± 1.7 to 18.7 ± 2.1 nmol/l reaching maximum at 9 months, never exceeding reference values. There was a slow but steady increase in prostate volume, not paralleled by an increase in serum prostate specific antigen (PSA) of similar magnitude. Serum PSA rose slightly over the first 24–36 months, then values stabilized at levels of 5–10% higher than baseline to rise again after 42 months. PSA never exceeded 4 ng/ml. The residual bladder volume decreased over the first 24 months and then stabilized. The scores on the International Prostate Symptoms Score decreased over the first 24 months and then stabilized. Hematocrit increased significantly and had reached its maximum values after 12 months. Over the 42-month study period, at any time point, nine patients had a hematocrit above 52%, the upper limit of normal. No specific measures were taken. An elevated hematocrit was never found at two occasions in the same patient.

#### Conclusions

Over a period of 42 months testosterone treatment with TU appeared safe. There was an increase in prostate size and PSA but not in bother.

### P152

#### Expression of ghrelin receptor GHSR-1a in mammalian spermatozoa and their response to ghrelin *in vitro*

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In this study, we attempted to demonstrate the expression of GHSR-1a receptor and possible effects of its activation in human, rat and boar spermatozoa.

For demonstration of GHSR-1a immunocytochemical, immunofluorescence and western blotting techniques were applied. The effects of receptor activation was tested in a series of *in vitro* incubations of spermatozoa with or without addition of ghrelin and the effects were evaluated using confocal microscopy and flow cytometry.

GHSR-1a was shown, with both immunohistochemical and immunofluorescence techniques, in rat epididymal and boar ejaculated spermatozoa in which profiles of acrosome and head membrane was marked by the reaction and the expression was confirmed by western blotting. However, we failed to demonstrate GHSR-1a expression in human ejaculated spermatozoa neither with immunohistochemical nor western blotting techniques. *In vitro* ghrelin affected rat and boar spermatozoa producing a dose dependent rapid (s) and prolonged (to 20 min) increase in intracellular  $Ca^{2+}$  concentration, higher percentage of spermatozoa showing progressive movement and increase in number of spermatozoa with acrosome reaction induced by ionophore. Interestingly, a response of human spermatozoa to ghrelin could be recorded as a short lasting (s) increase in intracellular  $Ca^{2+}$  concentration and poor changes in sperm motility and acrosome reaction. It is concluded that while the effects of ghrelin in rat and boar sperms arose as a result of GHSR-1a activation those in human spermatozoa probably indicate the peptide action through different than GHSR-1a receptor(s).

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## P153

### Lower serum testosterone and estradiol ( $E_2$ ) in adult men with unfused epiphyses due to unrecognized and untreated congenital hypogonadotropic hypogonadism: evidence for an $E_2$ threshold for bone maturation in men

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#### Introduction

At puberty, the raise of serum estradiol ( $E_2$ ) (after testosterone conversion) is needed to fuse epiphyses and to complete bone maturation in boys. Owing to severe hypogonadism and very low circulating testosterone, adult men with congenital hypogonadotropic hypogonadism (CHH) may present with unfused epiphyses and continuing linear growth if androgen deficiency is unrecognized and untreated. In order to establish the minimal amount of sex steroids needed to ensure bone maturation, we prospectively studied 28 caucasian men first diagnosed as CHH in adulthood (a very rare clinical condition) and we compared 11 adult CHH men (mean age  $\pm$  s.d.:  $22.7 \pm 6.1$ ) with fused epiphyses to 17 adult CHH men (mean age  $\pm$  s.d.:  $21.8 \pm 4.3$ ) with unfused epiphyses.

#### Methods

Serum testosterone,  $E_2$ , LH, FSH were assayed. Bone age, target height, Tanner stage, anthropometrical measurements (height, arm span, upper (U) and lower (L) segments) and bitemporal volume (b-TV) were calculated.

#### Results

Bone age, testosterone,  $E_2$ , Tanner stage, b-TV were significantly lower in HH men with unfused than with fused epiphyses ( $P < 0.001$ ). Height, arm span, the arm span/height and the U/L ratios, and the difference between patient's height and his target height were significantly greater in HH men with unfused than with fused epiphyses ( $P < 0.001$ ). All patients with unfused epiphyses had  $E_2 < 15$  pg/ml and all patients with fused epiphyses had  $E_2 > 20$  pg/ml, while testosterone resulted partially overlapped in the two groups.

#### Conclusion

A threshold of 20 pg/ml exists for serum  $E_2$  above which epiphyseal closure and bone maturation may be reached in men. Setting this threshold is challenging for targeting some kind of treatment for short or tall stature in boys, like aromatase inhibitors or androgens respectively. Unfused epiphyses, tall stature and eunuchoid skeleton all depend from circulating estrogens rather than androgens not only in genetic diseases due to congenital estrogen deficiency.

## P154

### Erectile dysfunction in endocrine patients (pituitary diseases)

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#### Aim of the study

Assessing prevalence and severity of erectile dysfunction in patients with pituitary diseases.

#### Materials and methods

International Index of Erectile Function (IIEF) and Sexual Health Inventory for Men (SHIM) questionnaires were used to assess erectile dysfunction (ED) in 83 patients having pituitary diseases (31 patients had hypopituitarism, 11 diabetes insipidus, 10 prolactinomas, 11 nonfunctional adenomas, 16 operated acromegaly, 4 GH deficiency). The presence and severity of erectile dysfunction were quantified by using IIEF questionnaire scores for erectile function (IIEF-FE) validated by Altho and Juliano in 2006. Correlation between plasma testosterone levels and severity of ED was analyzed by using Pearson correlations.

#### Results

Erectile function was most severely affected in patients with hyperprolactinemia, with a mean IIEF-FE score of 5.7, and among them 60% had severe dysfunction, 30% moderate, 10% mild dysfunction. Plasma testosterone levels were in inverse relation with prolactin levels. Acromegalic patients had severe impairment of EF, mean IIEF-FE 9.2, and weak correlation with testosterone levels ( $r^2 = 0.235$ ). ED severity decreases progressively in patients presenting nonfunctional adenomas, mean IIEF-FE 12, followed by hypopituitarism, mean IIEF-FE 12.45, GH deficiency having a mean IIEF-FE 15 and diabetes insipidus IIEF-FE 18.5. Patients having nonfunctional adenomas showed a strong direct correlation between testosterone levels and IIEF-FE score ( $r^2 = 0.286$ ). In patients with hypopituitarism IIEF test showed a high prevalence of severe ED, 41.9%, displaying a weak correlation with testosterone levels ( $r^2 = 0.054$ ). In GH deficient patients IIEF-FE score shows moderate ED, close to the mean value of the other subjects. Patients with diabetes insipidus showed elevated prevalence of ED, 63.7%, but most of them had mild to moderate ED. There was no correlation between testosterone levels and severity of ED.

#### Conclusions

The prevalence and severity of ED are different between the groups studied. The most severe erectile dysfunction was seen for prolactinomas, followed by acromegaly, nonfunctional adenomas, hypopituitarism, GH deficiency and diabetes insipidus.

## Neuroendocrinology

### P155

#### Short-term balneotherapy is associated with changes in salivary cortisol levels

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Since ancient times, physicians have speculated that balneotherapy (therapeutic bathing in medicinal and thermal springs) has a stress-relieving effect, but this has not yet been scientifically established. The aim of this study was to evaluate the stress-relieving effects of short-term balneotherapy in a controlled trial by measuring salivary cortisol as a sensitive stress marker. Forty-nine healthy probands were randomised into three intervention groups. Group one performed bathing in a thermal spring (Bad Loipersdorf, Styria Thermal Region, Austria), group two relaxed in deckchairs, and group three performed progressive muscle relaxation. In each group, the intervention lasted for 25 min. Saliva samples were collected immediately after getting up in the morning. Immediately before and after intervention saliva samples were taken again and participants rated their subjective relaxation level on a quantitative scale. Salivary cortisol was determined by ELISA. Additionally, the following psychological tests were employed: Perceived Stress Scale, Recovery-Stress Questionnaire, Symptom list. One-way ANOVAs for repeated measures were performed to detect changes in salivary cortisol and subjective stress ratings between groups. In all three groups, saliva cortisol decreased ( $F = 23.532$ ,  $P < 0.001$ ) and subjective relaxation ratings increased ( $F = 132.178$ ,  $P < 0.001$ ) after intervention. Groups did not significantly differ concerning the reduction of salivary cortisol. Interestingly, the increase of study participants' subjective level of relaxation was significantly higher in the balneotherapy group ( $F = 5.216$ ,  $P = 0.009$ ). These findings suggest both an objective and subjective stress-relieving effect associated with short-term balneotherapy. With respect to changes in saliva cortisol levels other stress reduction interventions seem to produce similar effects but may not be experienced as similar beneficial as balneotherapy.

**P156**

**Character of macrovascular complications as per the Uzbekistan National Register of patients with the combined diabetes mellitus and diabetes insipidus**

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**Aim**

To study character of macrovascular complications in combination of diabetes mellitus (DM) and diabetes insipidus (DI).

**Materials and methods**

Within the period from 2006 to 2010, we examined 2208 cases of DI among them 106 DM–DI combinations. The patients had their endocrine and neurologic statuses assessed with blood and urine density investigation, including complete urinalysis and Zimnitsky urine count, RIA and measurement of pituitary hormones (GH, STH, LH, FSH, IGF1, prolactin, cortisol, etc.), ECG, echocardiography, Doppler ultrasound, Turkish saddle CT, eye fundus examination and assessment of vision fields.

**Results**

In 2010, the Uzbekistan National Register included 2208 patients with DI, 106 of which (4.8%) had DM–DI combination, 22 cases registered in Tashkent. The combination in question mutually burdens clinical presentation aggravating outcomes of both diseases and resulting in early cardio-vascular complications, high incidence of myocardial infarctions and strokes serving as the evidence. DM is a significant cardio-vascular risk factor in patients with diabetes insipidus. When added it results in increase of arterial hypertension and atherosclerosis, making myocardial infarctions (in 12.2 vs 5%) and cerebral circulation disturbances (in 88.4 vs 22.5%) more frequent. Progressing atherosclerosis with heart and brain vessels stenosed, myocardial contraction reduced and left ventricle overloaded, because of increase in the blood minute volume due to DI, underlie the complications of the combination. The fact is confirmed by presence of cardiac insufficiency in most patients with DI.

**P157**

**Clinical presentation peculiarities of constitutional puberty delay in boys**

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**Aim**

To study clinical presentation peculiarities of constitutional puberty delay in boys.

**Materials and methods**

We examined 54 boys (mean age 13.8 years) with constitutional puberty delay (CPD). All examinees underwent general clinical examination as well as biochemical and hormonal investigations (levels of GH, LH, FSH, free testosterone, prolactin, cortisol, TSH, etc.), having their bone age and anthropometric parameters by Tanner-Whitehouse measured and their sexual development stage by Tanner assessed.

**Results**

Most frequently the patients presented with vertigo (32%), fatigue (30%), headaches (29%), general weakness (28%), memory reduction (25%), irritability (24%), sleep disorders (17.4%), etc. As to degree of manifestation growth and puberty delay was observed in 17%, growth, puberty and speech delay in 4.3%, delay in growth, puberty, speech and psycho-motor development being found in 1.06% of the patients. Nocturnal enuresis (45%), chronic pyelonephritis (31%), neurotic asthenia (30%), vegeto-vascular dystonia (28%), chronic tonsillitis (23%) and chronic hepatitis (10%) were among the comorbidities to name.

**Conclusions**

Multiple clinical presentation peculiarities are typical of constitutional puberty delay necessitating further detailed study.

**P158**

**Evaluation of the questionnaire for revealing of GH deficiency in adults with various pituitary adenomas**

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**Objective**

To estimate the value of the questionnaire QoL – AGHDA, KIMS for revealing of GH deficiency (GHD) in adults for estimation of the condition of patients with various pituitary adenomas.

**Material and research methods**

We examined 72 adult patients with pituitary adenomas and GHD from 2008 till 2009. From them there were 30 men (41.6%), and 42 women (58.3%). Middle age was 38.0 years.

**Research methods included**

i) Clinical, ii) instrumental (perimetry, electrocardiogram, CT/MRI of sella turcica, etc.), iii) hormonal blood tests (GH, IGF1, LH, FSH, TSH, ACTH, prolactin, etc. iv) biochemical blood tests (HDL, LDL, cholesterol, TG, calcium, etc.), v) the questionnaire of quality of a life – QoL – AGHDA, KIMS.

**Research results**

Our patients were divided into 4 groups: 1st group – 39 (54.1%) patients which received conservative therapy (bromocriptin, cabergoline), 2nd group – 21 (29.1%) patients undergone THE, 3rd group – 4 (5.5%) patients which received radiation therapy, 4th group – 8 (11.1%) patients undergone THE and radiation therapy.

Quality of life assessment on the basis of questionnaire of GHD QoL – AGHDA in 72 patients of the 4 groups revealed, that in the 1st group mean score was 16.3 points, in the 2nd – 13, in the 3rd – 22.5, and in the 4th – 16.3 points. So, in all groups the mean score QoL – AGHDA was above admissible norm (normally < 11 points). The highest mean score (22.5 of 25) was revealed among patients of the 3rd group.

**Conclusion**

The Questionnaire QoL – AGHDA of GHD revealing in adults is a reliable method of estimation of quality of life and psychological status of adult patients with GHD before treatment.

**P159**

**Frequency of secondary GH deficit in patients with acromegaly undergone radiation or surgical treatment**

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**Objective**

To investigate frequency of secondary GH deficit (SGHD) in patients with acromegaly in the Republic of Uzbekistan (RUz).

**Material and methods**

There are 366 patients with acromegaly registered in RUz. We observed 15 patients with acromegaly with SGHD, among them 3 male and 12 female with middle age 51.6 years. All patients undergone following tests: pituitary hormones levels (GH, IGF1, ACTH, prolactin, TSH, FSH, LH, etc.), blood glucose, biochemical blood indices, ECG, pituitary MRI/CT, densitometry, visual fields, and others. Total hypophisectomy (THE) was performed to 4 patients, pituitary radiation therapy (PRT) – to 8 patients, THE + PRT – to 1 patient. Two patients undergone medical treatment.

**Study results**

We established that mean level of GH was significantly decreased (by 0.4 ± 0.01 ng/ml). IGF1 level were also significantly decreased (97.5 ± 7.3 ng/ml). All patients had deficit of 2 or 3 stimulating hormones, including SGHD.

We revealed post radiation hypopituitarism in 8 patients (53.3%), post operative hypopituitarism in 4 (26.6%), and combined hypopituitarism – in 1 (6.6%) patient. In 2 patients (13.3%) hypopituitarism has developed after medical treatment.

**Conclusion**

According to preliminary data, SGHD in patients with acromegaly may be the criterion of the process remission.

**P160****Hormonal disorders in patients with acromegaly in Khorezm region, Republic of Uzbekistan**Rikhsieva Urmanova<sup>1,2</sup>, Sh O Khodjaev<sup>1,2</sup>, Sh A Eshmetov<sup>1,2</sup>, M A Rakhmetova<sup>1,2</sup>, F S Khodjaeva<sup>1,2</sup> & B A Azizov<sup>1,2</sup><sup>1</sup>Republican Specialized Scientific-Practical Medical Center of Endocrinology under the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan; <sup>2</sup>Tashkent Medical Pediatric Institute, Tashkent, Uzbekistan.**Aim**

To study spectrum of hormonal disorders in patients with acromegaly, residents of Khorezm region, Republic of Uzbekistan.

**Materials and methods**

We examined 22 acromegalics (9 men and 13 women, mean age 39.8 years), residents of Khorezm region, Republic of Uzbekistan. All patients were clinically and biochemically examined with their hormonal parameters, such as levels of GH, LH, FSH, IGF1, prolactin, cortisol and TSH measured, ECG, perimetry and pituitary CT/MRI performed. In 15 examinees (68.2%) the latter procedure helps reveal pituitary macroadenomas.

**Results**

Mean levels of hormones were found as follows: GH 48.9 ng/ml, LH 3.5 mIU/l, FSH 2.9 mIU/l, IGF1 756 nmol/l, cortisol 320 nmol/l, prolactin 22.8 ng/ml. Two of the patients underwent surgery; three persons received radiotherapy and 17 are still on drug therapy preferentially with dopamine agonists.

**Conclusions**

Results of investigations indicate the decompensate in most patients examined.

**P161****Incidence of diabetes mellitus and diabetes insipidus combination in the Republic of Uzbekistan**Z Yu Khalimova<sup>1,2</sup>, Yu M Urmanova<sup>1,2</sup>, D A Alieva<sup>1,2</sup> & V Aripova<sup>1,2</sup><sup>1</sup>Republican Specialized Scientific-Practical Medical Center of Endocrinology under the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan; <sup>2</sup>Tashkent Medical Pediatric Institute, Tashkent, Uzbekistan.**Aim**

To study clinical-epidemiological peculiarities of diabetic macroangiopathies in diabetes mellitus (DM) and diabetes insipidus (DI) combination.

**Materials and methods**

We examined 2208 cases of DI registered in the RUz in 2006–2010, 106 DM–DI combinations among them. DM–DI combination prevalence, calculated for 100 000 of adult and infant population, was assessed analysis of data on patients registered at the endocrine ambulances. The patients had their endocrine and neurologic statuses assessed with blood and urine density investigation, including RIA and measurement of pituitary hormones, ECG, echocardiography, Doppler ultrasound, CT/MRI, etc.

**Results**In 2010, the Uzbekistan National Register (UzNR) included 2208 patients with DI, 106 of which (4.8%) had DM–DI combination, 22 cases registered in Tashkent. There were 49 men (46.2%) and 57 women (53.7%) among 106 patients with DM–DI combination to show no gender differences. Mean age was 37.6 ± 2.6 years. The disease duration in most patients ( $n=58$ , 53.7%) was 5–10 years, the longest one being 48. Age group of 40–49 years included patients with the DM–DI combination most of all ( $n=38$ , 35.5%). In 2010, no lethal cases were registered.**Conclusions**

The UzNR system for DI patients allows appropriate DI form diagnosing, disease severity assessing, therapy correcting as well as long-term patients' following-up and predicting of the disease outcomes. DI prevalence was 7.9 cases per 100 000 of population with sex ratio 1:1.1. In 2006–2010, DI incidence among other endocrine pathologies in RUz was 0.46 and 0.37% respectively.

**P162****Incidence of puberty delay risk factors in boys**Yu M Urmanova<sup>1,2,3</sup> & U Kh Mavlonov<sup>1,2,3</sup><sup>1</sup>Republican Specialized Scientific-Practical Medical Center of Endocrinology under the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan; <sup>2</sup>Bukhara State Medical Institute, Bukhara, Uzbekistan; <sup>3</sup>Tashkent Medical Pediatric Institute, Tashkent, Uzbekistan.**Aim**

To study significance of risk factors in puberty delay in boys.

**Materials and methods**

We examined 54 boys (mean age 13.8 years) with constitutional puberty delay (CPD). All examinees underwent general clinical examination as well as biochemical and hormonal investigations (levels of GH, LH, FSH, free testosterone, prolactin, cortisol, TSH, etc.), having their bone age and anthropometric parameters by Tanner-Whitehouse measured and their sexual development stage by Tanner assessed.

**Results**By their sexual development the boys could be attributed to stage I by Tanner in the average, though the III one is normal. Mean testicular volume was 4.98 ± 3.6 ml. As to mean levels of hormones, free testosterone was 1.21 nmol/l, LH and FSH being respectively 2.7 and 3.4 mIU/l, GH measuring 2.3 ng/ml, TSH 2.4 mIU/l, cortisol 106.5 ng/ml, thyroxine 96.9 nmol/l and triiodothyronine 1.7 ng/ml. The boys' mean height was 125.5 cm (SDS > -2). The findings above demonstrated delay in activation of hypothalamo-pituitary-gonads system. Familial low stature ( $n=45$ , 84%), perinatal traumas ( $n=23$ , 42.5%), brain injuries ( $n=19$ , 29.6%), BMI > 30 ( $n=16$ , 29.6%), complicated pregnancy in mother ( $n=15$ , 28%), smoking ( $n=5$ , 9.2%), frequent stresses ( $n=15$ , 28%) and colds ( $n=5$ , 9.2%) were found to predominate among the risk determinants of CPD.**Conclusions**

Constitutional puberty delay in the examinees has been found determined by familial low stature, brain injuries, perinatal traumas, BMI &gt; 30 and frequent stresses.

**P163****Structure of neuroendocrine disorders rate in patients with different pituitary adenomas (retrospective analysis)**

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**Aim of the research**

To reveal neuroendocrine disorders rate in patients with different pituitary adenomas retrospectively.

**Patients and methods**

We analyzed 81 patients records with different pituitary adenomas from 2000 to 2009. Of 81 patients 23 were men (28.4%) and 58 women (71.6%). Mean age of male patients was 37.2 years-old whereas in women this was 34.5 years-old.

1st group of patients was with Cushing's disease (CD) – 33 (40.7%), 2nd group was with non-functioning pituitary adenoma (NFPA) – 43 (53%), third group was with prolactinoma – 4 (4.9%) and fourth group with acromegaly 1 (1.2%). All patients underwent clinical assessment including hormonal evaluation, pituitary and adrenals imaging (CT, MRI), vision tests, densitometry etc.

**Results**

The most common complaints in examined patients were headache – 64 patients (79%), dysmenorrhea in 30 (52%) of 58 women, artralgia in 34 patients (41.9%), heartache in 32 patients (39.5%), lack of energy in 29 patients (35.8%), dizziness in 24 patients (29.6%), eye ache in 20 patients (24.6%), bitemporal hemianopsia in 20 patients (24.6%), dry mouth in 12 patients (14.8%), constipation in 11 patients (13.5%), poliuria in 11 patients (13.5%), hair loss in 11 patients (13.5%), galactorrhea in 10 patients (12.3%), vision deterioration in 9 patients (11.1%), vision loss in 2 patients (2.4%), exterior scotoma in 2 patients (2.4%) etc.

**Conclusions**

Thus, retrospective analysis of patients with different pituitary adenomas showed variety of both clinical and neuroendocrine disorders.

**P164****Sexual desire in female-to-male transsexual persons**

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**Objective**

The current study aims to describe sexual desire in female-to-male transsexuals post SRS (sex reassignment surgery) using a validated questionnaire. The association between serum androgen levels and the intensity and frequency of sexual desire are examined. The data are compared to those of male-to-female transsexual persons.

**Design**

Cross sectional study.

**Methods**

Female-to-male transsexual persons, post SRS, ( $n=48$ ) completed a questionnaire measuring sexual desire (Sexual Desire Inventory). Also, participants were asked additional questions on current and past sexual desire, frequency of masturbation and sexual intercourse in the past month. Serum levels of testosterone and SHBG were measured on fasting morning serum samples. Data from our previous study on sexual desire in 62 male-to-female transsexual persons using the same validated questionnaire was used as reference population.

**Results**

In retrospect, the majority of the participants (73.9%) reported an increase in sexual desire after cross sex hormone treatment and SRS. No associations between levels of testosterone and scores of solitary and dyadic sexual desire were found. Solitary sexual desire scores were significantly correlated with frequency of masturbation ( $r=0.835$ ;  $P=0.0001$ ), whereas frequency of sexual intercourse with a partner was not correlated with dyadic nor solitary sexual desire ( $P=0.515$ ;  $P=0.221$ ). Female-to-male transsexual persons scored significantly higher on sexual desire scores than male-to-female transsexual persons ( $P=0.0001$ ).

**Conclusion**

Most female-to-male transsexual persons report on a marked increase of sexual desire after testosterone treatment and SRS. No associations between levels of testosterone and measures of sexual desire were found. Sexual desire is significantly higher compared to male-to-female transsexual persons.

**P165****Role of IGF1 system in human longevity. A study in a wide population of centenarians and centenarians' offspring**

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Increasing evidence has recently accumulated suggesting that IGF1 system is involved in the regulation of longevity. While in animal models alterations of IGF1 signalling increase life expectancy, in humans there are contradictory data. Centenarians are an extraordinary model to study human longevity, however, they present a number of drawbacks: rarity, frailty due to extreme age and lack of a control group of the same age. The availability of an age-matched control group is crucial in studies evaluating IGF system, considering that age impacts on IGF1 production. Centenarians' offspring, for whom it is possible to obtain an appropriate control group (subjects of same age born from not long-lived parents), represent a new model of longevity, showing a lower morbidity and mortality. The aim of the present study was to investigate the role of IGF1 in the modulation of longevity. We recruited: 106 centenarians, 192 centenarians' offspring and 80 offspring of both non long-lived parents (controls) living in Northern Italy. No significant difference in age was observed between centenarians' offspring and controls. Serum total IGF1 levels and IGF1 bioactivity, using an in-house KIRA assay, were evaluated in all subjects. Mean serum total IGF1 values were significantly lower in centenarians compared to centenarians' offspring and controls (both  $P<0.0001$ ). IGF1 levels were lower in centenarians' offspring than controls ( $P=0.002$ ). Circulating IGF1 bioactivity were lower in centenarians and their offspring than in controls ( $P<0.001$  and  $P=0.002$ , respectively), while no significant difference was observed between centenarians and centenarians' offspring. Interestingly, these differences in total IGF1 and IGF1 bioactivity between centenarians' offspring and controls remained significant after correction for age, gender and BMI (both  $P=0.001$ ). In conclusion, these data strongly support a role of IGF1 system in the modulation of human aging and longevity.

**P166****Evaluation of insulin secretion and sensitivity and lipid profile in GH deficient children before and at the end of rhGH therapy**

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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 lipids after the end of GH therapy.

**Subjects and methods**

Aim of our study was to evaluate changes in insulin secretion, sensitivity and lipid profile in children with isolated GHD longitudinally followed during rhGH treatment. We measured lipid profile, glucose and insulin levels at fasting and after an oral glucose tolerance test (OGTT) in 24 GHD children at 4 times: i) before starting of GH therapy (BT); ii) during the last year of therapy (T1); iii) 6 months (T6) and iv) 12 months (T12) after stopped therapy. They were compared at T12 with 28 healthy puberty matched subjects.

**Results**

No subjects showed dysglycemia or hypertension. Basal glucose levels were lower at BT but similar among the last 3 times. Fasting insulin, insulin and glucose levels at each point after OGTT were progressively lower at T6 and T12 compared to T1 ( $P<0.02$ ). HOMA-IR index was higher, and Matsuda and QUICKI indexes were lower at T1 respect to T6 and T12 ( $P<0.02$ ) without returning to the BT prepupal levels. A compensatory insulin secretion was recorded at T1 as HOMA-B and insulinogenic index both of which progressively decreased at T6 and T12 respect to T1 ( $P<0.04$ ) returning to BT levels. The disposition index was unchanged from BT to T12. HDL-cholesterol decreased after stopped therapy ( $P<0.04$ ). All the parameters were similar to controls at T12.

**Conclusions**

Insulin sensitivity decreased during rhGH therapy without the onset of dysglycemia and was associated with a compensatory insulin secretion after OGTT and without changes of the disposition index. Insulin and glucose secretion progressively restored after stopping treatment being similar to controls in the next 12 months.

**P167****Immune status and functional activity of blood neutrophils in patients with active acromegaly**

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The chronic GH and IGF1 excess causes a specific changes in immune system due to presence of its receptors on T-, B-lymphocytes, granulocytes. The aim of this study was to evaluate the subpopulation composition and chemiluminescence's indices of neutrophils in patients with active acromegaly.

Thirty-three patients with active acromegaly (11 men and 22 women, mean age  $51.7 \pm 11.7$  years) and 53 healthy subjects sex- and age-matched with the patients were enrolled in this study. The analysis of lymphocytes subset pattern were performed by indirect immunofluorescence, using of the FITC-marked monoclonal antibodies to CD3, CD4, CD8, CD16, CD19, CD25 and CD95. The De Sole P. method and 'CL3606M' chemiluminescent analyzer for the initial and zimozan-induced chemiluminescence of blood granulocytes were used.

The patients with acromegaly had significantly increased levels of CD8<sup>+</sup> (median 47.0 vs 28.0%;  $P<0.001$ ), CD16<sup>+</sup> (39.0 vs 17.00%;  $P<0.001$ ), CD19<sup>+</sup> (31.0 vs 17.01%;  $P=0.030$ ), CD25<sup>+</sup> (37.0 vs 19.0%;  $P=0.001$ ) and CD95<sup>+</sup>-cells (20.05 vs 8.5%;  $P=0.004$ ), decreased levels of leukocytes (4.25 vs 5.62;  $P=0.001$ ), CD3<sup>+</sup>- lymphocytes (43.0 vs 65.0%;  $P<0.001$ ) and CD4<sup>+</sup>/CD8<sup>+</sup> index (median 1.04 vs 1.54;  $P=0.001$ ). The initial and zimozan-induced chemiluminescence of neutrophils revealed the significant extension of curve area ( $P<0.001$ ). Also, the increasing of maximum intensity index by zimozan-induced chemiluminescence ( $P=0.003$ ) was observed.

The whole T-lymphocytes quantity decreasing, the high level of cytotoxicity T-cells concentration, which expressed the receptors to interleukin-2 and FasL in patients with acromegaly were revealed. The results demonstrate that in

acromegaly developing the population and subpopulation imbalance of immune cells, with the increasing of activated lymphocytes and functional activity of phagocytic cells.

## P168

### Dexamethasone suppression test (BDST) and bilateral inferior petrosal sinus sampling (BIPSS) in diagnosis of ACTH: dependent hypercortisolism

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#### Background

BIPSS is the most reliable procedure for differentiation Cushing's disease (CD) from ectopic ACTH secretion (EAS).

#### Aim

To determine the comparability of 8-mg dexamethasone suppression test (BDST) and BIPSS for differential diagnosis ACTH-dependent hypercortisolism.

#### Materials and methods

We present 6 patients with confirmed ACTH-dependent hypercortisolism. All of them BDST (8 mg dexamethasone at 2300 h) and BIPSS (with a cut-off central/peripheral ACTH-ratio of 2.0 or more) have been made. Also MRI and CT scan were performed.

#### Results

In 3 of 6 cases plasma cortisol suppression was more than 50% after BDST and pituitary adenoma was seen in MRI. Only in 1 of this 3 cases of CD a center/periphery-ACTH gradient was more than 2. Interestingly that the gradient was on the opposite to the location of pituitary microadenoma side. It was probably because of left sinus (on the side of adenoma location) contraction during catheterization. In other 2 of this 3 cases of CD a center/periphery-ACTH gradient was <2: in 1 case it was sinus anomaly (the absence of the right petrosal sinus), in other – may be attributed to technical factors.

In 1 of 6 case plasma cortisol suppression was 53.6% in BDST and no adenoma on MRI. However, a center/periphery-ACTH gradient was 15, 2. So CD was diagnosed.

In 2 of 6 cases plasma cortisol suppression in BDST were 3.5 and 54% respectively. MRI and CT show no abnormalities. After BIPSS the center/periphery-ACTH gradient was 1.06 and 1.27 respectively. EAS was diagnosed.

#### Conclusion

It should take into account data from all possible exam for the differential diagnosis of different forms of ACTH-dependent hypercortisolism.

## P169

### The NAD- and NADP-dependent blood lymphocytes dehydrogenases activity in acromegaly

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GH and IGF1 regulate plastic and synthetic intracellular lymphocytes process. We aimed to investigate the NAD- and NADP-dependent dehydrogenases activity in blood lymphocytes in patients with active acromegaly.

Thirty-three patients (11 men and 22 women, mean age  $51.7 \pm 11.7$  years), with the 6–10 years active phase duration of acromegaly, and 53 age- and gender matched healthy controls were determined the activity of NAD(P)-dependent dehydrogenases in blood lymphocytes. The activity of glycerol-3-phosphate dehydrogenase (Gly3PhD), glucose-6-phosphate dehydrogenase (Glu6PhD), lactate dehydrogenase, NADH-glutamate dehydrogenase (NADH-GDH), NADPH-glutamate dehydrogenase (NADPH-GDH), NAD(P)-isocitrate dehydrogenases, NAD(H)-malate dehydrogenases and glutathione reductase (GR) were measured using of biochemiluminescence method. The concentrations of somatotropin (GH) and IGF1 were measured by ELISA.

Chronic excess of GH and IGF1 in acromegaly associated with the high levels activity of Glu6PhD ( $P < 0.001$ ) and MDH ( $P < 0.001$ ), also, reduce the activity of GR ( $P < 0.001$ ), NADH-GDH ( $P = 0.005$ ) and NADPH-GDH ( $P < 0.001$ ). The correlation analysis revealed the reverse interaction between GH and NADH-GDH activity ( $r = -0.52$ ,  $P = 0.014$ ) and between IGF1 and NADPH-GDH activity ( $r = -0.56$ ,  $P = 0.007$ ).

Glu6PhD is the key enzyme of phosphogluconate pathway, MDH characterize the intensity of substrate flux on concluding steps of Krebs cycle. The increasing activity of GR in patients with acromegaly is the sign of glutathione-dependent antioxidant system insufficiency. Thus, the lymphocytes metabolism in acromegaly is remarkable for high level of plastic and aerobic respiration process, but the decreasing activity of glutathione-dependent antioxidant system.

## P170

### Metabolic encephalopathy and quadriparesis induced by severe hyponatremia in the patient with Sy Sheehan

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Sy Sheehan or postpartal pituitary necrosis presents with symptoms and signs of hypopituitarisms. It is usually a consequence of complicate delivery followed by intensive uterine bleeding. Diagnosis can be delayed years after delivery, up to 20 years.

We present a case of 51-year-old female patient. She was admitted in the department of neurology in the Emergency center, Belgrade, with disturbance of consciousness. Few days before she felt leg weakness and numbness, become sleepness and develop disturbance of consciousness. There was no serious illness in the previous history. She born four children, the last delivery was 10 years ago and it was complicated with prolonged bleeding. After that there was no lactation or restoring of menstrual cycles, body hair disappeared in the following years.

In the moment of administration patient was somnolent, dehydrated, body hair were absent, breasts atrophic, TA 100/80 mmHg. Neurologic status showed quadriparesis. EEG was performed showing diffuse encephalopathic changes with irritative focal locus on the left side. NMR of endocranium revealed empty sella with thickening of stalk. Laboratory analyses showed severe hyponatremia 116 mmol/l, normocytic anemia Hb 85, RBC 2.83, MCV 88. Other routine analyses were without changes.

We made a diagnosis of hypopituitarism and patient was transferred to Metabolic unit of EC. Hormone analyses confirmed diagnosis: FT<sub>4</sub> <5.1, TSH 0.22, cortisol/0800 h/344.2, ACTH 17.2, IGF1 30.2, FSH 11.6, LH 1.8, LTH/pulled blood/308, estradiol 36.9, insulin 30.2.

Therapy was started with administration of isotonic saline solutions and substitution with hydrocortison, several days later L-thyroxine was added. After few days patient felt much better, electrolyte disorders was corrected and she was able to walk 3 weeks later. After 2 months on the control, hormonal analyses were corrected.

We present a patient with Sy Sheehan and severe hyponatremia that leads to metabolic encephalopathy and quadriparesis. Substitution treatment with Hydrocortison and L-thyroxine diminished all signs of illness.

## P171

### Effects of orexin-monoaminergic interactions on vasopressin secretion in rat neurohypophyseal cell cultures

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#### Introduction

The effects of dopamine (DA), serotonin (5-HT), histamine (HA) adrenaline (ADR), noradrenaline (NADR) and K<sup>+</sup> administration on vasopressin (VP) secretion were studied in 13–14 days cultures of rat neurohypophyseal (NH) cells, and it was examined whether orexin (ORX) can modify the VP release enhancement induced by these monoaminergic compounds.

#### Methods

An enzymatic dissociation technique was used to prepare the rat NH cell cultures. The VP contents of the supernatants were determined by RIA.



## Results

Following administration of ORX-A or ORX-B in increasing doses ( $10^{-10}$ – $10^{-4}$  M), significant changes were not observed in the VP levels (pg VP/mg protein; average  $\pm$  S.E.M.) of the supernatant media (control:  $50.14 \pm 0.9$ ; ORX-A:  $51.6 \pm 0.67$ ; ORX-B:  $52.30 \pm 0.86$ ). VP level substantially increased after NADR ( $222.98 \pm 2.45$ ), ADR ( $208.68 \pm 2.36$ ) or 5-HT ( $194.9 \pm 2.69$ ) treatment, while the enhancing effects of DA ( $166.74 \pm 2.33$ ), HA ( $143.56 \pm 13.75$ ) or  $K^+$  ( $123.77 \pm 2.51$ ) administration were more moderate. Preincubation with ORX-A or ORX-B reduced the NADR ( $197.06 \pm 3.35$ ;  $202.14 \pm 1.61$ ) or HA-induced ( $107.30 \pm 1.63$ ;  $110.12 \pm 2.03$ ) VP level increases. Following ADR, 5-HT or DA administration, the VP level enhancement was slightly decreased ( $195.32 \pm 2.22$ ;  $181.36 \pm 1.8$ ;  $158.22 \pm 0.89$  in ORX-A;  $194.62 \pm 1.61$ ;  $181.16 \pm 2.3$ ;  $160.94 \pm 0.53$  in ORX-B preincubation), though the differences proved to be significant ( $P < 0.05$ ), but the VP concentrations of the supernatant media remained above the control level. There was no significant difference in decreasing effect between ORX-A and ORX-B. ORX had no influence on the VP level increase induced by  $K^+$ , which causes non-specific hormone secretion. ORX-A or B did not induce any changes in VP release following monoaminergic treatment.

## Conclusions

The results indicate that the changes in VP secretion induced by the monoaminergic system can be directly influenced by the ORX system. The interactions between the monoaminergic and ORX systems regarding VP secretion occur at the level of the posterior pituitary.

## P172

### Age related distribution of the endothelial nitric oxide synthase gene G894T polymorphism: relationship with metabolic-endocrine and cognitive features

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## Background

Genetic variants of the endothelial nitric oxide synthase (eNOS) gene have been reported to be associated with vascular disease. We hypothesized that G894T polymorphism might trigger many of the endocrine-metabolic cognitive changes related to aging.

## Study design

One hundred and eighty subjects aged 55–80+ years with moderately cognitive impairment (MCI) (MMSE < 28) (lot 1), 140 age-matched subjects without cognitive impairment (MMSE > 28) (lot 2) and 150 healthy subjects under 55 years were studied. The biochemical and hormonal profiles and eNOS gene G894T polymorphism evaluated.

## Results

The frequencies of genotypes and alleles of G894T polymorphism according to age showed considerable differences among lots. The genotype and allele frequencies deviated from the Hardy–Weinberg equilibrium in subjects over age of 55 years ( $P < 0.001$  /lot 1 and  $P = 0.04$  /lot 2) compared with lot 3 under 55 years that was in HW equilibrium. The percentages of both G allele and GG genotype significantly decreased while T allele and TT genotype increased (OR = 2.3;  $P < 0.001$  and OR = 4.2;  $P < 0.001$ ) in lot 2 compared to lot 3. In the MCI group (lot 1) the tests for association showed risk allele G (OR = 2.4;  $P = 0.018$ ); the percentages of both G allele and GG genotype significantly increased. GG genotype was significantly associated with cortisol, PRL, T<sub>3</sub>, T<sub>4</sub>, FT<sub>4</sub> (OR = 2.87; 95% CI = 1.7–4.8;  $P < 0.001$ ) and more significantly with Glu, C-LDL, apoB, CRP and IGF1 (OR = 4.6; 95% CI = 2.5–8.3;  $P < 0.001$ ).

## Conclusion

The findings suggest that there is a substantial difference in the distribution of gene G894T polymorphism between population under and over 55 years and that it is involved in aging process related to cognitive performance and endocrine changes. These results show an interaction between the G894T polymorphism and its phenotypes in conferring a higher susceptibility to the endocrine changes involved in healthy aging.

## P173

### Glucose homeostasis in GH deficient adults not treated with GH: more than 10 year follow-up

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## Study objective

We investigated prevalence of impaired glucose homeostasis in a large cohort of adult patients with GH deficiency (GHD), not treated with GH after more than 10 years of follow-up.

## Design

Seventy-one adult GHD patients (30 males, 41 females; mean age  $48.3 \pm 2.0$  years, mean BMI  $28.8 \pm 0.7$  kg/m<sup>2</sup>, GH deficient for  $10.2 \pm 1.2$  years) were evaluated. Ninety percent of patients had multiple pituitary hormone deficiencies which were replaced. Fasting plasma glucose, HbA1c and oral glucose tolerance test (OGTT) were analyzed at baseline and after additional 2 years of follow-up. Peak glucose and insulin levels during OGTT, area under the curve (AUC) for glucose and insulin during OGTT, index of basal insulin resistance (HOMA-IR) and the whole body insulin sensitivity index (WBISI) were analyzed.

## Results

At baseline, 58 unreplaced (with GH) GHD patients exhibited no abnormalities in glucose metabolism, 10 patients (14%) had impaired glucose tolerance and 3 patients (4%) had diabetes mellitus (1 patient treated with insulin, 2 patients treated with oral agents). After additional 2 years of follow-up, there were an increase in body weight (from  $80.4 \pm 2.5$  to  $83.2 \pm 2.5$  kg,  $P = 0.006$ ) BMI (from  $28.8 \pm 0.7$  to  $30.3 \pm 0.84$  kg/m<sup>2</sup>,  $P = 0.004$ ), basal insulin level (from  $13.8 \pm 1.1$  to  $18.6 \pm 2.3$  mIU/L,  $P = 0.001$ ), peak insulin level during OGTT (from  $103.5 \pm 10.7$  to  $120.3 \pm 11.8$  mIU/L,  $P = 0.05$ ) and AUCinsulin (from  $6622 \pm 654$  to  $8558 \pm 879$  mIU/l per 120 min,  $P = 0.004$ ). HOMA-IR increased after 2 years (from  $2.8 \pm 0.2$  to  $3.7 \pm 0.4$ ,  $P = 0.003$ ), while WBISI decreased (from  $6.7 \pm 0.7$  to  $2.4 \pm 0.3$ ,  $P = 0.001$ ), indicating the significant increase in insulin resistance.

## Conclusion

Prevalence of impaired glucose tolerance after 10 years of follow-up of adult GHD patients without GH therapy was 14%, while 4% had overt diabetes mellitus. During additional 2 years of follow-up, insulin sensitivity decreased due to an increase in body weight and ageing.

## P174

### A raised GR $\alpha$ / $\beta$ expression ratio in monocytes of schizophrenic and bipolar patients

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## Purpose

Individuals with mood disorders and schizophrenia (SCZ) exhibit altered function of the HPA axis in response to stress. The glucocorticoid receptor (GR) plays an important role in negative feedback regulation of the HPA-axis. There are two protein isoforms, GR $\alpha$  and GR $\beta$ , which have activating and blocking activity respectively. In line with aberrant expression of GR mRNA in the brains of depressed, SCZ and bipolar (BD) patients (1), lymphocytes of depressed, SCZ and BD patients showed reduced response to dexamethasone (2, 3). Furthermore, we previously detected a pro-inflammatory gene expression signature in monocytes of SCZ and BD patients. We hypothesized that the expression of GR $\alpha$  and GR $\beta$  might be altered in monocytes of SCZ and BD patients resulting in steroid resistance in the monocytes of these patients coupled to the pro-inflammatory monocyte state.

## Research design and methods

GR $\alpha$  and GR $\beta$  expression was investigated in monocytes of 32/22 SCZ/BD patients and 34/22 age/gender-matched healthy controls (HC) using quantitative PCR (qPCR). In a next step the Spearman rank correlation was calculated to assess the correlation between GR $\alpha$ , GR $\beta$  and the other inflammatory genes. The GR $\beta$ / $\alpha$  ratio was calculated to assess a possible glucocorticoid resistance in the monocytes of these patients.

## Results

Up-regulated expression of GR $\beta$  was detected in 69/45% of the SCZ/BD patients, respectively. Down-regulated expression of GR $\alpha$  was detected in 53/41% of the SCZ/BD patients, respectively. Therefore, the GR $\beta$ / $\alpha$  ratio calculation revealed an increased ratio in SCZ > BD > HC (Table 1). Evaluation of qPCR data further revealed that increased expression of GR $\beta$  positively correlated with the expression of the previously described pro-inflammatory genes up-regulated in monocytes of SCZ and BD patients. Expression of GR $\alpha$  negatively correlated to

the expression of the pro-inflammatory monocyte genes in SCZ patients, but this correlation was lost in BD patients.

**Table 1** GR  $\beta/\alpha$  ratio

	Mean	s.d.	P value
SCZ patients	11.7	22.1	0.003
Healthy controls	3.6	4.4	
BD patients	1.6	0.9	0.032
Healthy controls	1.1	0.5	

#### Conclusions and perspectives

Our results indicate that GR $\beta$  expression is significantly up-regulated in pro-inflammatory monocytes of SCZ patients whereas GR $\alpha$  is down-regulated. The same results were found in BD patients, though to a lesser extent. The correlation analysis suggests that up-regulated GR $\beta$  expression leads to glucocorticoid insensitivity and is closely linked to the pro-inflammatory expression pattern in monocytes of all investigated groups. The down-regulated GR $\alpha$  expression is closely linked to loss of glucocorticoid sensitivity, but only in the SCZ and HC groups, this process seems to be uncoupled in BD patients.

## P175

### The role of food choice in the effects of a high-fat high-sugar diet on leptin sensitivity

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#### Background

Rats on a free-choice (fc) diet of saturated fat and liquid sugar in addition to chow (fcHFHS) overeat persistently, and show increased hypothalamic neuropeptide Y and decreased melanocortin signaling, which reflects a hunger state despite hyperphagia. This response is specific for a fcHFHS diet, because when given the choice between fat and chow (fcHF) or between liquid sugar and chow (fcHS) hyperphagia is not observed. Because leptin signaling is important for the regulation of feeding behavior, we determined whether rats on a fcHFHS diet become leptin resistant, and whether this depends on the free-choice component. Methods

Rats were subjected to a chow, fcHF, fcHS or fcHFHS diet. After either i.p. or i.c.v. injections of leptin or vehicle food intake was determined. Another group was subjected to chow, fcHFHS or a non-choice HFHS (ncHFHS) diet and the extent of leptin resistance was determined by i.p. injections of leptin or vehicle. The ncHFHS diet consisted of pellets containing the same ingredients and the same percentages for fat, sugar and chow as consumed by rats on a fcHFHS diet. Results

After i.p. injection of leptin, rats on a chow, fcHF or fcHS diet significantly reduced food intake, whereas rats on a fcHFHS diet showed leptin resistance. On the contrary, upon i.c.v. injection, the fcHFHS group did respond to leptin and showed increased sensitivity when food intake was measured over 24 h. In the second experiment, again we observed leptin resistance to i.p. injections in rats on a fcHFHS, while the rats on a ncHFHS diet remained sensitive to leptin and significantly reduced their food intake.

#### Conclusion

The fcHFHS diet results in leptin resistance peripherally but not centrally. Furthermore, the choice component is important for the peripheral leptin resistance that results from the fcHFHS diet.

## P176

### Hypothalamic neuropeptide Y controls the hepatic secretion of VLDL triglycerides in rats via the sympathetic nervous system

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During fasting neuropeptide Y (NPY) neurons in the mediobasal hypothalamus are activated to increase food intake and to conserve energy. Under conditions of

food deprivation, lipid metabolism plays an important role in providing fuel for muscle. We investigated if central NPY affects VLDL-TAG secretion directly via the autonomic nervous system. We measured VLDL-TAG secretion in Wistar rats by an intravenous bolus of tyloxapol to inhibit uptake of triglycerides by peripheral tissues. In the absence of chylomicrons from the gut, the increase in plasma triglycerides reflects VLDL-TAG secretion by the liver. First, we investigated the acute effect of i.c.v. infusion of NPY (1  $\mu\text{g}/\mu\text{l}$ ; bolus 5  $\mu\text{l}/5$  min, followed by 5  $\mu\text{l}/\text{h}$ ) on VLDL-TAG secretion after a selective sham or sympathetic liver denervation. Second, we investigated the effects of overnight fasting (19 h fast) in sham, parasympathetic, sympathetic, or total liver-denervated animals. Finally, we tested the effects of fasting and liver denervation in rats treated neonatally with monosodium glutamate (MSG), which is an established model for chronic NPY depletion from the arcuate nucleus. We report that hepatic sympathetic innervation mediates the stimulatory effect of i.c.v. NPY on VLDL-TAG secretion. During fasting, when hypothalamic NPY tone is physiologically high, an intact hepatic sympathetic innervation is necessary to maintain VLDL-TAG secretion. Finally, in MSG treated rats VLDL-TAG secretion could not be maintained during fasting while hepatic denervation did not yield any additional effect. Our findings show that the release of hypothalamic NPY during fasting stimulates triglyceride secretion by the liver via hepatic sympathetic input. By inference, an elevated hypothalamic NPY tone may increase sympathetic tone as well as plasma TAG in pathophysiological conditions such as the metabolic syndrome.

## P177

### Inverse association of dehydroepiandrosterone sulfate with stroke severity and poor functional outcome in a female stroke population

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#### Introduction

DHEAS is among the most abundant sex steroid products of the adrenal gland. DHEAS levels have been repeatedly associated with cardiovascular disease, but data concerning cerebrovascular disease, especially in women, is lacking. In this study, we aimed to investigate the role of DHEAS in a female population suffering an acute stroke.

#### Patients and methods

We studied 302 consecutive female patients presenting with an acute stroke hospitalized in two tertiary Hospitals of Athens, Greece, 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). Besides basic biochemical investigation and lipid profile testing, a hormonal panel, including DHEAS,  $\Delta 4$ -androstenedione, testosterone and sex-hormone binding globulin, was performed in all subjects 2–5 days after stroke.

#### Results

A significant inverse association was found between DHEAS levels and stroke severity on admission, as expressed by NIHSS ( $r = -0.153$ ,  $P 0.01$ ); DHEAS remained a significant determinant of stroke severity in the multivariate model ( $r = -0.137$ ,  $P 0.02$ ) independently of systolic blood pressure, HDL levels, atrial fibrillation and hemorrhagic stroke subtype.  $\Delta 4$ -androstenedione and testosterone levels were significantly associated with 1-month mortality in the univariate analysis ( $P 0.021$  and  $0.019$  respectively). DHEAS levels were inversely associated with poor outcome, i.e. combined severe handicap ( $mRS \geq 4$ ) and death, 1 month post-stroke ( $P 0.019$ ), although this did not remain significant in the multivariate model.

#### Conclusions

DHEAS levels are significantly associated with the severity of stroke on admission and short-term functional outcome among female stroke subjects. Further studies may clarify the role of DHEAS in cerebrovascular disease and the possible underlying mechanisms.

## P178

### Normosmic congenital hypogonadotropic hypogonadism due to *TAC3/TACR3* mutations: characterization of neuroendocrine phenotypes and novel mutations

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#### Introduction

*TAC3/TACR3* mutations have been reported in normosmic congenital hypogonadotropic hypogonadism (nCHH). In the absence of animal models, studies of human neuroendocrine phenotypes associated with neurokinin B and NK3R receptor dysfunction can help to decipher the pathophysiology of this signaling pathway. Our objective was to characterize novel *TAC3* mutations and to analyze neuroendocrine profiles in nCHH patients with *TAC3/TACR3* biallelic mutations.

#### Results

From a cohort of 352 CHH, we selected 173 nCHH patients and identified eight *TACR3* variants (one frameshift, four missense and three nonsense mutation) in five adults (four sporadic cases, one familial). Molecular analyses, modeling and functional studies demonstrated the pathogenic nature of four novel variants. Three patients with *TAC3/TACR3* biallelic mutations had an apulsatile LH profile and low-frequency  $\alpha$ -subunit pulses. Using the same assays, we found a statistically significant higher mean FSH/LH ratio in 11 patients with *TAC3/TACR3* biallelic mutations than in nCHH patients with biallelic mutations in *GPR54/KISS1R* ( $n=4$ ), *GNRH1* ( $n=2$ ) or *GNRHR* ( $n=11$ ), and nCHH patients with no identified mutations ( $n=32$ ) or mutations in *KAL1* ( $n=19$ ), *FGFR1* ( $n=17$ ) or *PROK2/PROKR2* ( $n=14$ ) ( $P<0.0001$ ). Pulsatile GnRH administration to three patients harboring *TAC3/TACR3* mutations increased  $\alpha$ -subunit pulsatile frequency and reduced the FSH/LH ratio.

#### Conclusion

The gonadotropin axis dysfunction associated with nCHH due to *TAC3/TACR3* mutations is related to a low GnRH pulsatile frequency leading to a low frequency of  $\alpha$ -subunit pulses and to an elevated FSH/LH ratio. This ratio might be useful for pre-screening nCHH patients for *TAC3/TACR3* mutations.

## P179

### X-linked adrenoleukodystrophy: an intersection between Endocrinology and Neurology

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#### Introduction

X-linked adrenoleukodystrophy (X-ALD) is an important cause of primary adrenocortical insufficiency (PAI) in men. It's characterized by impairment in peroxisomal degradation of very-long-chain-fatty-acids (VLCFA), leading to its accumulation on central nervous system, adrenal cortex and testes.

Adrenomyeloneuropathy, the most common phenotype of X-ALD, coexists with PAI in up to 70% cases.

#### Case report

A 28-year-old previously healthy male, with acute impairment of muscular strength, was referred to Neurology's Department. Neurologic examination revealed a spastic paraparesis, generalized hyperreflexia, distal hypoesthesia and ataxic gait.

Cranial MRI displayed contrast enhancement at right internal capsule, suggesting active demyelination.

His clinical condition grew worse along the following 10 years and he further developed spastic tetraparesis, urinary incontinence and behavioural changes. Meanwhile, his nephew was diagnosed as having X-ALD. The patient's plasmatic VLCFA levels were assessed, he was classified with the same diagnosis and then referred to Endocrinology's Department.

He was normotense and without hyperpigmentation. Plasmatic glucose, sodium and potassium levels were normal. The tetracosactrin-stimulation-test showed high basal level of ACTH (360 pg/ml; RR:9-52) and a subnormal cortisol response (basal-23.2; 60' after-22.7  $\mu$ g/dl). The total testosterone was 6.4 ng/ml (RR:2.7-11), renin 14  $\mu$ U/ml (RR:7-76) and aldosterone 21 pg/ml (RR:40-310). Later, at the age of 39, diabetes' diagnosis was settled and treated with insulin. He presented negative diabetes-specific-autoantibodies and a good C-peptide reserve (glucagon test: basal C-peptide-2.4; 6' after-6.8 ng/ml).

He has a 5-cm thyroid nodule (whose FNA result was 'benign'), in waiting for surgery.

#### Conclusion

X-ALD is a clinically heterogeneous disease.

The reported case was diagnosed due to advanced neurologic disease; a low adrenal reserve was also found.

In conclusion, adrenal reserve must be regularly assessed in X-ALD in order to early diagnose PAI and avoid an adrenal crisis triggered by an eventual stress. Likewise, an early diagnosis of X-ALD in PAI might prevent progression to neurologic disability.

## P180

### Targeting PKC in human pancreatic neuroendocrine tumor cells

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The currently curative therapy for pancreatic endocrine tumours (PET), accounting for <3% of pancreatic tumors, is complete surgical resection, which is achieved in the minority of cases. Most tumors are diagnosed in a late stage, especially in endocrine-inactive forms, indicating the need for further medical therapy. The serine-threonine protein kinase C (PKC) family plays central regulatory roles in several cellular processes, including cell proliferation. PKC signaling and PI3 kinase/Akt pathway, targets of several PKC inhibitors, have been indicated as the most dysregulated in PET, suggesting a possible application for PKC inhibitors in medical therapy of unresectable disease. We aimed to investigate the antiproliferative and antisecretory effects of a novel PKC inhibitor (PI) in the human pancreatic endocrine cancer cell line, BON1, and in a group of six human PET in primary culture. Cell viability was investigated by ATPlite kit, apoptosis by caspase 3/7 assay, GSK3- $\beta$  phosphorylation and chromogranin A (CgA) secretion by ELISA, PKC $\beta$ II localization by immunofluorescence, CgA expression by western blot. We found that PI suppresses cell proliferation by inducing apoptosis, and reduces phosphorylation of GSK3- $\beta$  (Ser9), a downstream target of PKC pathway and a pharmacodynamic marker for PIs. Moreover, treatment with the compound induces translocation of PKC $\beta$ II from the cytoplasm to the nucleus, and reduces CgA expression and secretion. These data support the hypothesis that the compound reduces cell viability by inducing apoptosis, with a mechanism likely involving GSK3 $\beta$  signaling. In addition, PI inhibits BON1 secretory activity, suggesting that PIs might represent a possible medical treatment of human pancreatic neuroendocrine tumors.

## P181

### GH enhances breast cancer chemoresistance by inhibiting JNK-mediated apoptosis

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GH and insulin-like growth factor I (IGF1) are known to promote breast carcinogenesis. Breast cancer (BC) incidence is not increased in female acromegalic patients, but mortality is greater as compared to general population. We previously demonstrated that GH/IGF1 excess influences BC response to therapy, possibly accounting for the increased mortality. We indeed showed that GH and IGF1 induce cell proliferation of a BC cell line, the MCF7 cells, providing protection towards the cytotoxic effects of doxorubicin (D). GH effects are direct and not mediated by IGF1, since they are apparent also in the presence of an IGF1 receptor blocking antibody and disappear in the presence of the GH receptor antagonist Pegvisomant (Peg). The aim of the present study was to evaluate the possible mechanisms implicated in the protective action of GH towards the cytotoxic effects of D in the BC cell line, MCF7. We investigated the combined effects of GH and D on MCF7 cell cycle, apoptosis, glutathione-S transferase (GST) activity and JNK transcriptional activity. We found that GH does not revert D-induced accumulation of MCF7 cells in G2/M phase of the cell cycle. However, GH significantly reduced both basal and D-induced MCF7 cell apoptosis, an effect nearly completely blocked by Peg. We therefore explored the involvement of GST, which activation protects DNA against damage by anticancer drugs. However, GH treatment significantly reduced GST activity in

MCF7 cells, as well as Peg, possibly indicating a greater susceptibility of Peg-treated cells to DNA damage. In addition, GH reduced basal and D-stimulated JNK transcriptional activity. These data altogether indicate that GH directly induces resistance to chemotherapeutic drugs by protecting the cells from apoptosis by reducing JNK transcriptional activity. Our data further support the hypothesis that GH excess might hamper BC treatment, possibly resulting in an increased mortality.

## P182

### Glycoprotein pituitary hormones in the cerebrospinal fluid of patients with pituitary adenomas

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#### Introduction

The concentrations of pituitary hormones in the cerebrospinal fluid (CSF) are much lower than their serum counterpart.

#### Aim

To study the CSF access for pituitary glycoprotein hormones (GPH) in patients with pituitary adenomas (PA).

#### Patients and methods

In 221 cases submitted to transphenoidal surgery for PA (85 acromegaly – ACM, 92 nonfunctioning pituitary adenomas – NFA, 44 prolactinomas – PRM) the surgical specimen was immunostained for expression of FSH, LH and TSH using avidin-biotin technique. In these patients (221) and a control group without endocrine diseases (79), in serum and CSF simultaneously sampled, the FSH, LH and TSH were assayed by fluoroimmunoassay with europium.

#### Results

In all types of pituitary tumors the mean CSF levels of GPH, i.e. (FSH  $3.4 \pm 0.56$  IU/l, LH  $1.94 \pm 0.28$  IU/l and TSH  $1.78$  mU/l  $\pm 0.47$ ) were much higher than in the control group:  $0.56 \pm 0.08$ ,  $0.39 \pm 0.03$ ,  $0.01 \pm 0.03$  respectively ( $P < 0.05$ ). The serum levels do not follow this pattern.

The tumors with positive immunostaining for FSH (12.21%), LH (9.5%), TSH (4.5%) do not influence more the CSF levels, but the size of the tumor does (mean diameters  $30.4$  mm  $\pm 0.17$  NFA,  $28.1$  mm  $\pm 0.23$  PRM,  $19.9$  mm  $\pm 0.13$  ACM). The intrasellar tumors ( $n=41$ ), without contact to the CSF, showed greater CSF hormone levels than controls, but less than suprasellar expanded tumors (SSE  $n=123$ ). The SSE are larger. They showed contact with the CSF space but even in that group the GPH-immunopositive tumors were not associated with higher CSF levels than GPH-immunonegative ones. This suggests that glycoprotein hormones, that originate in pituitary cells, are not directly released into the CSF, but, probably, through local blood vessels.

#### Conclusion

In conclusion, in the presence of pituitary adenomas, pituitary glycoprotein hormones (from tumor or normal pituitary cells) pass easier in the CSF. Very probably the retrograde blood flow of the portal vessel system is influenced by the tumor size.

## P183

### Hypothalamo-pituitary-adrenal axis function in posttraumatic stress disorder

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#### Introduction

Posttraumatic stress disorder (PTSD) is anxious disorder that occurs following exposure to trauma. In the last two decades, numerous studies showed that dysregulation of hypothalamo-pituitary-adrenal axis (HPAA) is a core feature of the PTSD. Still, results are heterogeneous and often conflicted with one another. Therefore, we conducted strictly controlled study in order to characterize relationship between ACTH and cortisol secretion in patients with chronic war-related PTSD.

#### Methods

We examined HPAA basal function (ACTH and cortisol concentrations measured hourly from 2200 h to 0900 h) and sensitivity (overnight low-dose (0.5 mg) dexamethason suppression test-DST<sub>0.5 mg</sub>) in men with chronic war-related PTSD (group PTSD,  $N=59$ ) in comparison to healthy non-traumatized men (group Healthy,  $N=53$ ). Participants with depressive disorder and other major psychiatric and somatic comorbidities were excluded from the study. There were no group differences in age (PTSP: 44.1 (9.3) years, Healthy: 42.2 (9.7) years;  $P=0.28$ ) and body mass index (PTSP: 26.9 (3.6) kg/m<sup>2</sup>; Healthy: 27.0 (3.6) kg/m<sup>2</sup>,  $P=0.92$ ).

#### Results

During the time period from 2200 h to 0900 h, PTSD had lower ACTH ( $P=0.043$ ) and the same cortisol concentration ( $P=0.37$ ) which resulted in lower ACTH/cortisol ratio ( $P=0.029$ ) in comparison to Healthy group. There were no group differences either in ACTH secretion amplitude ( $P=0.63$ ) or in cortisol secretion amplitude ( $P=0.24$ ). In comparison to Healthy, PTSD showed enhanced cortisol suppression during DST<sub>0.5 mg</sub> ( $P=0.030$ ).

#### Conclusion

Results of our study implicate that patients with chronic war-related PTSD without somatic comorbidities and depression, in comparison to healthy non-traumatized subjects of the same gender, age and body mass index, have concomitantly enhanced HPA axis sensitivity to cortisol at the level of anterior pituitary and enhanced adrenal reactivity to ACTH. It is yet to be determined which of these disturbances is primary and which is compensatory.

## P184

### Vasopressin and vasopressin receptor expression in the rodent retina

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Vasopressin acts through G protein-coupled receptors on the cardiovascular and renal system to induce vasoconstriction and antidiuresis. Vasopressin also has important functions within the CNS influencing cognition, olfaction and a range of complex social interactions and behaviours. In addition, vasopressin has been reported in the mammalian retina and here we investigated the location and expression of vasopressin and vasopressin receptors (V1a, V1b, and V2) in the rat retina.

Transgenic Wistar rats expressing vasopressin-enhanced green fluorescent protein (vasopressin-eGFP) and wild-type controls (1–6 months of age;  $n=7$ ;  $n=5$ ) provided the retinal tissue. This was either fixed and processed for immunostaining to reveal the location of vasopressin containing neurons, or was snap frozen on dry ice and processed for mRNA extraction and cDNA production to investigate vasopressin, V1a, V1b and V2 receptor expression. Retinal sections (200–350  $\mu$ m) were used for live cell imaging of eGFP positive cells and to enable the extraction of mRNA from single cells for subsequent RT-PCR.

Live and fixed retinas imaged using epifluorescent and confocal microscopy ( $n=4$ ;  $n=5$ ) showed the cell bodies of vasopressin-eGFP neurons to be located primarily within the inner nuclear layer of the retina with projections to the output ganglion cell layer. A small number of eGFP positive cells were also located in the ganglion cell layer. The specific distribution and structure of the neurons are consistent with the cells being amacrine in nature. Whole retinas were found to express significant amounts of vasopressin, V1a, and V1b receptor. Single cell PCR revealed that eGFP labelled cells expressed message for vasopressin and the V1a receptor.

In conclusion, the rat retina contains vasopressin positive neurons and message for V1a and V1b receptors. Further investigation will determine whether this is consistent with a physiological role for retinal vasopressin.

## P185

### Serum leptin levels in patients with Alzheimer's disease

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#### Introduction

Leptin receptors have been identified in many peripheral tissues as well as the CNS including the hippocampus, which is particularly vulnerable in Alzheimer's

disease (AD). Animal data shows that leptin may be implicated in the pathophysiology of AD. The aim of this study was to examine if there is any differences in serum leptin levels between patients with AD 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 ICD-10 system.

Blood samples were frozen at  $-80^{\circ}\text{C}$  until analysis. Leptin levels were measured using a human leptin ELISA kit.

Differences in leptin 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 leptin 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  $80.53 \pm 6.03$ , mean body weight  $71.49 \pm 8.33$ ) and from the control group 78 were women and 17 men (mean age  $79.27 \pm 6.86$ , mean body weight  $70.23 \pm 6.73$ ). Linear regression revealed that sex was associated with serum levels of leptin ( $P < 0.001$ ). The levels of leptin in men ( $10.48 \pm 8.28$ ) were significantly lower than in women ( $25.07 \pm 22.27$ ,  $P < 0.0001$ , Mann-Whitney *U*).

Serum leptin levels were, also, significantly lower in patients with AD compared to normal controls ( $17.89 \pm 23.59$  in AD patients versus  $26.82 \pm 17.77$  in normal controls,  $P < 0.0001$ , Mann-Whitney *U*).

#### Conclusion

Our study, in accordance with the findings of studies in animal models, provides evidence that leptin may be implicated in the pathophysiology of AD.

## P186

### Estrogen signaling in the hypothalamus regulates glucose metabolism in female rats

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#### Background

Previous studies reported that direct injection of estrogen ( $E_2$ ) into different hypothalamic nuclei affects food intake, body weight and locomotor activity. We hypothesized that  $E_2$  signaling in the hypothalamus regulates glucose metabolism independently of circulating estrogen levels.

#### Methods

We investigated the effects of hypothalamic estrogen administration on basal glucose production as well as on hepatic and peripheral insulin sensitivity. We selectively modulated estrogen concentrations in the systemic circulation and in the brain using intrahypothalamic reverse microdialysis, stable isotope dilution and selective hepatic sympathetic or parasympathetic denervation in female rats after ovariectomy (OVX).

#### Results

OVX caused a 17% decrease in plasma glucose as compared to intact animals.  $E_2$  administration either in the hypothalamic paraventricular (PVN) or ventromedial (VMH) nucleus restored plasma glucose to levels seen in intact animals ( $P < 0.01$ ), without affecting plasma  $E_2$ . Administration of propyl-pyrazole-trisphenol (PPT), a selective agonist of estrogen receptor (ER)  $\alpha$ , in the PVN or VMH increased plasma glucose concentrations by 10 and 20% respectively, as compared to the vehicle group. Administration of diarylpropionitrile (DPN), a selective ER $\beta$  agonist, in the PVN increased plasma glucose concentrations by 25%, while administration in the VMH did not affect plasma glucose.  $E_2$  administration in the VMH, but not in the PVN, resulted in hepatic insulin resistance.  $E_2$  administration in both the PVN and VMH resulted in peripheral insulin resistance. Sympathetic hepatic denervation blunted the effect of  $E_2$  in the VMH on hepatic insulin sensitivity.

#### Conclusion

Our findings demonstrate that the modulatory role of estrogen in glucose metabolism is partly mediated via the hypothalamus and sympathetic signaling to the liver. Within the hypothalamus the PVN and VMH have differential roles in  $E_2$ 's effects on glucose metabolism.

## P187

### The role of the Diphereline (Triptoreline) test in the etiological diagnosis of gonadal dysfunction

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#### Objective

To assess the usefulness of the GnRH analogue Diphereline (Triptoreline) in the evaluation of central gonadal dysfunction in children and adults.

#### Methods

A 18-month retrospective study of dynamic testing of the hypothalamic pituitary gonadal axis in 36 patients, 10 prepubescent children ( $< 13$  years for girls and 14 years for boys) and 26 postpubescent subjects. The children were investigated for disorders of sexual development while the adults presented with clinical hypogonadism. In order to assess the response of the gonadal axis to the GnRH analogue Diphereline, 100  $\mu\text{g}$  were subcutaneously injected. FSH, LH were sampled basally and after 4 h, while testosterone/ estradiol were sampled after 24 h.

#### Results

Of the 26 postpubescent patients, 3 men presenting pituitary adenomas were shown to have hypergonadotropic hypogonadism, 15 patients had hypogonadotropic hypogonadism of probable pituitary origin and 8 had hypothalamic disorders. In the pituitary group the results showed a diminished response to the GnRH test in FSH, LH and the periphery, significant only for FSH ( $1.31 \pm 1.46$  to  $2.72 \pm 3.04$  IU/l,  $P = 0.02$ ). In the group with hypothalamic hypogonadism there was a significant FSH increase from an average value of  $2.07 \pm 1.10$  to  $12.62 \pm 10.75$  IU/l ( $P = 0.02$ ), LH from  $0.97 \pm 0.93$  to  $17.68 \pm 16.62$  IU/l ( $P = 0.02$ ), while the peripheral answer was an increase in testosterone from  $2.24 \pm 1.84$  to  $3.10 \pm 1.61$  ng/ml and an increase in estradiol from  $14.68 \pm 3.45$  to  $19.36 \pm 13.51$  pg/ml ( $P = \text{NS}$ ). The response to GnRH was significantly higher for the hypothalamic than the pituitary group for both LH ( $P = 0.03$ ) and FSH ( $P = 0.04$ ), consistent with the original etiological hypothesis and pathophysiological mechanism. All three patients with hypergonadotropic hypogonadism had high basal LH or FSH levels, with further increase after stimulation, without reaching statistical significance. In the prepubescent group, aged 4–13 years, the subgroup aged  $< 10$  had an increase in LH and FSH only, while the older children had both central and peripheral responses, consistent with gonadal maturation. A similar response in a 7-year-old girl helped establish the diagnosis of precocious puberty. There was no response in a 13-year-old boy with Prader Willi syndrome.

#### Conclusion

Although not diagnostic in itself, GnRH testing can help shed light on the aetiology of hypogonadotropic hypogonadism syndromes in complex clinical settings. Diphereline can be used as a surrogate test.

## P188

### Glucocorticoid receptor gene polymorphisms are associated with disease course during pregnancy and the post-partum period in rheumatoid arthritis

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#### Background

The mechanism underlying the spontaneous improvement of rheumatoid arthritis (RA) during pregnancy and the subsequent *post-partum* flare is incompletely understood. The glucocorticoid receptor (GR) polymorphisms Bcl1 and 9 $\beta$  are associated with an increased and decreased glucocorticoid sensitivity respectively. We examined the relation between carriership of these functional GR polymorphisms and disease course during pregnancy and *post-partum* in RA.

#### Methods

One hundred and forty participants of the PARA study (pregnancy-induced amelioration of rheumatoid arthritis), a prospective study investigating the natural improvement during pregnancy and the postpartum flare in women with RA, were studied. During pregnancy, patients were evaluated each trimester and *post-partum* at 4–6, 12 and 26 weeks using a standardized 28-joint count for swelling and pain. Disease activity was scored using the DAS28-CRP-3. All patients were genotyped for GR polymorphisms Bcl1 and 9 $\beta$ . Data were analyzed using a mixed linear model, comparing carriers of the minor allele with non-carriers of

each polymorphism with respect to improvement during pregnancy and *post-partum* flare. The area under the curve (AUC) was calculated for both time periods, reflecting total disease activity.

#### Results

During pregnancy, carriers of the Bcl1 polymorphism had a lower disease activity compared to carriers of the 9 $\beta$  minor allele. These differences were most pronounced in patients treated with glucocorticoids. Non-carriers had a disease course between those of Bcl1 and 9 $\beta$  carriers. *Post-partum*, disease activity increased in carriers of the 9 $\beta$  polymorphism, whereas carriers of the Bcl1 minor allele had less RA reactivation. The AUC of disease activity in the *post-partum* period was significantly lower for Bcl1 carriers when compared with 9 $\beta$  carriers.

#### Conclusions

Functional GR polymorphisms may influence RA disease course during and directly after pregnancy. Carriers of the glucocorticoid-sensitive GR polymorphism BC11 may benefit most from treatment with exogenous glucocorticoids.

## P189

### Cabergoline influence on antioxidative protection enzymes activity in complex therapy in patients with non-functioning pituitary adenoma (NFPA)

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#### Aim

To evaluate influence of cabergoline on LAO and BAS processes in treatment of patients with NFPA.

#### Materials and methods

We included 64 patients with NFPA in the study. Mean age was  $43.6 \pm 3.84$  years (range 30–55 years). Of 64 patients 26 (40.6%) were men and 38 (59.4%) were women. Duration of the disease from diagnosis based on MRI constituted 1–15 years. Severity criteria used in the evaluation were LH, FSH and prolactin (PRL) growth as well as progression of neurological and vision problems.

#### Results and discussion

Before treatment FSH, LH and PRL levels in patients with NFPA were 18.3, 20.9, and 50.4% ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ ) higher than in controls (10 healthy men and 10 healthy women). The same time there was noticed different concentration of CL and MDA which defined depending on the duration of the disease and tumor size. We revealed different variants of BAS enzymes activity such as SOD, CT, GP and GR. In patients with NFPA with the duration of the disease from 8 to 15 years LAO high intensity associated with inhibition of BAS enzymes. 0.25 cabergoline use during 3 months associated with inhibition of FSH, LH, 15.8% ( $P < 0.05$ ) decrease in PRL levels.

#### Conclusion

Cabergoline increases treatment effect in patients with NFPA by decreasing pituitary hormones levels such as FSH, LH and PRL. However, it practically does not have influence on BAS enzymes activity such as SOD, CT, GP and GR and this approves the add of antioxidative therapy to main treatment.

## P190

### The role of centrally applied ghrelin on hypothalamic AMPK activity and pituitary–adrenal responses in differently fed rats

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The pandemic increase of obesity, malnutrition and associated comorbidities encouraged intensive studies on energy balance control. Ghrelin is a brain-gut orexigenic hormone that is involved in both the short-term and long-term regulation of energy homeostasis. The aim of this research was to investigate the role of centrally applied ghrelin on the pituitary morphofunctional features and

corticosterone secretion in normal-fed, food-restricted and obese 8 weeks old Wistar male rats, as well as to examine changes in their hypothalamic AMP-activated protein kinase (AMPK) activity. After 4 weeks of normal feeding (NF group), progressive food restriction (FR group) and high-fat diet (HF group), rats received 5 daily injections of ghrelin and/or saline i.c.v. Blood samples, hypothalami and pituitaries were excised for further analyses 1 h after the last i.c.v. injection. Results showed that i.c.v. ghrelin had orexigenic effect in all examined groups, and it increased pituitary corticotroph cell volumes and their secretion. Corticosterone blood levels were elevated after central ghrelin treatment in NF and FR groups, while remained unchanged in HF group. Hypothalamic AMPK activity was increased in FR group and decreased in HF group when compared to NF group, and i.c.v. ghrelin treatment increased AMPK phosphorylation in NF and HF groups, while had no effect in FR group. In conclusion, centrally applied ghrelin had significant effect in energy balance regulation, mostly under preserved energy homeostasis and increased hypothalamic AMPK activity in normal-fed and obese rats.

## Pituitary

### P191

#### The treatment with dopamine agonists are not associated with increased prevalence of cardiac-valve regurgitation in patients with prolactinomas and acromegalia

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#### Background

Dopamine agonists are first-line agents for the treatment of prolactinomas and sometimes are used in patients with acromegalia. There is evidence that these drugs, especially cabergoline, are associated with an increased risk of new cardiac valve regurgitation in patients treated for Parkinson's disease.

#### Objective

Our objective was to evaluate the prevalence of cardiac valve regurgitation in patients with prolactinomas and acromegalia treated with dopamine agonists.

#### Design and settings

An observational, case–control study was conducted at a University Hospital.

#### Patients

81 treated patients, 37 with microprolactinomas, 31 with macroprolactinomas and 13 with acromegalia; 61 were treated with cabergoline, 16 with bromocriptine and 4 with quinagolide. We used an age-matched control group of fourteen patients diagnosed of prolactinomas before starting treatment with dopamine agonists.

#### Results

Patients receiving a cumulative dose above the media were compared with those receiving a cumulative dose below it. The prevalence of cardiac-valve regurgitation of aortic (19.5 and 15%,  $P = 0.77$ ); mitral (19.5 and 22.5%,  $P = 0.79$ ); and tricuspid (9.8 and 12.5%,  $P = 0.73$ ); valves was similar. When treated patients were compared with control patients, the prevalence of cardiac-valve regurgitation of aortic (17 and 0%,  $P = 0.12$ ); mitral (21 and 14.3%,  $P = 0.72$ ); and tricuspid (11.1 and 14.3%,  $P = 0.66$ ); valves was also similar. There were no cases of pulmonic regurgitation in all patients.

#### Conclusion

The prevalence of cardiac-valve regurgitation was similar in patients with higher and lower cumulative doses of dopamine agonists. The result was the same in treated patients when compared with control patients. This study shows results which coincide with some publications. Nevertheless, it also found data that differ from other studies.

## P192

### Adipocytes as a source of increased circulating levels of NAMPT/visfatin in active acromegaly

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#### Background

Nicotinamide phosphoribosyltransferase (NAMPT)/PBEF/visfatin is a widely expressed cytokine with various effects on glucose and lipid metabolism, cell

survival, and inflammation. Despite a favorable body composition profile, acromegalic patients present insulin resistance, increased cardiovascular risk, and higher incidence of secondary tumors.

#### Aim

Based on its relation to glucose and lipid metabolism and inflammation we hypothesized that NAMPT could be related to the metabolic disturbances in patients with acromegaly.

#### Methods

Body composition, glucose metabolism (glucose, insulin, HOMA-IR, HOMA-B), leptin and NAMPT levels were measured in 47 patients with active acromegaly (26 women, 21 men) and 25 matched control subjects. *In vitro* effects of GH/IGF1 on NAMPT expression in peripheral blood mononuclear cells (PBMC), hepatocytes, osteoblasts, human subcutaneous (SCA) and visceral (VA) adipocytes were studied.

#### Results

NAMPT was markedly increased in acromegaly in both sexes ( $P < 0.01$ ). NAMPT levels correlated, before and following adjustment for age, gender and leptin, negatively with total body fat mass (% fat:  $r = -0.35$ ,  $P = 0.03$ ) and arms fat mass (% fat:  $r = -0.37$ ,  $P = 0.02$ ) and positively with total lean mass (% lean:  $r = 0.35$ ,  $P = 0.03$ ) and arms lean mass (% lean:  $r = 0.42$ ,  $P = 0.008$ ). No correlation between NAMPT and glucose metabolic parameters was demonstrated. *In vitro* studies revealed that GH increases NAMPT expression during differentiation of subcutaneous (SCA) and visceral (VA) preadipocytes and in mature SCA, but not in mature VA, PBMC, osteoblasts or hepatocytes.

#### Conclusions

NAMPT levels are increased in active acromegaly with no relation to glucose metabolism. Our *in vitro* findings may suggest that GH directly could induce NAMPT expression in SCA, and the positive correlation of NAMPT with lean mass suggests that skeletal muscles could be another source of NAMPT in active acromegaly.

## P194

### Immunopositive ACTH cells in juvenile female rats after treatment with estradiol

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It was shown that estrogens decreased the POMC gene expression level in female rats. The aim of the present study was to examine the morphometric parameters of immunohistochemically labeled ACTH cells in juvenile female rat pituitaries after estradiol dipropionate (EDP) treatment. The females in juvenile period (16th day) were divided into two groups, each of seven animals. The experimental group received five injections of EDP (i.p. 0.25 mg/kg b.w.) every second day from the 4th to 14th day after birth. The controls were treated by the same schedule with equivalent volume of sterile olive oil. All animals were sacrificed 24 h after the last treatment. Immunopositive ACTH-producing cells were studied using the peroxidase-anti-peroxidase immunohistochemical procedure. The cell volume, nuclear volume and volume density of ACTH cells were determined by the stereological method. The significance of differences between mean values was estimated by Student's *t*-test. In juvenile females treated with EDP absolute and relative pituitary weights were significantly increased ( $P < 0.05$ ) by 446.7 and 447.4% respectively, in comparison with the controls. The ACTH cell and nuclei volume as well as their volume density were significantly decreased by 6.4, 33.3 and 46.2% respectively, compared to the controls. In juvenile females immunohistochemically labeled ACTH cells were stellate shaped with the cytoplasmic processes among neighbouring cells, localized between the capillaries. The nuclei follow the shape of the cell body. Small, specific secretory granules were distributed mainly at the periphery of the cytoplasm. The shape and localization of ACTH immunoreactive cells in EDP-treated female rats were not significantly changed in comparison with controls. In conclusion, the application of EDP caused the significant decrease of morphometric parameters of ACTH cells in juvenile female rats.

## P193

### Pituitary mycosis complicating a Cushing's macroadenoma

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#### Introduction

A 59-year-old gentleman with longstanding poorly controlled type 2 diabetes mellitus, obesity, hypertension, obstructive sleep apnoea, depression and type 2 respiratory failure was seen in diabetes review clinic and noted to have truncal obesity, moon facies and wasting of the proximal muscles.

#### Investigations

Urinary free cortisol was 782 nmol/24 h (NR < 200). Midnight cortisol was 595 and 532 nmol/l on consecutive days. After low dose dexamethasone suppression test cortisol was 580 nmol/l with ACTH of 130 nmol/l, and cortisol was 159 nmol/l after high dose test. MRI showed a pituitary macroadenoma occupying the pituitary fossa and most of the sphenoid sinus. Trans-sphenoidal hypophysectomy was carried out, and cortisol post-operatively was 60–142 nmol/l. Histology found strong ACTH staining (Ki67 index < 3%), and unexpectedly, fungal elements indicating significant infection. The patient was given 4 weeks voriconazole.

Post-operatively regular hydrocortisone was prescribed. The patient lost 20 kg in weight, antihypertensives and insulin requirements reduced by 50% and HbA1c improved from 11.4 to 7.9%. Follow-up scan showed a good surgical result.

#### Conclusions

There is increased incidence of aspergillosis and fungal infections in Cushing's syndrome. It is postulated this is due to several effects of glucocorticoids, including reduced lymphocytic activity and proliferation, impaired neutrophilic phagocytosis and impaired macrophage formation of nitric oxide. In addition, glucocorticoids have been shown to directly increase growth of fungal hyphae. Primary pituitary aspergilloma is rarely reported in the literature, all of which have been diagnosed post-operatively and related to sphenoidal sinusitis. Sphenoid sinus mass secondary to cryptococcosis and other species have also been reported. If identified, treatment of fungal granuloma should be carried out before removal of pituitary adenomas, to prevent intracranial spread.

## P195

### A case of an ectopic prolactinoma

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The most common cause of hyperprolactinemia is a prolactinoma in women after pregnancy is excluded. A 34-year-old female presented to our clinic with a 1.5 year history of secondary amenorrhea, galactorrhea and malaise. Prolactin (PRL) level was found to be 151.89 ng/ml. Pituitary imaging was reported to be normal. An examination of the patient revealed that PRL level was still high so the dose of cabergoline was further increased and subsequently, bromocriptine was added to the treatment. There was no reduction in PRL levels in controls. A scanning was performed to look for an ectopic focus. Abdominal computerized tomography revealed a heterogenous mass lesion originating from the uterus. Octreotide scintigraphy was performed and we observed an involvement consistent with the mass in the uterus. The patient underwent abdominal total hysterectomy. PRL dropped to 0.4 ng/ml the next day after the operation. The pathology result was a low-grade malignant mesenchymal tumor. Prolactin was found to be immunohistochemically negative. However, galactorrhea disappeared postoperative and PRL levels are still low. Elevated levels of PRL, resistant to bromocriptine and cabergoline, rapidly returned to normal after hysterectomy, which obviously indicates that hyperprolactinemia was associated with the myoma of the uterus. Other than pituitary PRL secretion, ectopic hyperprolactinemia may be caused by the presence of a pituitary tissue in the ectopic focus or, as in our case, secretion of dopamine antagonist or PRL stimulating factors from the tumor tissue, which is unable to detect.

**P196****Coping strategies in patients after treatment for functioning or non-functioning pituitary adenomas**

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**Context and objective**

Coping strategies may affect quality of life (QoL), which is decreased in patients after treatment for Cushing's disease, acromegaly, or non-functioning macroadenomas (NFMA). We aimed to explore coping strategies in these patients, since this has never been done before.

**Design**

A cross sectional study.

**Subjects**

We included patients treated for Cushing's disease ( $n=42$ ), for acromegaly ( $n=80$ ), and for NFMA ( $n=61$ ). These patients were compared with three reference populations: an a-select sample from the Dutch population ( $n=712$ ), patients with chronic pain ( $n=59$ ), and patients receiving primary care psychology services ( $n=525$ ). Furthermore, the three patient groups were compared with each other. Coping strategies were assessed by the Utrecht Coping List. The protocol was approved by the Medical Ethics Committee.

**Results**

Compared to the a-select sample, patients with pituitary adenomas reported less active coping ( $P<0.0001$ ), sought less social support ( $P<0.0001$ ), and reported more avoidant coping ( $P=0.008$ ). In contrast, patients treated for pituitary adenomas reported somewhat better coping strategies than patients with chronic pain and those with psychological disease. When patients with different pituitary adenomas were compared, patients treated for Cushing's disease sought more social support than patients treated for NFMA ( $P=0.035$ ).

**Conclusions**

Patients treated for pituitary adenomas display different and less effective coping strategies compared with healthy controls. A targeted intervention might help to stimulate patients to use a more active coping strategy and to seek social support, instead of an avoiding coping strategy. This might, in turn, improve their QoL.

**P197****Polycystic ovary syndrome and prolactinoma association**

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**Introduction**

Prolactinoma account for 40% of all pituitary tumors. It is the most common cause of hyperprolactinemia. Infertility, galactorrhea and hirsutism are the significant symptoms. Polycystic ovary syndrome affects the 5–10% of the women in reproductive age. The most common symptoms are irregular menses, hirsutism and infertility. However, prolactinoma and PCOS association is rare. A case of PCOS and prolactinoma is reported.

**Case**

A 21-year-old woman was admitted to internal medicine outpatient clinic for irregular menses and hirsutism for 2 years. Physical examination, revealed hirsutism (Ferriman-Gallwey score 12 points). Her laboratory results were as follows: prolactin level 70 ng/ml (range: 1.8–20.3 ng/ml), testosterone 21.9 pg/ml (normal <12 pg/ml). TSH, free  $T_3$ , free  $T_4$ , kidney and liver function tests, estrogens, DHEAS levels were normal. Pelvic ultrasonography was correlated with polycystic ovary syndrome. Pituitary MRI showed 5×6 mm microadenoma. Carbergolide 0.25 mg (Dostinex) once a week and drospiridone/etinilestradiol (Yasminelle) daily, were started.

After 6 months of carbergoline and drospiridone/etinilestradiol treatment their prolactin and testosterone levels were normal and no adenoma was detected in pituitary MRI.

**Discussion**

The patient was admitted to our clinic because of irregular menses, hirsutism, pelvic US result and pituitary MRI lead to PCOS and prolactinoma association diagnosis.

In 15–20% of the PCOS patients mild elevated prolactin levels can be functionally found without prolactinoma. Persistent high prolactin levels in PCOS of reproductive age women, prolactinoma must be taken into account after excluding causes such as drugs and hypothyroidism.

**Conclusion**

PCOS and prolactinoma association should be taken into account in PCOS cases with mild hyperprolactinemia.

**P198****Effects on metabolic parameters after 12-month treatment with a new once-a-week sustained release recombinant growth hormone (LB03002) in adult patients with GH deficiency (GHD)**

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**Introduction**

GH substitution in GH deficiency (GHD) must be subcutaneously administered daily. Recently, a new sustained release formulation of GH (LB03002) has been developed which has to be injected only once per week. As a sub-study to the double-blind, randomized, placebo-controlled, multicenter, phase-III-study we performed this prospective study to evaluate influences of the new GH formulation on metabolic parameters and the hormones leptin and ghrelin in adult patients with GHD.

**Methods**

Eleven patients with GHD (4f/7m, median age 58 years (29–69 years)) without any GH therapy for at least 6 months were included in the study. Eight patients were treated with LB03002 for 12 months, three patients started with placebo for 6 months and were then switched to LB03002 for another 6 months. A 3 h oral glucose tolerance test (OGTT) was performed at study entry and at study end. Additionally, IGF1, lipid parameters including cholesterol, LDL, HDL, and triglycerides, leptin, ghrelin, HbA1c and C-peptide were measured. Body composition was evaluated by dual-energy-X-ray-absorptiometry, waist/hip (WHR) and waist/height (WHtR) ratio by tape.

**Results**

Multiple of upper limit of normal (xULN) of IGF1 (0.23 (0.09–0.4) vs 0.71 (0.4–1.04),  $P<0.01$ ), WHR (0.98 (0.86–1.04) vs 1.01 (0.86–1.05),  $P<0.05$ ) and ghrelin levels (119.8 ng/l (67.7–266.6) vs 137 ng/l (67–289.5),  $P<0.05$ ) were significantly higher and fat mass (34.7% (20.4–49.2) vs 32.4% (16.7–48.5),  $P<0.05$ ) and leptin levels (11.2 µg/l (3.3–55.7) vs (7.05 µg/l (2.4–54.3),  $P<0.05$ ) were significantly lower at study end. Glucose and insulin levels during OGTT as well as insulin resistance (HOMA-IR), insulin sensitivity (ISI),  $\beta$ -cell function (HOMA- $\beta$ ), C-peptide and HbA1c were not statistically significantly different before and after GH substitution, neither were BMI, WHtR, bone-mineral-density (BMD) and lipid parameters.

**Conclusion**

Substitution with LB03002 showed statistically significant reduction of fat mass, which leads to reduced leptin levels and increased ghrelin levels but does not seem to influence glucose and lipid metabolism.

**P199****Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency**

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**Objective**

Few studies have determined the effects of more than 5–10 years of GH replacement in adults on bone mass and density.

**Design/patients**

In this prospective, single-centre, open-label study, the effects of 15 years of GH replacement on bone mineral content (BMC) and bone mineral density (BMD) were determined in 126 hypopituitary adults (72 men) with adult onset GH deficiency (GHD). Mean age was 49.4 (range 22–74) years at study start. BMC and BMD were measured using dual X-ray absorptiometry (DXA).

**Results**

The mean initial GH dose of 0.63 (S.E.M. 0.03) mg/day was gradually lowered and after 15 years the mean dose was 0.41 (0.01) mg/day. The mean serum insulin-like growth factor 1 (IGF1) SDS increased from -1.69 (0.11) at baseline to 0.63 (0.16) at study end ( $P<0.001$  versus baseline). The 15 years of GH replacement induced a sustained increase in total body BMC (+5%;  $P<0.001$ ) and BMD (+2%;  $P<0.001$ ). Lumbar (L2–L4) spine BMC was increased by 9% ( $P<0.001$ ) and BMD by 5% ( $P<0.001$ ). In femur neck a peak increase in BMC and BMD of 7 and 3% respectively was observed after 7 years (both  $P<0.001$ ). After 15 years, femur neck BMC was still 5% above the baseline value ( $P<0.05$ ), whereas femur neck BMD had returned to the baseline level. In most variables reflecting bone mass and density, men had a more marked response to GH replacement than women.

**Conclusions**

Fifteen-year GH replacement in GHD adults induced a sustained increase in total body and lumbar (L2–L4) spine BMC and BMD. In the femur neck, BMC and BMD increased progressively up to 7 years and then slightly, but not fully, decreased towards baseline values.



## P200

### Effects of 3-year GH replacement therapy on bone mineral density in younger and elderly adults with adult onset GH deficiency

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#### Context

The effect of GH replacement in elderly GH deficient (GHD) adults on bone mineral density (BMD) is not known.

#### Objective

In this prospective, single-centre, open-label study, the effects of 3-year GH replacement were determined in 45 GHD adults above 65 years of age and in 45 younger GHD patients with a mean age of 39.5 (s.e.m. 1.1) years. All patients had adult onset disease and both groups were comparable in terms of number of anterior pituitary hormonal deficiencies, gender, body mass index (BMI), and waist:hip ratio. BMD was measured using dual energy X-ray absorptiometry (DXA).

#### Results

The mean maintenance dose of GH was 0.24 (0.02) mg/day in the elderly patients and 0.33 (0.02) mg/day in the younger GHD patients. At baseline, absolute values of total body and lumbar (L2–L4) spine BMD were similar in both study groups whereas femur neck BMD was lower in the elderly as compared to the younger patients (0.91 (0.02) vs 1.02 (0.02) g/cm<sup>2</sup>;  $P < 0.01$ ). However, in the elderly GHD patients, z-scores at baseline were approximately zero. After 3 years of GH replacement, total body BMD was unchanged compared to baseline in both study groups. The 3-year GH replacement significantly increased lumbar (L2–L4) spine BMD and femur neck BMD by 4 and 2%, respectively, in the elderly patients and by 5 and 3%, respectively, in the younger patients. There were no statistical differences between groups in the treatment responses. At study end, femur neck BMD was still lower in the elderly patients.

#### Conclusions

This study shows that GH replacement increases lumbar (L2–L4) spine and femur neck BMD in elderly adults with adult onset GHD. This supports that GH replacement is useful in elderly GHD patients.

## P201

### Evaluation of aortic stiffness in the acromegaly patients with remission

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#### Objective

Cardiovascular diseases (CVD) are the most important cause of mortality in acromegaly patients. Noninvasive assessment of aortic stiffness which can be used as a predictor of atherosclerosis, may be of benefit in acromegaly patients for early diagnosis of CVD. We evaluated the aortic elasticity parameters of acromegalic patients in remission and compared with healthy controls.

#### Methods and results

Study subjects consisted of 20 patients with acromegaly in remission (12 females, 8 males, mean age 43.7 ± 11.5 years) and 20 age-matched controls (12 females, 8 males, mean age 38.7 ± 6.4 years). Parameters of aortic elasticity such as aortic strain, aortic dispensability and stiffness index beta were measured by echocardiography and calculated by using standard formulas. Although aortic strain rate was lower in acromegaly group than control subjects it was not statistically different (13.1 ± 7.0 vs 16.6 ± 4.1,  $P = 0.064$ ). However it was found that aortic stiffness beta index was significantly higher and dispensability was significantly lower in acromegaly patients compared to control subjects (respectively; 4.0 (1.2–12.3) vs 2.6 (1.4–5.8),  $P = 0.009$  and 0.5 (0.2–1.9) vs 0.8 (0.4–1.5),  $P = 0.006$ ). Systolic and diastolic blood pressure, diameters of aorta measured in systole and diastole, total and LDL cholesterol levels were found to be effective independent variants contributing to aortic stiffness.

#### Conclusion

The assessment of aortic stiffness by echocardiography in terms of parameters mentioned above, were found to be statistically different in patients with

acromegaly who were in remission. According to these results conducting to increased aortic stiffness, it can be suggested that cardiovascular risk increased in acromegaly patients and ongoing even in remission period.

## P202

### Baseline characteristics and effects of 10 years of GH replacement therapy in adults previously treated with pituitary irradiation therapy

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#### Context

Little is known of the importance of previous irradiation therapy for baseline characteristics and responsiveness to GH replacement in GH deficient (GHD) adults.

#### Objective/design/patients

In this prospective, single-centre, open-label study, the effects of 10-year GH replacement on metabolic variables and safety were determined in 18 GHD adults that had previously received pituitary irradiation therapy and 18 non-irradiated GHD patients. All patients had adult onset disease and complete deficiency of anterior pituitary hormones. Both groups were comparable in terms of gender, body mass index (BMI), and waist:hip ratio.

#### Results

At baseline, the GHD patients previously treated with pituitary irradiation therapy had higher serum levels of triglycerides (TG) and insulin and lower serum HDL-cholesterol level than non-irradiated patients (all  $P < 0.05$ ). The 10-year GH replacement improved serum lipid profile in both study groups, and in both groups variables reflecting glucose homeostasis responded similarly to GH replacement. At study end, serum HDL-cholesterol level was still lower in the GHD patients that had previously received pituitary irradiation therapy whereas serum TG and insulin levels were similar in both groups. During the 10-year GH replacement, four patients previously treated with pituitary irradiation therapy had cardiac events (two fatal) and one had a non-fatal cerebral infarction whereas only one non-fatal vascular event occurred in the non-irradiated patients.

#### Conclusions

GHD patients that have previously received pituitary irradiation therapy display a more severely impaired metabolic profile at baseline compared with non-irradiated patients. GH replacement can partly, but not fully, reverse these metabolic aberrations. This may be of importance for the more marked cardiovascular morbidity observed in this group.

## P203

### Effects of dopamine agonist on heart and lung in patients receiving dopamine agonist due to hyperprolactinemia

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#### Introduction

Dopamine agonists such as cabergoline and bromocriptine are used in the treatment of hyperprolactinemia to provide normoprolactinemia, to improve gonadal functions and to decrease the size of tumour if there is. Besides common side effects, fibrosis is another rare side effect of dopamine agonists. Long-term use of these drugs has been reported to cause fibrotic changes in heart valves. In addition, there are a few case reports about dopamine agonist-related pleural and pulmonary fibrosis.

#### Patients and methods

In this study, we investigated whether cabergoline and bromocriptine cause fibrosis in heart valves and lungs. The study included 50 (47 females and 3 males) patients treated with cabergoline and/or bromocriptine for hyperprolactinemia and 30 (26 females and 4 males) healthy subjects. In all subjects, transthoracic echocardiography, pulmonary function tests and high resolution computed tomography of the chest were performed.

#### Results

In the patient group, the mean cabergoline dose was 139 ± 104.53 mg while the mean bromocriptine dose was 3877.77 ± 3418.04 mg. The mean treatment duration was 35.26 ± 23.24 months. Compared to controls, mild tricuspid regurgitation and mitral regurgitation were significantly more frequent in patients taking cabergoline and bromocriptine. Pulmonary fibrosis developed in nine (18%) patients, but statistically there was no difference between the two groups.

**Conclusion**

Transthoracic echocardiography may be useful for early detection of cardiac valvular fibrosis in patients treated with dopamine agonists. Also, pulmonary fibrosis should be kept in mind and symptomatic patients should be evaluated using radiological methods.

**P204****Efficacy of transsphenoidal surgery in treatment of Cushing's disease: factors influencing postsurgical outcome**

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The study analyses direct postoperative and long-term results of surgical treatment of ACTH-secreting pituitary adenomas in 158 patients, operated between 2004 and 2009. There were 143 (91%) female and 15 (9%) male. The mean age of the patients was 36 years, s.d. 10.7. In all patients the diagnosis of Cushing's disease was confirmed by high dose dexamethasone suppression test. Preoperative hormonal evaluation: ACTH (0800 h), pg/ml (Me-78.5 (50.5 (25th percentile)-115.6 (75th percentile)), ACTH (11 pm), pg/ml (Me-69.9 (43.2-109)), cortisol (0800 h), nmol/l (Me-707.5 (553-884)), cortisol (2300 h), nmol/l (Me-584.5 (425-725)), urinary free cortisol (UFC), nmol/l (Me-1352 (936.7-2387.5)).

In the early postoperative period morning serum cortisol level <140 nmol/l was achieved in 69%; normalization of morning and midnight serum cortisol and UFC - in 86%. In 6 months postoperative normalization of UFC level was 79%, in 12 months - 81.3%, in 24 months - 71%. Normalization of UFC level and morning serum cortisol level during low-dose dexamethasone test <50 nmol/l was reached in 72.2, 50 and 55.6% after 6, 12 and 24 months after surgery respectively.

Morning serum cortisol level in the early postoperative period correlated with serum cortisol level in 6 ( $P=0.00014$ ) and 12 ( $P=0.0071$ ) months after operation, and also with UFC level in 6 ( $P=0.000007$ ), 12 ( $P=0.0017$ ) and 24 ( $P=0.017$ ) months after operation. Such factors as longer duration of the disease ( $P=0.04$ ), tumor invasiveness, ( $P=0.0087$ ), lower decrease rate of serum cortisol level immediately after surgery ( $P=0.000025$ , 0.002 and 0.005 for 6, 12 and 24 months after surgery respectively) and lower decrease rate of ACTH level immediately after surgery ( $P=0.006$ , 0.0003 and 0.00035 for 6, 12 and 24 months after surgery respectively) were shown to be predictors for unfavourable postsurgical prognosis. Age, adenoma size and detection of mitosis on pathology in our study did not influence surgical outcome.

**P205****LHRH-antagonist cetrorelix may reduce postmenopausal flushing**

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**Background**

Estrogen replacement therapy is the most effective treatment for postmenopausal (PMP) flushing, but its use is often contraindicated. An effective and safe alternative is urgently needed.

**Aim**

To explore the effects of the LHRH receptor antagonist cetrorelix in women with severe PMP flushing who have a mean daily flush score > 15.

**Methods**

Open-label treatment with cetrorelix 250 µg twice a day in nine women with severe PMP flushing, for a period of 4-6 weeks. The response to treatment was evaluated by monitoring serum gonadotropin levels, flush scores, and quality of life.

**Results**

At baseline, the mean daily flush score was  $35.9 \pm 2.0$  (range 29-44). All subjects demonstrated a decrease in serum LH and FSH during treatment, but premenopausal levels of both gonadotropins were reached in only two subjects. The mean daily flush score decreased by  $39.6 \pm 8.4\%$  ( $P < 0.005$ ).

**Conclusion**

Severe PMP flushing can be reduced by LHRH receptor blockade. The data suggest that a longer treatment period is required to capture the maximal effect.

**P206****O-6 methylguanine-DNA methyl transferase (MGMT) immunorexpression in GH secreting pituitary adenomas and it's correlation with Ki-67 labeling index (Ki-67 LI)**

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**Background**

Currently, multiple treatment options are available for the treatment of acromegaly. However, cure is obtained only in 50% of patients with macroadenomas after surgery. Persistent tumor enlargement occur in 2.2% of the patients treated with somatostatin analogs and in 1.6-2.9% of the patients treated with pegvisomant. The nuclear antigen Ki-67 is related to growth potential and is also a major prognostic indicator for pituitary adenomas. Studies demonstrated that, the mean Ki-67 LI was high in invasive tumors that could not be cured by surgery. Tumors with high Ki-67 LI are also less responsive to somatostatin analogs. Recent reports suggest the utility of temozolomide in the management of aggressive pituitary adenomas and carcinomas, resistant to conventional treatments. The response to temozolomide is inversely correlated with tumoral expression of MGMT.

**Purpose**

To evaluate MGMT immunorexpression in GH secreting pituitary adenomas, in an effort to predict the likelihood of response to TMZ, and to correlate MGMT immunorexpression with Ki-67 LI.

**Methods**

Our material consisted of 36 GH secreting pituitary adenomas (21 female, 15 male, mean age  $42.5 \pm 10.5$ ), operated at our center between 2003 and 2010. Immunostaining for Ki-67 and MGMT was performed using avidin-biotin-peroxidase complex method. Immunoreactivity was evaluated microscopically and recorded as percentage of nuclear Ki-67 and MGMT immunostaining. MGMT immunorexpression scored as 0=none, 1=<10%, 2=<25%, 3=<50%, 4=>50%.

**Results**

Staining for MGMT was <10% (score 1) in 30 (83.3%), 10-25% (score 2) in 3 (8.3%), 25-50% (score 3) in 2 (5.6%) and >50% (score 4) in 1 (2.8%) of the tumors respectively. There is no correlation between Ki-67 LI with MGMT immunoreactivity ( $P > 0.05$ ).

**Conclusion**

Our data suggests that more than 90% of GH secreting pituitary adenomas express negative/low MGMT. Despite high Ki-67 LI, most of these patients may respond to TMZ, if conventional treatment fails.

**P207****Mechanism of anterior pituitary gene regulation by LHX3 in paediatric combined pituitary hormone deficiency**

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LHX3, a member of the LIM-homeodomain family of developmental transcription factors, is required for establishment of mammalian anterior pituitary hormone-secreting cell types as well as the formation of specialized neurons of the nervous system. Paediatric patients with pituitary insufficiency are sometimes diagnosed with combined pituitary hormone deficiency disease (CPHD). This disease can be linked to mutations in essential pituitary developmental transcription factor genes, including *LHX3*.

Various mutations in *LHX3* have been found to cause CPHD and we recently created a mouse gene knock-in model of CPHD patients whose *LHX3* gene encodes a truncated protein, resulting from an early termination signal at residue 224 (W224Ter). This mutation differs from other *LHX3* mutations in that the patients (and mouse model) do not present with nervous system defects but have pituitary insufficiency. Therefore, we hypothesized that the carboxyl-terminus (C-terminus) of LHX3 is necessary for pituitary development but not nervous system development. We have previously reported that the C-terminus of LHX3 contains activation and repression domains required for pituitary gene activation. In order to understand the mechanism of LHX3-mediated pituitary gene regulation, we have characterized co-regulatory proteins that interact with the C-terminus of LHX3. An affinity purification screen of pituitary proteins identified members of the inhibitor of histone acetyltransferase (INHAT) transcriptional repression complex as interacting with the LHX3 C-terminus.

Protein–protein interaction assays indicated that LHX3 binds to specific members of the INHAT complex. Deletion analyses demonstrated that the C-terminus of each INHAT subunit was essential to bind to LHX3. Chromatin immunoprecipitation (ChIP) assays indicate that INHAT components are present with LHX3 at the gene loci of predicted LHX3 pituitary gene targets. The INHAT complex also repressed LHX3-dependent pituitary gene expression in functional assays. This study provides further insight into the mechanism governing anterior pituitary gene regulation by regulatory transcription factor proteins.

## P208

### High risk of central adrenal insufficiency in GH deficient long-term survivors of childhood acute lymphoblastic leukaemia

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#### Introduction

Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy and accounts for 25% of all childhood cancer. The survival rate is now 85% which emphasizes the importance of long-term treatment complications. ALL patients treated with cranial radiotherapy (CRT) and chemotherapy are particularly at risk for GH deficiency (GHD), but little is known about central adrenal deficiency. Partial ACTH deficiency is often asymptomatic, but it may lead to deleterious consequences during stress.

#### Methods

Out of 44 (21 women) ALL patients (median 25 years, range 19–32), treated with 24 Gy CRT (18–24) and chemotherapy, 37 were tested with an insulintolerance test (ITT) to evaluate GH and cortisol secretion. All were GHD based on the GH response ( $<3 \mu\text{g/l}$ ). The normal cortisol cut-off level was  $\geq 500 \text{ nmol/l}$ . Basal serum ACTH- and cortisol-levels were investigated in all 44 ALL patients and 44 matched population controls.

#### Results

Fourteen out of 37 (38%) ALL patients had subnormal cortisol response to an ITT (257–478 nmol/l) while there was no significant difference in basal serum cortisol levels between 44 patients and controls ( $P > 0.3$ ). ALL patients had significantly lower serum ACTH levels compared to their controls ( $P = 0.04$ ), being significantly lower among the women ( $P = 0.03$ ), only. There was a significantly positive correlation between peak serum cortisol after ITT and basal cortisol ( $P < 0.001$ ,  $r = 0.6$ ), and the GH peak after ITT ( $r = 0.3$ ,  $P = 0.04$ ).

#### Conclusion

Thirty-eight percent of former ALL patients with GHD, treated with CRT, have central adrenal insufficiency 20 years after ALL diagnosis. About 500 ALL survivors have been subjected to this therapy in Sweden (pop. 9 millions), and with the corresponding numbers in other countries. This is of great concern, particular as GHD masks the presence of a hidden central adrenal insufficiency.

## P209

### Rationale and design of a global hyponatraemia registry

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#### Background

Hyponatraemia (HN) is the most common electrolyte disorder of hospitalised patients with a frequency approximating 15%. HN is associated with multiple co-morbidities as well as increases in length of stay (LOS), rehospitalisation, cardiovascular events, and death. This novel HN registry represents the first large-scale international effort to document the impact of HN on clinically relevant outcomes.

#### Methods

The HN registry is an ongoing, global, multi-centre, prospective, observational study designed to collect data on hyponatraemic patients in the hospital setting. HN is defined as serum sodium  $[\text{Na}] \leq 130 \text{ mmol/l}$ . Objectives of the registry are to: i) obtain demographic data on HN patients; ii) demonstrate comparative effectiveness of available HN treatments; and iii) define and compare resource utilization in the management of HN patients. Out of a total of 2500 patients world-wide, up to 1000 patients with HN secondary to SIADH will be enrolled throughout Europe.

#### Results

Primary end points are: i) change in  $[\text{Na}]$  from beginning to end of treatment or discharge and ii) LOS from the time of diagnosis. Secondary end points includes:

i) time to a  $[\text{Na}]$  increase of  $\geq 5 \text{ mmol/l}$  or time to  $[\text{Na}] \geq 130 \text{ mmol/l}$ ; ii) effectiveness of individual therapies in correcting  $[\text{Na}]$ ; and iii) effectiveness of individual therapies in achieving improvement in symptoms. Impact of HN on resource utilization will be measured by LOS and duration of intensive care unit stay.

#### Conclusion

The HN registry will add valuable information to the current literature on SIADH-related HN and will provide important insights into the efficacy of current and emerging treatment options for HN and their impact on clinically relevant outcomes.

## P210

### Screening of Cushing's syndrome and Acromegaly in Japanese diabetic patients

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#### Introduction

Recent reports showed that 2–9.4% of diabetic patients have Cushing's syndrome (CS) including its mild form, subclinical CS (subCS), but there is no report about screening of acromegaly in diabetic patients. Either 1-mg or 0.5-mg dexamethasone suppression test (DST) is used as the screening test of adrenal CS or Cushing's disease (CD). The aim of the present study was to screen CS including CD and acromegaly in Japanese diabetic patients.

#### Design, patients, and measurement

We prospectively evaluated consecutive 225 diabetic patients who were admitted in our affiliated hospitals from November 2008 to March 2010. No patient had clear acromegalic or Cushingoid features. Screening was performed using by 0.5-mg and 1-mg DST as well as measurement of serum IGF1 concentration. The threshold of adequate suppression after either DST was set at 80 nmol/l.

#### Results

Thirty-six patients failed to suppress cortisol after 0.5-mg DST. One showed positive result in 1-mg DST. Twenty-four patients demonstrated additional diagnostic examinations. Five and one patient met Japanese subclinical CD (sub CD) and subCS criteria respectively, while none of these patients had a positive imaging study. On the other hand, in eighteen patients, the serum IGF1 levels were exceeded age- and sex-specific range. In one woman, definitive diagnosis of acromegaly was made by a surgically proven pituitary GH-producing adenoma.

#### Conclusions

The present study showed that many diabetic patients have positive CS screening test, and considerable number of patients may have subCD or subCS despite no definitive image findings. Meanwhile, this study is the first demonstration that screening for acromegaly may be feasible in a cohort of diabetic patients. Early diagnosis and treatment of acromegaly may provide the opportunity to improve the prognosis of diabetes, therefore, we simply recommend to measure serum IGF1 levels in all diabetic patients to screen acromegaly.

## P211

### Gender-related differences in prolactinomas

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#### Introduction

It has been previously described that prolactinomas may have a more aggressive behaviour in men than in women.

#### Methods

We performed a retrospective study in 59 patients diagnosed of prolactinoma (19 men). The variables collected were: age at diagnosis, clinical presentation, tumor size and stage (Hardy classification) and treatment response. The results were compared between both genders.

#### Results

Age at diagnosis was 41 (18.9) years in men and 32.7 (12.5) in women ( $P = 0.051$ ). At the moment of diagnosis men had higher basal prolactin ( $P < 0.001$ ) and a bigger adenoma size ( $P < 0.05$ ). Macroprolactinomas were more frequent in men ( $P < 0.001$ ) and had a greater invasiveness ( $P < 0.05$ ). The most common clinical onset in men was headache (73.7%) and erectile dysfunction (68.4%), whereas in women was menstrual disorders (74.2%), galactorrhea (39.4%) and headache (39.4%). 89.5% of men and 37.5% of women had some kind of hypophysary hormonal deficiency ( $P < 0.001$ ). Complications such as visual defects and

haemorrhagic transformation were more prevalent in men ( $P < 0.05$ ). A delay of 29.8 (37.9) months in the diagnosis was observed in men whereas in women it was of 7.2 (10.7) months ( $P = 0.001$ ). There was a positive correlation between the delay at diagnosis and tumoral stage ( $P = 0.001$ ) but not with the tumor size ( $P = 0.068$ ). During the follow-up with medical treatment, imaging techniques showed: tumor resolution in 81.8% of women and 47.4% of men ( $P = 0.01$ ), reduction in 50% of women and 15.8% of men ( $P = 0.02$ ) and an increase 31.3% of men and 3% of women ( $P < 0.01$ ). Prolactin took more time to normalize in men ( $P = 0.001$ ). Surgery was required in 31.6% of men and only 3% of women ( $P < 0.01$ ).

#### Conclusions

Clinical behaviour of prolactinomas in men is more aggressive, this could be due to a delay in the diagnosis. Women presented a faster and better response to medical treatment.

## P212

### Efficacy and safety of the use of the recombinant GH receptor antagonist pegvisomant (PGV) in acromegaly. Evaluation of its use in 13 patients treated in a pituitary disorders unit

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#### Introduction

The recombinant GH receptor antagonist pegvisomant (PGV) is actually used for the treatment of acromegaly when surgery and medical therapy with somatostatin analogues (SSA) have failed. Long term safety and effectiveness need to be evaluated.

#### Material and methods

Retrospective analysis of clinical data from all patients treated with pegvisomant in a pituitary disorders unit.

#### Results

Thirteen patients are being treated with PGV in our unit. Medium time between diagnosis and beginning of PGV treatment is  $90.71 \pm 92.75$  months. Basal size of the tumours was  $23 \pm 1.60$  mm (80% macroadenomas). Before starting with PGV therapy, 63.6% of the patients had surgery and also 63.6% had radiotherapy. All the patients had been previously treated with SSA and two patients have combination treatment (SSA and PGV). Medium time of PGV treatment was  $45.4 \pm 19.62$  months (9–72). Medium dose of maintenance is  $15 \pm 8$  mg/24 h (5–30). Medium IGF1 values measured at the beginning and after treatment stabilization were  $873 \pm 551$  (432–1769) vs  $234 \pm 139$  (116–464) ( $P < 0.01$ ). Normal IGF1 values ( $< 300$ ) were obtained in 72.7% of the cases, being necessary a mean time of treatment of 6.6 meses (2–36 months) and a mean dose of 15.8  $\pm$  4.9 mg. Tumor size or remaining tumor did not increase in any patient. There was clinical improvement in 100% of the cases. No side-effects have been found and we have found only one case of lipodistrofia.

#### Conclusion

Treatment with pegvisomant seems to be effective, safe and well-tolerated in our experience.

## P213

### Hypothalamic tumor in a patient with Pendred syndrome

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#### Introduction

Pendred syndrome is a rare autosomal recessive disease and the classic triad is congenital sensorineural hearing impairment, goiter and impaired iodine organification with abnormal perchlorate discharge test, but hypothalamic tumors are not a feature of this syndrome.

#### Case report

We report a case of a 32-year-old female with Pendred syndrome confirmed by genetic testing and perchlorate discharge test, with goiter, hypothyroidism treated with levothyroxine (125  $\mu$ g/day), sensorineural deafness and mental retardation. The patient presented a 3-month history of bilateral galactorrhea, without menstrual irregularity, no headaches, no visual disturbances, neither other symptoms. Endocrinological studies revealed hyperprolactinemia (basal prolactin = 147 ng/ml), without other anterior pituitary dysfunction. Brain magnetic resonance imaging (MRI) identified a hypothalamic tumor (17 mm), isodense on T1 and hyperdense on T2 imaging, with enhance after contrast injection, and discreetly thickened pituitary stalk, without other changes. Visual fields testing were normal.

In the absence of visual field abnormalities and endocrine dysfunction other than hyperprolactinemia, an expectant policy of no intervention and periodic follow-up was indicated (MRI scan at 12 month intervals).

She started bromocriptine (2.5 mg/day) with favorable biochemical response and normalization of prolactin levels.

During 8 years of follow-up, the patient remains asymptomatic, without galactorrhea and the size of the hypothalamic mass stable in annual MRI scan.

#### Conclusion

The case is presented in the light of its rarity.

## P214

### Multimodal treatment and minimally invasive surgery for 117 pituitary adenomas: a Croatian Pituitary Centre experience

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Studies that consolidate the results of medicamentous and surgical treatment of pituitary adenomas are scarce. Pure endoscopic endonasal transsphenoidal surgery is rather new technique.

The aim of this study is to report on early postoperative outcome of pure endoscopic endonasal transsphenoidal surgery and to present the results of multimodal treatment in patients with postsurgical failure.

We reviewed 117 consecutive patients who underwent pure endoscopic transsphenoidal adenectomy in Croatian reference Pituitary Centre over one year period (year 2007). Postoperative success was defined as normalization of hormonal excess, and was assessed 6 weeks after surgery. Residue in non-functional (NF) tumors were analysed by magnetic resonance 6 months postoperatively. The series consisted of 21 somatotrophic (GH) adenomas, 61 prolactinomas, four corticotrophic (ACTH) and 31 NF adenomas.

Biochemical criteria for early postoperative remission were obtained in 15 (71.4%) patients with somatotrophic adenoma, 55 (90.2%) patients with prolactinoma, three (75%) patients with corticotrophic adenoma and in 25 (80.6%) patients with NF adenomas. After medicamentous treatment (somatostatin and bromocriptine), gamma knife surgery in seven patients and reoperation in three patients, 4.7% patients with GH adenoma, 3.3% with prolactinoma and 12.9% with NF adenoma remained reluctant to treatment.

In conclusion, pure endoscopic technique is an effective method in pituitary surgery. Our results are similar to those in greater centres. In early postoperative failure multimodal treatment improves the remission rate. The multidisciplinary approach is the key to successful treatment of pituitary tumors.

## P215

### Illness perceptions, quality of life and self-reported physical symptoms in patients after long-term remission of Cushing's syndrome

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#### Context and objective

Illness perceptions pertain the pattern of beliefs patients develop about their illness. These views are determinants of behavior directed at the illness. Illness perceptions are determinants of quality of life (QoL). QoL remains impaired in patients after treatment of endogenous hypercortisolism, however, illness perceptions were never studied in these patients. The objective of the current study was to explore illness perceptions and its relation with QoL in patients treated for Cushing's syndrome.

#### Design

Cross-sectional study.

#### Subjects

We included patients in long-term remission of Cushing's syndrome ( $n = 52$ ), and compared them with five reference populations: vestibular schwannoma patients ( $n = 80$ ), patients with head and neck cancer ( $n = 68$ ), patients with acute ( $n = 35$ ) or chronic ( $n = 63$ ) pain, and patients with COPD ( $n = 171$ ). Illness perceptions were evaluated using the illness perception questionnaire-revised. The protocol was approved by the Medical Ethics Committee.

## Results

Patients after long-term remission of Cushing's syndrome reported more negative illness perceptions compared to reference samples. Patients with Cushing's syndrome scored distinctively worse compared to patients with vestibular schwannoma, patients with head and neck cancer, and patients with acute pain, and also reported more illness related complaints. There were also some differences in illness perceptions between patients with Cushing's syndrome and patients with chronic pain and patients with COPD, but there was no distinct pattern. Illness perceptions showed a strong correlation with QoL.

## Conclusion

Patients after long-term remission of Cushing's syndrome report more negative illness perceptions compared to several reference populations. Future studies have to assess whether self-management intervention programs will improve these illness perceptions and thereby QoL.

## P216

### Long-term treatment of acromegaly with pegvisomant (Somavert): cross-sectional observations from ACROSTUDY, a post-marketing, international, safety, surveillance study

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## Introduction

Somavert is a GH receptor blocker, which inhibits hepatic production of IGF1. While it has been approved for the treatment of acromegaly since 2003 there are few data regarding its effects in everyday clinical practice.

## Methods/design

ACROSTUDY is an open-label, international, prospective, non-interventional, post-marketing surveillance study monitoring the long-term safety and efficacy of Somavert.

## Results

As of December 31, 2009 data from 1288 patients (51% men) were available. The majority of patients came from Germany (31%), France (15%), Spain and Italy (both 14%). Mean (s.d.) age (years) of patients at diagnosis of acromegaly was 42 (13.5) and at Somavert start, 50 (14.0). Mean (s.d.) duration of Somavert treatment was 3.7 (1.9) years. Before Somavert start, other treatments were reported as follows: medical therapy and surgery in 43.5% of patients; medical therapy, radiation and surgery in 24.4%; medical therapy only in 18.3%; surgery only in 4.2%; radiation and surgery in 2%; medical therapy and radiation in 1.6% and only radiation in 0.1%. In 5.9% of patients no previous treatment was reported. At Somavert start ( $n=839$ ) 86% of patients were reported as having increased IGF1 concentrations. After 1 year of treatment ( $n=769$ ), at a mean dose 14.8 mg/day, in 57%, normal IGF1 concentrations were reported. Subsequent IGF1 normalization rates were reported as follows: 2 years ( $n=705$ ), mean dose 15.6 mg/day - 60%; 3 years ( $n=563$ ), mean dose 15.9 mg/day - 60%; 4 years ( $n=406$ ), mean dose 16.8 mg/day - 63%; 5 years ( $n=253$ ), mean dose 17.5 mg/day - 63%.

## Conclusions

The majority of patients experienced normalization of IGF1 on relatively low doses of Somavert. The normalization rate was lower than in clinical trials. This might be explained by insufficient upward dose titration, less rigorous, frequent monitoring and lower patient compliance than in clinical trials, longer follow-up, and unselected patients with severe acromegaly, resistant to other medical treatments.

## P217

### Pituitary gland function following craniocerebral trauma III° in younger patients

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## Objects

Based on the recently published literature, the incidence of single- or multi-axis anterior pituitary insufficiency following severe craniocerebral injury was between 33 and 68%. The mean patient age was between 36 and 45 years.

## Methods

Using a prospective and standardized protocol, we investigated anterior and posterior pituitary function following craniocerebral injury III° (determined by Glasgow Coma Score) in 35 young patients 14-45 years of age (mean patient age was 25 years). The combined anterior pituitary function test (CRH, TRH, GnRH, GHRH-arginine) as well as measurement of plasma and urine osmolality was performed no earlier than 3 months following the craniocerebral injury (19.8 months on average).

## Results

We found isolated ACTH, GH and FSH/LH secretion insufficiency in 8.7, 5.7 and 5.7% of cases respectively (cumulative percentage = 20.1%). Combined adrenocorticotrophic and somatotrophic axis insufficiency was observed in 2.9% of cases. Thyrotrophic and lactotrophic function remained intact. No diabetes insipidus. The prevalence of axis insufficiency correlated with the age at the time of craniocerebral injury (age <20 years 18%, 20-45 years 25%, age <30 years 20%, 30-45 years 27%).

## Conclusions

The relatively high prevalence of anterior pituitary insufficiency after severe craniocerebral trauma reported in the literature applies to older patients. These results must be treated with caution since few authors have used suitable and standardized methods for measuring anterior pituitary function.

Anterior pituitary insufficiency following craniocerebral trauma III° is only rarely observed in younger patients. Since adrenocorticotrophic insufficiency had the highest incident rate, we consider the testing of adrenocorticotrophic function during follow-up after craniocerebral trauma III° as being of particular importance.

## P218

### Macroprolactinomas in 38 children and adolescents: sex differences and long-term results of medical treatment

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## Objectives

Long-term results of medical treatment of macroprolactinoma in children and adolescents (<20 years).

## Patients and methods

Retrospective analysis of all the cases of macroprolactinoma occurring in children and adolescents, medically treated in two university centers.

## Results

38 patients, 25 girls 13 boys, age at diagnosis:  $15.7 \pm 2.8$  years (only two cases before 12 years). At diagnosis, 2/3 presented with headaches, 1/3 visual disturbances, 1/2 abnormal pubertal development, 1/4 growth failure, amenorrhea (primary or secondary) in 22/25 girls. Mean serum prolactin was  $2700 \pm 6145$  µg/l, maximum diameter of the adenoma was  $22 \pm 12$  mm. There were sex differences (boys/girls): age 14/16.6 years ( $P=0.0016$ ), size of adenoma 28/18 mm ( $P=0.019$ ), prolactin level 5176/1368 µg/l ( $P=0.016$ ), growth failure 6/3 ( $P=0.027$ ; OR=3.6), pituitary deficiency 7/2 ( $P=0.003$ ; OR=13.4). First line treatment was dopamin agonist in all cases: cabergoline (22), bromocriptine (14) quinagolide (2) leading to normalisation of prolactin in 74% of the patients, 50% decrease of the volume of the adenoma in 58%, gonadal function normalised in 78% (10/25 girls had normal pregnancy). The patients resistant to medical treatment had surgery (9), radiotherapy (3) or high and prolonged dose of dopamin agonists (6). At this time, only one is not controlled with tumoral expansion symptoms. After a median 8 years follow-up, 1/3 of the patients had sequelae (boys/girls 8/5,  $P=0.014$ ), predictive factor was initial diameter of the adenoma ( $P=0.022$ ), >20 mm.

## Conclusion

This is the largest cohort of macroprolactinomas under 20 years. Sex differences are the same than in adult population with larger tumors in boys, leading to more frequent sequelae. This study confirms that development of prolactinomas is associated with puberty. Long term medical treatment in children and adolescents is effective and safe in 3/4 of the patients but must be completed by surgery or radiotherapy in a few resistant cases.

**P219****Medical treatment of an aggressive non-secreting pituitary tumor and non-secreting pituitary carcinoma: a report of two cases**

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**Introduction**

Since aggressive pituitary tumors are rare, there are no clear guidelines considering treatment of these tumors. A wide range of treatments have been used, with various success.

**Case report 1**

A 58-year old lady presented with sudden severe headache and ptosis of the left eye. MRI revealed an expansive intrasellar, suprasellar and bilateral parasellar process with extension to the skull base which was inaccessible for neurosurgery. Pituitary function was intact, with IGF1 slightly above the normal range. A transphenoidal biopsy was performed, which revealed a pituitary tumor with accompanying necrosis, negative on immunohistochemical staining for pituitary hormones. Octreotide scintigraphy confirmed the presence of somatostatin receptors in the tumor. Treatment with octreotide LAR and cabergoline was initiated. After 3 years of treatment, the patient is in a very good shape with normal pituitary function and reduced size of the tumor on control MRI.

**Case report 2**

A 55-year-old patient had been transcranially operated because of a large non-secreting pituitary adenoma. Because of tumor regrowth, two reoperations followed 7 and 12 years after the first. Pathohistology revealed a pituitary tumor with central necrosis and bone infiltration. At that point elevated levels of serum alkaline phosphatase were observed. Bone scintigraphy revealed numerous osteoblastic metastases, which were confirmed to originate from the pituitary tumor by bone biopsy. The presence of somatostatin receptors in the tumor and metastases was confirmed by octreotide scintigraphy. After 2 years of treatment with zoledronic acid and octreotide LAR (both once monthly), the patient is clinically stable with no further tumor growth on control MRI and the same status of bone metastases on scintigraphy.

**Conclusion**

We report an aggressive pituitary adenoma and a pituitary carcinoma that were successfully managed by medical treatment. This implies that in such cases medical treatment should be considered before proceeding to more aggressive treatment modalities.

**P220****Normal pregnancy in a woman with history of panhypopituitarism due to lymphocytic hypophysitis**V Dimarakis, C T Tsentidis, A Antoniou-Tsigos & A A Argyropoulos  
Nicaea General Hospital, Piraeus, Athens, Greece.**Introduction**

Lymphocytic hypophysitis is an uncommon autoimmune disease usually presenting during or just after pregnancy. It may be followed by panhypopituitarism. In such case is extremely rare for a woman to have a normal delivery again.

**Case report**

We report the case of a 28-year-old woman who had a successful delivery 2 years after lymphocytic hypophysitis which had resulted in panhypopituitarism. The young woman presented with amenorrhea, symptoms of secondary adrenal insufficiency and polyuria several months after her first labor. She reported severe headache which occurred 5 weeks after the labor and lasted for about 1 month. We performed a complete hormonal evaluation of the pituitary gland, which revealed posterior and anterior lobe insufficiency. The MRI findings were consistent with the diagnosis of lymphocytic hypophysitis. The patient was prescribed a full hormone replacement therapy, but after a few months she stopped the estrogen and progesterone therapy by herself. Five months later, she restored normal menses and after two more consecutive months she conceived again. The new pituitary gland hormonal evaluation revealed insufficiency of all other axes. She had a normal pregnancy and labor. She only needed a small increase in hydrocortisone and thyroxin dose at the third trimester.

**Conclusion**

It is possible for a patient with a history of lymphocytic hypophysitis to conceive and have a normal pregnancy again.

**P221****PANCH tumor in a female patient**C T Tsentidis, A A Antoniou-Tsigos, V D Dimarakis, G S Georgakopoulou, P I Prokvas & A A Argyropoulos  
Nicaea General Hospital, Piraeus, Athens, Greece.**Case report**

We present the case of a patient with somatotroph adenoma combined with neuronal choristoma Pituitary Adenoma Neuronal Choristoma (PANCH). A female patient, 44 years of age, manifested with acromegaly type features since 1993, while mentioning also headaches, amenorrhea, and peripheral type visual disturbances since 1997. The clinical examination revealed an obese woman with typical acromegalic features. The laboratory evaluation before pituitary surgery showed a greatly increased IGF1 value of 995 ng/ml (normal values: 101–303) and basal GH value of 13.3 ng/ml (normal values: 0–5), while for the rest frontal pituitary lobe a low basal value of FSH 1.4 mIU/ml (normal value: 3–8) and LH 0.1–mIU/ml (normal values: 1–7) was noted, with normal values of PRL 4 ng/ml (normal values: 1.9–25), TSH 0.8 µIU/ml (normal values: 0.3–4.0) and T4 9.3 µg/dl (normal values: 4.5–12.5). The first pituitary surgery took place in 2/5/01. The histological evaluation of the resected pituitary section revealed a chromophobe somatotroph pituitary adenoma with sparse granulation, combined with neuronal choristoma (PANCH tumor), immunocytochemical positive for GH and focally for PRL. Because of acromegaly remission, a second and a third pituitary surgery followed at 5/6/02 and 13/11/03. She also received conventional radiotherapy in total dose of 54 Gy from 22/9 to 4/11/04. At present, she manifests thyrotroph, lactotroph and gonadotroph cell failure with normal IGF1 levels and the remaining pituitary tumor is stable in size. We present this case report because of its rarity (~0.5% of the hypophyseal tumors).

**P222****On the effect of CRH and dexamethasone on *Pomc* synthesis and ACTH secretion in rat pituitary primary cultures**M F Cassarino<sup>1</sup>, L Pagliardini<sup>2</sup>, F Cavagnini<sup>1,2</sup> & F Pecori Giraldi<sup>1,2</sup><sup>1</sup>Chair of Endocrinology, University of Milan, Milan, Italy; <sup>2</sup>Ospedale San Luca, Istituto Auxologico Italiano IRCCS, Milan, Italy.

*Pomc* synthesis and ACTH secretion by the anterior pituitary are controlled by two major, opposing players namely CRH and glucocorticoids. Most studies were performed on the AtT-20 mouse corticotroph tumor cell line or *Pomc*-transfected cells.

**Aim**

Aim of the present study was to evaluate the effect of CRH and DEX on *Pomc* expression and ACTH secretion in normal rat corticotropes *in vitro*.

**Methods**

Rat anterior pituitary primary cultures were incubated with 10 nM CRH or 10 nM DEX for 4, 24, 48, 72, 96 and 120 h prior to medium and RNA collection. *Pomc* gene expression was evaluated by real time PCR and ACTH was measured by IRMA.

**Results**

CRH stimulated ACTH secretion at 4 h and the effect was maintained for 120 h; stimulation of *Pomc* synthesis was evident after 24 h and increased progressively over time. DEX clearly inhibited ACTH secretion starting at 4 h, but had no effect of *Pomc* gene expression at any time point.

ACTH (ng/ml)	4 (h)	24 (h)	48 (h)	72 (h)	96 (h)	120 (h)
Control	0.5±0.03	2.2±0.68	4.2±0.28	5.4±0.54	7.0±0.44	8.4±0.52
CRH	2.9±0.025	8.3±0.49	14±0.96	22±1.04	27±2.23	33±2.76
DEX	0.4±0.03	1.3±0.71	2.1±1.63	2.4±0.34	3.2±0.40	2.9±0.26
<i>Pomc</i> vs <i>Hprt</i>						
Control	1.0±0.1	1.0±0.1	1.0±0.2	1.0±0.1	1.0±0.1	1.0±0.1
CRH	1.2±0.1	1.3±0.1	1.3±0.1	1.6±0.1	2.0±0.1	2.8±0.3
DEX	1.2±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.3±0.1	1.1±0.1

**Conclusions**

The present study shows that CRH stimulates ACTH synthesis and secretion by normal corticotropes while dexamethasone blunts ACTH release but appears to have no effect on *Pomc* synthesis. This data contrasts with results obtained in tumoral corticotropes or transfected cells and points to the need for studies on normal corticotropes in order to better understand HPA axis physiology.

**P223****KIT protein expression and mutational status of *KIT* gene in pituitary adenomas**O Casar-Borota<sup>1,2</sup>, S L Fougner<sup>4,6</sup>, J Bollerslev<sup>3,6</sup> & J M Nesland<sup>3,5</sup><sup>1</sup>Department of Laboratory Medicine/Pathology, Umeå University Hospital and Department of Medical Biosciences, Umeå University, Umeå, Sweden;<sup>2</sup>Department of Genetics and Pathology, Uppsala University Hospital, Uppsala, Sweden; <sup>3</sup>Faculty of Medicine, University of Oslo, Oslo, Norway; <sup>4</sup>Research Institute for Internal Medicine, Oslo, Norway; <sup>5</sup>Division of Pathology, Oslo University Hospital, Oslo, Norway; <sup>6</sup>Section of Specialized Endocrinology, Oslo University Hospital, Oslo, Norway.**Introduction**

The proto-oncogene *KIT* (CD117) is widely expressed in neoplastic tissues. Gain-of-function mutations of the *KIT* gene were found in some types of leukaemia, gastrointestinal stromal tumours, germinal cell tumours and rarely in other malignancies. Studies on the *KIT* protein and gene in different tumours have been intensified by the availability of imatinib mesylate, *KIT*/PDGFRA inhibitor.

**Methods**

We have immunohistochemically investigated *KIT*-expression in 252 pituitary adenomas. Mutational status of exons 9, 11, 13 and 17 of *KIT* gene has been examined in the tumours with membranous *KIT* expression and in a minority of tumours with cytoplasmic expression. Detailed clinicopathological correlation was performed for somatotroph adenomas.

**Results**

52.4% of adenomas showed cytoplasmic and 8.3% membranous *KIT*-expression. We found differences in *KIT* expression between NFPAs, somatotroph and corticotroph adenomas with the highest proportion of adenomas with membranous expression (17.2%) among the somatotrophinomas. No mutations in the examined exons were found.

**Conclusions**

i) The results suggest a pathogenetic role of *KIT* in a subset of pituitary adenomas. ii) The membranous expression of *KIT* may thus suggest a potential role for *KIT* targeting therapy. iii) Despite the high proportion of somatotroph adenomas with membranous expression of *KIT* in our study, they are probably less attractive candidates for imatinib mesylate therapy due to absence of mutation in the examined exons of *KIT*, no correlation with clinical data and high proportion of tumours with high *sstr2A* expression among the *KIT*-reactive tumours. iv) No mutation in exons 9, 11, 13 and 17 of *KIT* gene was detected. Search for mutations in other exons of the *KIT* gene as well as in kinases other than *KIT*, such as *PDGFR* would be of interest.

**P224****Long-term effects of radiotherapy on cardiovascular risk factors in acromegaly**C L Ronchi<sup>1,3</sup>, E Verrua<sup>1,2</sup>, E Ferrante<sup>1,2</sup>, G Bender<sup>3</sup>, E Sala<sup>1,2</sup>, A Lania<sup>2</sup>, M Fassnacht<sup>3</sup>, P Beck-Peccoz<sup>1,2</sup>, Bruno Allolio<sup>3</sup>, Anna Spada<sup>1,2</sup> & Maura Arosio<sup>2,4</sup><sup>1</sup>Unit of Endocrinology and Diabetology, Fondazione IRCCS Ospedale Maggiore Policlinico, Milan, Italy; <sup>2</sup>Department of Medical Science, University of Milan, Milan, Italy; <sup>3</sup>Unit of Endocrinology, University Hospital of Wuerzburg, Wuerzburg, Germany; <sup>4</sup>Unit of Endocrine Diseases, S. Giuseppe Multimedica Hospital, Milan, Italy.

Radiation therapy is a useful adjuvant tool for the management of difficult acromegalic patients. Its effects on cardiovascular morbidity are still unknown. Aim of the study was to investigate the long-term effects of radiotherapy on metabolic parameters and cardiovascular risk factors.

A total of 42 acromegalic patients (11 M and 31 F, age: 55 ± 12 years, RT group), cured after conventional RT (CRT, n=31) or radiosurgery by gamma-knife (GKRS, n=11), have been retrospectively evaluated (median follow-up: 16.5 years, range: 2-40). Fifty-six sex- and age-matched acromegalic patients cured after surgery alone served as control group. GH/IGF1 secretion, anterior-pituitary functions, metabolic parameters and cardiovascular risk factors (BMI, fasting glucose and insulin, 2 h-OGTT glucose, HOMA-IR, HOMA-B%, HbA1c, lipid profile, blood pressure) and the occurrence of new cardiac or cerebrovascular events have been investigated.

The median time from disease remission was similar in the RT and control group (10.6 vs 13.4 years, *P* NS). GH and IGF1 levels were also comparable. As expected, the percentage of patients with pituitary failure was higher in RT patients than in controls (86 vs 30%, *P*<0.0005). The number of obese,

hypertensive, and dyslipidemic subjects increased over time only in the RT group, whereas the glucose response to the OGTT and the percentage of subjects with glucose alterations improve only in control group. Despite these findings, a similar number of patients in the two groups had major cardiac or cerebrovascular events (4/42, two IMA and two TIA, vs 3/56, one stroke and two TIA). No differences were found between CRT and GKRS subgroups. In conclusion, previous RT seems to be associated with a worse metabolic profile in acromegalic patients studied after a long-term follow-up. Nevertheless, a direct link between RT and cardiovascular events remains to be proven.

**P225****Effect of GH as add-on treatment in severe fibromyalgia syndrome. Results from the IIIb, CT27560 placebo-controlled, multicenter trial**Guillem Cuatrecasas<sup>1</sup>, Cayetano Alegre<sup>2</sup>, Joaquim Fernandez Sola<sup>3</sup>, Maria Jose Gonzalez<sup>2</sup>, Mary Lage<sup>4</sup>, Gemma Sesnilo<sup>5</sup>, Enrique Granados<sup>6</sup> & Manuel Puig Domingo<sup>7</sup><sup>1</sup>Endocrinology Department, CM Teknon, Barcelona, Spain; <sup>2</sup>Rheumatology Department, Institut Universitari Dexeus, Barcelona, Spain; <sup>3</sup>Internal Medicine Department, Hospital Clinic Universitari, Barcelona, Spain; <sup>4</sup>Endocrinology Department, Hospital Clinico Universitario Santiago, Santiago Compostela, Spain; <sup>5</sup>Endocrinology Department, Institut Universitari Dexeus, Barcelona, Spain; <sup>6</sup>Merck SL, Madrid, Spain; <sup>7</sup>Endocrinology Department, Hospital Clinic Universitari, Barcelona, Spain.**Introduction**

Functional GH deficiency has been described in fibromyalgia (FM). The efficacy of GH as add-on treatment has been suggested in small studies, but little is known in larger and homogeneous populations.

**Design**

Patients (120) were enrolled in a multicenter, randomized, placebo-controlled trial for 18 months (NCT00933686). FM impact questionnaire (FIQ) > 75 (severe), duration of FM > 18 months, and stability of the standard therapy (amitriptyline + opioids + SSRI) > 12 months were required. Adult GH deficiency and insensitivity were ruled out with an ITT test and IGF1-generation test respectively. In the first 6 months (blind-phase), Group A received 0.006 mg/kg per day of GH s.c. (further titrated based on IGF1) and group B, placebo, both added to standard therapy. Placebo arm was then switched to GH from 6 to 12 months (open-phase), and an extension study from 12 to 18 months was carried-on after GH withdrawal. Number and intensity of tender points, FIQ and subscales, Quality of Life test (EQ5D) with visual analogic scale (VAS) were evaluated at different time-points.

**Results**

At 12 months, 53% of the patients in group A (GH continuously) reached < 11 positive tender points (cut-off for diagnosis) versus 33% in group B (6 months of GH) (*P*<0.05). Significant improvements were also seen comparing FIQ (*P*=0.01), subscales (*P*<0.05) and EQ5D (*P*<0.05). In group A, 56.5% patients reached > 30% improvement in VAS and 39.1% > 50%. Medication withdrawal in the extension study phase worsened all the scores within each group from the 1st month of follow-up (*P*<0.05). Carpal tunnel syndrome was the most prevalent related adverse event (17%) and no discontinuations were seen.

**Conclusions**

This is the larger and longer placebo-controlled trial performed so far in FM. Added GH is comparable to some labelled drugs in term of pain reduction, FIQ and VAS and further shows a sustained pattern of action.

**P226****Tumor occurrence or recurrence after five year GH replacement therapy**

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GH replacement is widely used in adults with hypopituitarism, but its effect on tumor occurrence and pituitary tumor recurrence is unknown. Furthermore, in literature there are scant with short follow-up time. The available data do not seem to suggest that rhGH replacement increased the incidence of regrowth of pituitary tumor and of cancer in adults with GHD, provided that IGF1 concentrations remain within the normal range for age.

The aim of our study was to evaluate the tumor occurrence and pituitary tumor recurrence in 84 adult patients with GHD (42 females, 42 males; range 16–75; 11 CO, 73 AO), admitted at our Department from 1995 to 2002 with the following diagnoses: 48 adenomas (34 clinically functionless pituitary tumors, 3 corticotropinomas, 9 prolactinomas, 2 somatotropinomas), 16 craniopharyngiomas, 1 dysgerminoma, 1 arachnoid cyst, 1 tumor gigantocellular, 17 idiopathic GHD. Diagnosis of GHD was performed after 6–12 months of the treatment for primary disease. All GHD patients were replaced with rhGH.

New tumors were reported in 2 (2.4%) patients during rhGH replacement (one colon carcinoma was diagnosed in a patients after 3 years, one breast carcinoma was diagnosed in another patient after 5 years). In 2 patients the occurrence of new tumor was found after the cessation of rhGH replacement (one uterus carcinoma and one hepatocarcinoma after 4 year of rhGH discontinuation). Recurrence of pituitary tumors was reported in 8 (12.5%) patients: 4 (8.3%) pituitary adenomas and 4 (25%) craniopharyngiomas.

In conclusion, the recurrence rate of pituitary tumor and occurrence of new tumors in our population do not appear to be increased compared with published data. However, longer follow-up regarding recurrences and secondary neoplasms remains essential.

## P227

### Pituitary function in type 2 diabetic men with good or poor glycaemic control

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#### Introduction

Several data suggest that male hypogonadism occurs frequently in men with type 2 diabetes mellitus (T2DM) but underlying pathophysiological mechanism remains partially unknown. In order to investigate the short term effect of hyperglycaemic state on hypothalamic–pituitary–gonadal axis we prospectively studied 18 men with T2DM younger than 55 years at the time of first diagnosis of T2DM. We compared men with HbA1c  $\geq 9.0\%$  with men with HbA1c  $\leq 9.0\%$  at baseline at baseline when glycaemic control was wrong and after a short and long period of treatment when a good glycaemic control had been obtained.

#### Methods

Basal serum fasting glucose, insulin, fructosamine, HbA1c, total, HDL-, LDL-cholesterol, triglycerides, LH, FSH, total testosterone (T), estradiol and stimulated LH and FSH after a standard GnRH test.

#### Results

A positive percentage increase from baseline of testosterone was observed in men with HbA1c  $\geq 9\%$  at baseline after glycaemic control had been achieved, while in men with HbA1c  $< 9\%$  a minimal decrease of testosterone was recorded, the mean percentage increase from baseline of group with HbA1c  $\geq 9\%$  being significantly higher ( $P=0.018$ ), and it was associated with a positive trend of testosterone/LH ratio and a positive percentage increase from baseline of the response of LH to GnRH infusion in terms of area under the curve and peak.

#### Conclusion

Testosterone secretion by Leydig cells could be impaired in poorly controlled T2DM men and both a better glycaemic control at baseline and/or an improvement of the glycaemic control are associated with a better gonadal function in T2DM men.

## P228

### Non-traditional effects of GH: a survival factor for retinal ganglion cells

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#### Introduction

GH and its receptor (GHR) are expressed in retinal ganglion cells (RGCs) in the eyes of chick embryos, and in mouse, rat and human eyes. Within the retina,

exogenous GH has neuroprotective actions, mediated by caspase-dependent and caspase-independent mechanisms that may also be dependent upon IGF1 signaling. Conversely, the immunoneutralization of endogenous GH promotes apoptosis in the retina and in isolated RGCs. The functional relevance of retinal GH was further assessed in the present study, by determining the effects of retinal GH knockdown on cell survival in the retinas of developing chick embryos.

#### Methods

At embryonic day (ED) 4 of the 21 days incubation period, a specific GH siRNA or a non-silencing siRNA was intra-vitreally injected in 100  $\mu$ l into the right optic cup. Eyes were removed 24 h afterwards and examined for TUNEL-labelled apoptotic cells or for GH mRNA and IGF1 mRNA content, using real-time PCR.

#### Results

The intra-vitreal injection of the GH siRNA lowered the retinal GH mRNA content and IGF1 mRNA content by  $\sim 50\%$  within 24 h of injection. The withdrawal of retinal GH was accompanied by increased ( $P < 0.01$ ) apoptosis, particularly in clusters of retinal cells close to the optic fissure.

#### Conclusions

These results demonstrate that retinal GH has functional significance and it directly or indirectly (through IGF1) promotes cell survival in the developing chick neural retina. Retinal GH therefore has hitherto unknown autocrine or paracrine neuroprotective roles in retinal function.

## P229

### Clinical and subclinical apoplexy in nonfunctioning pituitary tumors: clinical features, management and outcome

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#### Background

Pituitary apoplexy occurs when a tumor undergoes acute hemorrhage, infarct, or both. This often leads to acute severe symptoms (clinical) but can also occur without them and diagnosed on CT/MRI, surgery, pathology (subclinical). To investigate clinical and subclinical apoplexy in nonfunctioning tumors ( $n=221$ ) from our database.

#### Design

Retrospective review of clinical presentation, tumor characteristics and outcome of 24 patients, 11 females and 13 males, with pituitary apoplexy.

#### Results

Subclinical apoplexy occurred in 15 and clinical in 9 patients. They represented 10.9% of nonfunctioning macroadenomas. Subclinical was more frequent on females (81.8 vs 46.2%). Age at diagnosis was similar (subclinical  $47.2 \pm 15.4$  vs  $46.3 \pm 16.2$  years in clinical). Symptoms duration in subclinical was  $24.4 \pm 45.3$  vs  $34.1 \pm 43.8$  months in clinical. Neurological, endocrinological and ophthalmological symptoms were present in 86.7, 80.0, 73.3% of subclinical and in 88.9, 55.6, 77.8% of clinical. Ophthalmoplegia in subclinical 6.7 vs 22.2% of clinical. There was suprasellar extension in subclinical 86.7 vs 100% in clinical, parasellar in 13.3 vs 33.3%, and infrasellar in 40 vs 66.7% respectively. Tumor diameter in subclinical was  $25.0 \pm 7.9$  vs  $30.0 \pm 11.0$  mm in clinical. Surgery was performed in all but three cases, none underwent radiotherapy. Endocrinological evaluation before and after treatment showed in subclinical deficits of GH 66.7 vs 57.1%, FSH/LH 76.9 vs 64.3%, TSH 46.2 vs 42.9%, ACTH 23.1 vs 37.7% and in clinical 100 vs 100%, 100 vs 85.7%, 40 vs 83.3%, 25 vs 85.7% respectively. Treatment was effective in improving vision (subclinical 57.1 vs 42.8% for clinical) and ophthalmoplegia. Disease progression occurred in subclinical 20.0 vs 11.1% of clinical, after a follow-up of  $8.2 \pm 4.4$  vs  $9.4 \pm 9.6$  years respectively.

#### Conclusions

Apoplexy occurred in 10.9% of nonfunctioning pituitary tumors with a higher rate of subclinical type. In clinical apoplexy, tumors were larger and male gender more frequent. Hypopituitarism and neuroophthalmological alterations were high and only the last one improved after treatment. Clinical apoplexy had higher rate of morbidity but lower disease progression. Although pituitary apoplexy is not total predictable it is important to be aware of neurological and endocrinological symptoms in order to perform earlier diagnosis.



## P230

### Comparison of GH suppression response after oral and intravenous glucose tolerance tests in healthy subjects

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#### Introduction

In this study, we aimed to compare GH values obtained in oral glucose tolerance test (OGTT) and intravenous glucose tolerance test (IVGTT) in healthy individuals.

#### Material and methods

Data of 18 healthy volunteers were analysed (10 male and 8 female). Firstly all subjects were evaluated with 75 g oral glucose tolerance test. In another day, intravenous glucose tolerance test was performed. Serum glucose, insulin and GH levels obtained during two tests were measured.

#### Results

Basal GH levels had a wide distribution ranging from 0.00005 µg/l (0.05 pg/ml) to 0.768 µg/l (768.92 pg/ml) (median 0.0145 µg/l). Mean nadir GH level during OGTT was 0.0376 µg/l (between 0.00011 and 0.387, median 0.0016, s.d.: 0.095, s.e.m.: 0.038) and it was obtained at 60th minute. Nadir GH level during IVGTT was observed at 10th minute and it was 0.112 µg/l (between 0.0005 and 0.770, median 0.0053, s.d.: 0.242, s.e.m.: 0.057). There was statistically significant difference between GH levels at 10th minute in IVGTT and at 60th minute in OGTT ( $Z=2.201$ ,  $P=0.028$ ). GH level at 10th minute in IVGTT was higher than GH level at 60th minute in OGTT.

#### Conclusions

Effect of gastroenteropancreatic pathway which may show individual differences can be eliminated by IVGTT. GH suppression by IVGTT may be used in the diagnosis and follow-up of patients with acromegaly. Higher nadir GH levels in IVGTT indicates that nadir GH levels suggested for remission in acromegalic patients are relatively low. Our results show that there may be need to revise the remission criteria for acromegaly.

## P231

### The reliability of GH stimulation tests in the diagnosis of childhood GH deficiency

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#### Objective

To evaluate the reliability of GH stimulation tests in multiple pituitary hormone deficiency (MPHD) and isolated GHD.

#### Materials and methods

In 19 children with MPHD, we analysed the results of insulin tolerance test (ITT), clonidine and sleep tests, anthropometric parameters, IGF1, bone age, pituitary hormone levels ± MRI. GH provocation tests were repeated after variable periods, mostly 6–24 months. The GH provocation tests were considered positive, if peak GH-value < 10 ng/ml. The results were compared with those obtained in 15 children with isolated GHD (with harmonic short stature, height-SDS < 3 s.d., reduced IGF1 and bone age, at least one GH stimulation test positive).

#### Results

In children with MPHD, the mean age at diagnosis was  $11.25 \pm 3.48$  years and mean height-SDS  $-5.00 \pm (-1.40)$  s.d. Thyroid and adrenal function were normalised before GH provocation tests. ITT and sleep tests were positive in all children, clonidine test was false-negative in 3 cases (15%). IGF1 before rhGH-therapy was decreased in 81.2% cases. Bone age before rhGH therapy was delayed with 1.4–9.25 years (mean:  $4.61 \pm 2.2$  years). In children with isolated GHD mean age at diagnosis was  $9.69 \pm 3.65$  years, mean height-SDS  $-4.00 \pm (-0.61)$  s.d., bone age delayed with  $3.41 \pm 1.58$  years. False-negative results were obtained in 4.7% of ITT, 15.7% of clonidine tests and 20% of sleep tests, thus GH stimulation tests were discordant in five children (1/3 of isolated GHD).

#### Conclusion

The results of GH stimulation tests were discordant in 15.8% of MPHD (due to false-negative clonidine test), but more frequently (in 1/3 of children) in isolated GHD, even if IGF1 and bone age sustained the presumed diagnosis. We consider that in isolated GHD at least two tests should be used, mainly if the first GH provocation test is below the cut-off level.

## P232

### Copeptin in the differential diagnosis of the polydipsia–polyuria syndrome: revisiting the direct and indirect water deprivation tests

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#### Background

The water deprivation test (WDT) with direct or indirect measurement of plasma vasopressin (AVP) is the method of choice for the differential diagnosis of the polydipsia–polyuria syndrome. In theory, direct measurement of AVP is highly attractive but hampered by technical difficulties.

#### Objective

To evaluate the utility of copeptin, a surrogate of AVP secretion, in the diagnostic work-up of the polyuria–polydipsia syndrome, and to compare its performance with the current diagnostic standard.

#### Setting and design

In two tertiary referral centres, 20 healthy subjects and 50 patients with polydipsia–polyuria syndrome underwent WDT with measurements of both plasma AVP and copeptin levels. The reference diagnosis was based on clinical information and treatment response.

#### Results

Twenty-two patients (44%) were diagnosed with primary polydipsia, 17 (34%) with partial central diabetes insipidus (DI), 9 (18%) with complete central DI, and 2 (4%) with nephrogenic DI. The indirect WDT led to a correct diagnosis in 35/50 patients (70%). The direct WDT with AVP or Copeptin measurement correctly diagnosed 23 patients (46%) or 36 patients (72%), respectively. Baseline copeptin values > 20 pmol/l identified patients with nephrogenic DI, and concentrations < 2.6 pmol/l indicated complete central DI. The ratio between delta copeptin (8 to 16 h) and serum sodium concentration at 16 h yielded optimal diagnostic accuracy, allowing to also discern partial central DI from primary polydipsia (sensitivity and specificity 87 and 100%, respectively).

#### Conclusion

Copeptin holds promise as a diagnostic tool in the polyuria–polydipsia syndrome improving significantly the diagnostic accuracy of the direct WDT.

## P233

### GH replacement therapy in elderly GH deficient patients: a systematic review

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#### Context

Recombinant human GH (rhGH) is indicated for the treatment of adult subjects with GH deficiency (GHD). However, conflicting data are available on the efficacy of rhGH treatment in elderly GHD patients.

#### Objective

To assess the efficacy of rhGH treatment in elderly GHD subjects.

#### Methods

We searched PubMed, Cochrane Library, Web of Science, and EMBASE.

#### Study selection

Eligible studies included GHD patients, aged > 60 years, treated by rhGH. Data extraction was performed by two reviewers independently.

#### Results

We found 11 eligible studies with a total of 534 patients. Only 2 studies had prospective, randomized, placebo-controlled study designs with a duration of rhGH treatment of 6 ( $n=15$ ) and 12 months ( $n=62$ ), respectively. Treatment with rhGH decreased total and LDL-cholesterol levels by 4–8 and 11–16%, respectively, but did not alter HDL or triglyceride (TG) levels. RhGH did not affect body mass index, but decreased waist circumference (by ~3 cm) and waist/hip ratio. RhGH did not consistently affect blood pressure or bone mineral density. RhGH increased lean body mass by 2–5% and decreased total fat mass by 7–10% in 4 studies, but did not affect body composition in 2 other studies. RhGH consistently improved quality of life parameters reflected in AGHDA-scores. There are no explicit data on elderly GHD patients aged > 80 years.

**Conclusion**

The effects of rhGH replacement in elderly subjects with GHD are relatively limited. In addition, there are no data on the efficacy and safety of rhGH treatment in GHD octogenarians.

**P234****Does GH replacement therapy reduce mortality in adults with GH deficiency? Data from the Dutch National Registry of GH Treatment in adults**

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**Introduction**

Adults with GH deficiency (GHD) have a decreased life expectancy due to cardiovascular diseases (CVD). Recombinant GH treatment has made replacement therapy an option in adults with GHD. Data on long-term efficacy and safety are limited and the implication for life expectancy remains to be established.

**Methods**

The Dutch National Registry of GH Treatment in Adults was founded to gain more insight into long-term efficacy and safety of GH therapy in GHD adults in The Netherlands. A treatment group ( $n=2229$ ), primary control group ( $n=109$ ), and secondary control group ( $n=356$ ) were retrospectively monitored with a median follow up of 6.1 year (interquartile range (IQR) 6.5). Standardized mortality ratios (SMR) for all-cause and cause-specific mortality were calculated. Expected mortality was obtained from cause, sex, calendar year and 5-year age-specific death rates obtained from death and population counts from the Central Bureau of Statistics.

**Results**

Of the 2694 patients 135 had died at a median age of 62.1 year (IQR 21.6). The SMR for all-cause mortality for men was 1.06 (95% confidence interval (CI) 0.81–1.40) and for women 1.66 (CI 1.23–2.23) in the treatment group. The risk on CVD mortality was increased in women (SMR 2.52; CI 1.57–4.06) and there was no increased risk on cancer mortality in this group. The SMR in the control groups were not significantly elevated. Excluding high-risk patients (possible malignant causes of hypopituitarism) lead to normalization of the SMR for all-cause mortality. The risk on CVD mortality in women remained elevated.

**Conclusion**

GH deficient women receiving GH treatment have a higher SMR for all-cause mortality than men, which normalized after exclusion of high-risk patients. The risk on CVD mortality remained increased in women. There is no increased risk on cancer mortality in GH treated adults compared to the background population.

**P235****Hormonal effects on cardiovascular function in patients with acromegaly**

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**Introduction**

Cardiovascular complications are the most relevant cause of mortality in patients with acromegaly.

**Aims**

To evaluate the effects of chronic excess of GH, IGF1 and PRL on blood pressure, heart rate and pulse in patients with active acromegaly.

**Subjects and methods**

Sixty-three patients (22 men and 41 women; aged 18–76 years) with macroadenoma of hypophysis (45 – somatotropinoma, 19 – somatomammotropinoma) were under investigation. Blood samples for GH, IGF1, prolactin (PRL), insulin (IRI) were taken in fasting state. Routine measurements of blood pressure (SBP and DBP), heart rate (HR) and pulse (P) were done. Disease activity was evaluated by means of OGTT according to the Consensus Conference criteria. Hypertension was diagnosed in the presence of DBP above 90 mm/Hg. Data are given as  $M \pm s.e.m.$  and multiple regression model equations.

**Results**

In patients with somatotropinoma ( $GH = 22.8 \pm 3.5$  ng/ml) and somatomammotropinoma ( $GH = 26.3 \pm 5.3$  ng/ml) vital signs varied in large (SBP (80 – 176, mmHg); DBP (58–116, mmHg); HR (48–95, bpm); P (50–95, bpm)). Arterial hypertension was recorded in 64.5%, tachycardia – in 12.5% and bradycardia – in 4.5% of patients. It was revealed significant associations of GH and IRI with cardiovascular signs, approximated by equations:  $SBP \approx 2.32 * GH + 2.13 * IRI$  ( $R^2 = 75.4\%$ ;  $P < 0.001$ );  $DBP \approx 1.48 * GH + 1.31 * IRI$  ( $R^2 = 75.4\%$ ;  $P < 0.001$ );  $HR \approx 1.06 * GH + 1.01 * IRI$  ( $R^2 = 70.4\%$ ;  $P < 0.001$ );  $P \approx 1.13 * GH + 1.19 * IRI$  ( $R^2 = 73.54\%$ ;  $P < 0.001$ ).

**Conclusion**

High prevalence of arterial hypertension may be part of metabolic complications of chronic GH and IGF1 excess mediated by hyperinsulinemia. Increased or decreased heart rate may be due to sympathovagal imbalance in patients with acromegaly.

**P236****Expression of canonical Wnt proteins and survivin in pituitary tumours**

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Wnt developmental pathways, have been implicated in a number of cancers. Wnt signaling proteins activate the B-catenin transcription factor which induces transcription of the oncogenes *Cyclin D1* and *Myc* (Luo *et al.* 2007). Elston *et al.* (2008) found that Wnt inhibitors were down-regulated in pituitary tumours. Overexpression of Survivin, an inhibitor of apoptosis, correlates with poor prognosis and drug resistance (Altieri 2003, Ghosh *et al.* 2007). Survivin also interacts directly with the aryl hydrocarbon receptor – interacting protein (AIP) mutations of which have been linked with increased susceptibility to pituitary tumour development (Kang & Altieri 2006). The aim of this study was to analyze the expression of B-catenin, Cyclin D1, Myc and Survivin in 40 pituitary adenomas (30 non-functional, 7 acromegalic, 2 prolactinomas, 1 Cushing's) collected at transphenoidal surgery and 6 normal controls using immunohistochemistry. The results indicate that B-catenin is membrane bound with no difference between normal and tumour tissue, although smaller tumours had higher expression ( $P < 0.05$ ). Cyclin D1 and Myc expression was nuclear and higher in tumour vs normal tissue ( $P < 0.05$ ). Myc expression also increased with lower age at diagnosis ( $P < 0.001$ ,  $R = 0.361$ ) while Cyclin D1 expression was higher in males than in females ( $P < 0.05$ ). Female patients also had overall younger age at diagnosis ( $P < 0.05$ ) and smaller tumours. Expression of survivin was very low in tumours and absent in normal controls. Therefore, involvement of the canonical Wnt pathway appears to be low, since B-catenin was not found located in the nucleus but Myc and Cyclin D1 proteins may play an important role in early pituitary tumorigenesis. Survivin appears to play a minor role owing to its almost complete absence in tumours with  $< 1\%$  of cells showing nuclear staining. Further analysis of the roles of Myc and Cyclin D1 in pituitary tumorigenesis is required.

**P237****Quality of life is impaired in association with the need for prolonged postoperative therapy by somatostatin analogs in patients with acromegaly**

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**Objective**

To assess the influence of use of long-acting somatostatin analogs on long-term health-related quality of life (HR-QoL) in relation to disease control in patients surgically-treated for acromegaly.

**Design**

Cross-sectional study in two University Medical Centers in The Netherlands.

**Patients**

One hundred and eight patients (47 m/61 f, mean age  $54 \pm 11$  years) with a minimal follow up period of 1 year after pituitary surgery for GH-producing adenoma as primary treatment. Transphenoidal surgery was performed in 101

(94%), a craniotomy in 7 (6%) patients. Subsequently, 46 (43%) received additional radiotherapy (RT) and 41 (38%) were on postoperative treatment with somatostatin analogs (SSTA) because of persistent or recurrent disease at the time of study. All subjects filled in standardized questionnaires measuring HR-QoL, depression and fatigue, in addition to the disease-specific AcroQoL. Disease control at time of the study was assessed by local IGF1 Z-scores.

#### Results

IGF1 Z-scores were slightly higher in patients treated with SSTA in comparison to patients without use of SSTA ( $0.85 \pm 1.52$  vs  $0.25 \pm 1.21$ ,  $P=0.026$ ), but the percentage of patients with insufficient control (IGF1 Z-score  $>2$ ) was not different (17 vs 9%,  $P=0.208$ ). Social status, full-time/part-time employment and co-morbidities, as well as scores for anxiety and depression were all comparable between both groups. Patients using SSTA reported poorer scores on most of the subscales of the RAND36 and on all subscales of the Multidimensional Fatigue Inventory-20. In addition, scores on the AcroQoL were worse in patients treated with SSTA. These associations remained in multivariate analysis after correction for confounders like IGF1 Z-scores, RT, and hormone substitution.

#### Conclusion

Quality of life is impaired in association with the need for prolonged postoperative therapy by somatostatin analogs in patients with acromegaly.

### P238

#### Improvement of acromegalic cardiomyopathy following pituitary apoplexy

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#### Introduction

Cardiovascular disease is present in up to 60% of patients diagnosed of acromegaly and is responsible of the major related morbidity and mortality.

#### Case report

A 79-year-old-woman with previous history of diabetes mellitus and hypertension was admitted for acute pulmonary edema. Transthoracic echocardiogram showed a severe left ventricular dysfunction with an ejection fraction of 20%, dilated left ventricle and left atrium with a moderate mitral regurgitation. On physical examination, she had rough features, with growth of acral parts, prognatism, macroglossia, diastema, acrochordons and goiter, suggestive of acromegaly, without any previous study. During the admission, she suffered a sudden episode of severe frontal headache with hypotension and hyponatremia. Pituitary apoplexy and acute secondary adrenal insufficiency was suspected and immediate treatment with intravenous steroids was initiated. MRI showed a heterogeneous intrasellar mass of  $2.3 \times 2.3$  cm with hemorrhagic component secondary to necrosis. Hormonal analysis revealed a hypogonadotropic hypogonadism, secondary adrenal insufficiency, suppressed TSH, normal FT<sub>4</sub> and low FT<sub>3</sub>. Serum levels of GH and IGF1 were within normal limits. Visual field was not valuable. She was discharged with the diagnosis of acromegalicardiomyopathy. Eight months later, she presents an improvement of her acromegalic features, with hypogonadotropic hypogonadism, secondary adrenal insufficiency and normal GH and IGF1 serum levels. The echocardiogram shows an improvement of the left ventricular volume and the systolic left ventricular function with an ejection fraction of 50% and mild mitral regurgitation.

#### Conclusions

An early and accurate diagnosis of acromegaly is important to prevent serious cardiac related complications.

Acromegalic cardiomyopathy may improve dramatically with the treatment of the disease or exceptionally following a pituitary apoplexy, as illustrated in this case.

### P239

#### Pituitary apoplexy as initial manifestation of pituitary tumors: two case reports

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#### Introduction

Pituitary apoplexy is rare (0.6 to 12.3%). Sudden expansion of pituitary tumor, from hemorrhage or infarction, may lead to permanent vision loss, paresis

or death. We describe two cases of apoplexy as the initial manifestation of pituitary tumor.

#### Case 1

Male, 30 years, healthy, observed in the emergency room (ER) complaining of headache, visual deficit and left ptosis for 3 days. He had left ptosis and bilateral paresis of the IV pair. Magnetic resonance imaging (MRI) showed 'sellar and suprasellar mass  $2.7 \times 2.4 \times 1.9$  cm, compatible with pituitary macroadenoma with intrasellar hemorrhage'. Laboratory findings: FSH 3.4 mIU/ml, LH 1.6 mIU/ml, total testosterone 1.1 ng/ml, TSH 0.082 mIU/ml (0.4–4), FT<sub>4</sub> 0.4 ng/dl (0.8–1.9); IGF1 104 ng/dl (117–329), prolactin, ACTH and cortisol were normal. Transsphenoidal surgery performed three days later. Neuropathology: necrosis of pituitary adenoma. He had complete resolution of visual deficit, maintenance of secondary hypothyroidism and decreased IGF1 but without gonadal and adrenal insufficiency. MRI, 2 months later showed tumor residue with 7 mm. Currently on levothyroxine 0.075 mg id.

#### Case 2

Male, 66 years, observed in the ER for headache, diplopia and right ptosis. Background: atrial fibrillation, hypertension and dyslipidemia, all medicated. Physical examination: right ptosis and ophthalmoplegia, total involvement of III, IV and VI pairs. MRI: 'sellar mass  $3.0 \times 1.9 \times 2.2$  cm compatible with pituitary macroadenoma'. Laboratory findings: FSH 4.7 mIU/ml, LH 3.5 mIU/ml, total testosterone 1.7 ng/ml, prolactin 121 ng/ml ( $<18$ ), without other insufficiency. Transsphenoidal surgery was performed 1 week later. Neuropathology: prolactinoma with hemorrhagic necrosis. After surgery, he presented partial improvement of neurological deficits. Prolactin 22 ng/ml, IGF1 68 ng/dl (117–329), without other hormonal insufficiencies. MRI: residue  $1.21 \times 1.46 \times 1.39$  cm. No need for hormonal substitution therapeutic.

#### Conclusions

These cases call attention to the unpredictability of acute pituitary apoplexy. It should be considered in all patients with neuro-ophthalmological deterioration and acute headache. Early diagnosis and timely surgical treatment leads to significant improvement in visual and hormonal deficits.

### P240

#### Detection of antipituitary antibodies using human and primate pituitary glands in patients with autoimmune thyroid disease and diabetes mellitus type 1

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Lymphocytic hypophysitis is a rare endocrine autoimmune disorder. Principal laboratory markers for diagnosis are antipituitary antibodies (APA). So far there are no standardized methods for the detection of APA and it is not completely clear which antigens are they targeted to as well. At present the indirect immunofluorescence technique is commonly used for their detection. For this purpose cryostat sections of pituitary gland are used. Commercially accessible sections are acquired from primate pituitary glands, especially from baboons.

#### Design

The aim was to compare the results of APA detection in human sera using both baboon and human pituitary frozen sections in the same immunofluorescence system. A total of 138 sera of patients treated for both autoimmune thyroid disease and diabetes mellitus type 1 were evaluated for the presence of APA using both antigens. No patient had clinical signs of hypopituitarism.

#### Methods

APA were assayed by indirect immunofluorescence using sections of monkey anterior pituitary (Euroimmune AG) and freshly frozen human pituitary sections (from autopsy of person with blood group 0, Rh-). No dilution of investigated sera was used.

#### Results

From 138 patients 5 patients were APA positive using monkey sections. None of them was APA positive with human pituitary sections.

#### Conclusion

The difference between human and primate sections can be possibly caused by the presence of human anti-animal antibodies and this false positivity can increase the number of positive results in screening. The methods of APA detection are still to be standardized.

**P241****Periodontal disease frequency in acromegalic patients**Sibel Bascil<sup>1</sup>, Hülya Serinsöz<sup>2</sup>, Eda Ertörer<sup>2</sup> & Neslihan B Tutuncu<sup>2</sup><sup>1</sup>Baskent University Faculty of Oral Diseases, Ankara, Turkey; <sup>2</sup>Baskent University Faculty of Medicine, Ankara, Turkey.

Periodontal disease frequency was screened and compared in 15 acromegalic patients with age and sex matched 50 healthy subjects. Periodontal evaluation included panoramic radiography, plaque and gingival index and depth determination and clinical parameters. Estimated disease duration was 2–10 years in acromegalic patients (8 female, 7 male patients, 52±13 years). Five patients on somatostatin analog therapy had inactive disease for at least 2 years and 10 patients had active acromegaly. Oral examination revealed macroglossia and prognathism in all patients. Gingivitis was found in 6 and chronic periodontitis was diagnosed in 9 patients. Presence of chronic periodontitis was found to be correlated with serum GH/IGF1 levels. Presence of macroglossia and impaired glucose metabolism were not found to be correlated with periodontal disease. Gingivitis incidence was similar with the general population in our healthy control group which reached to about 80%. On the other hand all of the acromegalic patients revealed gingivitis or periodontitis. Routine periodontal examination is strongly recommended for acromegalic patients as it is almost always a problem.

**P242****Relationship between GH activity and markers of inflammation: a cross-over study of healthy volunteers treated with GH and a GH antagonist for 3 weeks**M Andreassen<sup>1</sup>, J Frystyk<sup>2</sup>, J Faber<sup>1</sup> & L Ø Kristensen<sup>1</sup><sup>1</sup>Herlev Hospital Department of Internal Medicine O, University of Copenhagen, Copenhagen, Denmark; <sup>2</sup>Medical Research Laboratories, Clinical Institute and Medical Department M, Aarhus University Hospital, Aarhus, Denmark.**Introduction**

Observations in patients with GH disturbances and in normal populations have suggested that GH/IGF1 activity could have anti-inflammatory effects. On the other hand, *in vitro* studies have shown that IGF1 may stimulate the immune system.

**Methods/design**

The study population consisted of 12 healthy volunteers (mean age 36, range 27–49 years) treated with GH for 3 weeks (1st week 0.01 mg/day, 2nd 0.02 mg/day, 3rd 0.03 mg/day) and subsequently with a GH antagonist (pegvisomant) (1st week 10 mg/day, last 2 weeks 15 mg/day) or *vis a versa*. Wash-out 8 weeks. Circulating levels of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 6 (IL6), CRP, YKL-40, haptoglobin, orosomucoid and fibrinogen were measured after stimulation and inhibition of GH action.

**Results**

GH treatment compared to pegvisomant treatment led to an increase in serum IGF1 (median 393 (IQR 346–543) vs 91 (78–114) ng/ml,  $P=0.005$ ) and in TNF- $\alpha$  (1.44 (0.97–1.75) vs 1.08 (0.74–1.48) pg/ml,  $P=0.047$ ), haptoglobin 10.0 (8.0–18.5) vs 9.0 (6.8–13.8)  $\mu$ M,  $P=0.051$  and fibrinogen (10.8 (9.2–12.2) vs 8.5 (7.4–10.6)  $\mu$ M,  $P=0.005$ ). By contrast orosomucoid (16.0 (15.0–19.5) vs 22.0 (17.0–29.3)  $\mu$ M,  $P=0.005$ ) and CRP (0.64 (0.55–1.71) vs 1.43 (0.71–3.29) mg/l,  $P=0.037$ ) were reduced during GH treatment. IL6 and YKL-40 were unchanged. Compared to baseline there was an increase in serum GH after treatment with both GH (0.26 (0.13–0.51) vs 4.33 (1.81–7.25) ng/ml,  $P=0.005$ ) and pegvisomant (0.26 (0.13–0.51) vs 1.02 (0.34–3.48) ng/ml,  $P=0.013$ ). Serum pegvisomant was 8714 (3881–15666) ng/ml.

**Conclusions**

The data support that high GH/IGF1 activity stimulates the initial phase of the innate immune response evaluated by the pro-inflammatory cytokine TNF- $\alpha$ . However the opposing results on down stream acute phase proteins (fibrinogen and haptoglobin versus orosomucoid and CRP) support a complex interplay between GH/IGF1 action and the inflammatory cascade that does not allow simple conclusions on whether GH/IGF1 action has pro- or anti-inflammatory effects *in vivo*.

**P243****Diabetes mellitus as adjunctive risk factor for vertebral fractures in men with acromegaly**G Mazziotti<sup>1,2</sup>, A Bianchi<sup>3</sup>, M Gola<sup>1</sup>, T Porcelli<sup>1</sup>, L De Marinis<sup>3</sup> & A Giustina<sup>1</sup><sup>1</sup>University of Brescia, Montichiari-Brescia, Italy; <sup>2</sup>Azienda Ospedaliera Carlo Poma, Mantova, Italy; <sup>3</sup>Catholic University of Rome, Rome, Italy.

GH excess is considered as one of the causes of secondary osteoporosis with an increased risk of vertebral fractures in men and post-menopausal women with acromegaly. GH excess in acromegaly is also frequently associated with type 2 diabetes which is considered as a risk factor for fragility fractures in the general population. In this cross-sectional study, we evaluated whether type 2 diabetes may influence the prevalence vertebral fractures in acromegaly. Fifty-seven males with acromegaly (median age 47 years, range: 24–85) and 57 control males were evaluated for bone mineral density (BMD) by lumbar DXA and for vertebral fractures by a radiological and morphometric approach. Seventeen acromegalic patients were affected by type 2 diabetes. The prevalence of vertebral fractures was higher in acromegalic patients as compared with the control subjects (52.6 vs 10.5%;  $P<0.001$ ). Acromegalic patients with fractures had serum IGF1 values significantly higher, and the duration of active disease significantly longer than those found in patients who did not fracture. The prevalence of fractures was significantly higher in acromegalic patients with diabetes as compared to those without diabetes (76.5 vs 42.5%;  $P=0.02$ ), without significant difference in BMD-Z-score at lumbar spine. No significant difference in prevalence of vertebral fractures was observed between patients with controlled and those with uncontrolled diabetes. In the multivariate regression analysis, the correlation between diabetes and vertebral fractures was independent of activity of acromegaly (odds ratio 5.8, 95% CI 1.4–23.5;  $P=0.01$ ). In conclusion, this study suggests that type 2 diabetes mellitus may be an adjunctive risk factor for vertebral fractures in men with acromegaly according to the hypothesis that GH hypersecretion and insulin resistance may exert synergic negative effects on the skeleton. On clinical point of view, our results would suggest to perform a strict skeletal monitoring of acromegalic patients with type 2 diabetes.

**P244****Hyperprolactinemia is a risk factor for radiological vertebral fractures in post-menopausal women**G Mazziotti<sup>1,2</sup>, T Mancini<sup>3</sup>, M Mormando<sup>4</sup>, E De Menis<sup>5</sup>, A Bianchi<sup>4</sup>, M Doga<sup>1</sup>, L De Marinis<sup>4</sup> & A Giustina<sup>1</sup><sup>1</sup>University of Brescia, Montichiari-Brescia, Italy; <sup>2</sup>Azienda Ospedaliera 'Carlo Poma', Mantova, Italy; <sup>3</sup>General Hospital, San Marino, Italy; <sup>4</sup>Catholic University of Rome, Rome, Italy; <sup>5</sup>General Hospital, Montebelluna-Treviso, Italy.

Hyperprolactinemia may cause bone loss in pre-menopausal women and men. Data on fractures are scanty and it is still unclear which is the skeletal impact of hyperprolactinemia in post-menopausal women. The aim of this study was to evaluate the prevalence of vertebral fractures in post-menopausal women with hyperprolactinemia.

Forty post-menopausal women (median age 57 years, range: 47–81) with prolactin (PRL)-secreting adenoma and 119 control post-menopausal women (median age 58, range: 48–74) with normal PRL values were studied by morphometric assessment of vertebral fractures and DXA measurement of bone mineral density.

Vertebral fractures were shown in 22 women with PRL-secreting adenoma (55.0%) and in 38 controls (31.9%;  $P=0.009$ ). The difference in vertebral fractures between women with prolactinoma and controls was significant either in presence of osteoporosis ( $P=0.009$ ) or osteopenia ( $P=0.01$ ), whereas this difference was not significant with normal BMD T-scores. Among PRL-secreting adenoma, fractured patients were older ( $P=0.01$ ) and had lower BMD ( $P=0.002$ ), longer duration of disease ( $P<0.001$ ) and higher ( $P=0.04$ ) serum PRL values vs. patients who did not fracture. Fractures occurred more frequently ( $P=0.003$ ) in patients with untreated hyperprolactinemia versus patients treated with cabergoline whose frequency of vertebral fractures was comparable to that observed in control women. In multivariate analysis duration of disease maintained a significant correlation with vertebral fractures even after correction for age, treatment with cabergoline, BMD and serum PRL values.

Hyperprolactinemia is associated with high prevalence of vertebral fractures in post-menopausal women and its pharmacological treatment should always be considered in this clinical setting.

**P245****Efficacy of weekly pegvisomant monotherapy**

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**Introduction**

Daily administration of Pegvisomant normalizes IGF1 levels >71% of acromegalics (1). Few studies have assessed the efficacy of weekly administration. We assessed the efficacy of weekly pegvisomant for 12 months, after withdrawal of long-acting somatostatin analogs (SSA) in acromegalics previously controlled on combination therapy.

**Design**

Fifteen subjects (8 males, age 58 (35–80) (median (range)) years on combination therapy of high-dose SSA and weekly pegvisomant and IGF1 levels within the normal range for > 6 months, were enrolled. IGF1 at baseline was 0.62 (0.3–0.84) times the upper limit of normal (ULN) and the weekly dose of pegvisomant was 60 (30–80) mg. SSA treatment was stopped for 12 months. IGF1, HbA1c, GH and pegvisomant concentrations were assessed at baseline and there after every 6 weeks. When IGF1 levels increased, pegvisomant dose was increased by 20 mg weekly. Dosages were divided into two injections per week when pegvisomant exceeded 80 mg.

**Results**

Baseline pegvisomant and GH serum levels were 2988 (252–19 440) and 2.99 (0.19–15.95) µg/l respectively. After 12 months, in 73.3% of subjects IGF1 levels remained controlled under monotherapy of pegvisomant. One patient restarted SSA, during the study-period, due to an increase in signs and symptoms of acromegaly. After 12 months, IGF1 was 0.83 (0.30–1.75) ULN, while the cumulative weekly pegvisomant dose increased to 80 (50–120) mg. Pegvisomant and GH levels increased to 4356 (1116–21 276) and 6.36 (0.180–31.45) µg/l. Pegvisomant levels tended to be different between subjects in whom IGF1 remained normal and those who did not: 5544 (1116–21 276) vs 3848 (1296–4392) µg/l, although the weekly pegvisomant dose was the same. HbA1c decreased from 6.0 (5.1–9.2) to 5.9 (5.0–9.1)% ( $P=0.02$ ).

**Conclusion**

After cessation of SSA treatment in acromegalic subjects on combination therapy, weekly pegvisomant could control 73.3% of patients. Pegvisomant levels seems to be lower in patients with an elevated IGF1 than in patients with a normal IGF1, with similar subcutaneous administration dosages.

**P246****AcroBel-2: the Belgian follow-up survey on acromegaly: contribution of pituitary surgery and the use of pegvisomant to an improvement in disease control**

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Two nationwide surveys on acromegaly (AcroBel-1 and 2) have been performed in Belgium at an interval of five years, including centralized GH and IGF1 measurements in most patients. A normal IGF1 for age was observed in 56% of 311 vs 74% of 365 treated patients, in 2004 and 2009 respectively. Factors responsible for this improvement were investigated. A selection bias was unlikely as the follow-up rate was higher in patients with active (83%) or medically controlled disease (71%) compared to those with surgical remission (61%).

\* In 225 patients with sampling in each survey, a normal IGF1 was observed more frequently in AcroBel-2 (80%) than in AcroBel-1 (52%), due to a first or additional surgery ( $n=20$ ; remission in 45%), long-term effects of previous radiotherapy ( $n=84$ ), initiation or intensification of medical therapy, including pegvisomant ( $n=21$ ; control in 67%).

\* In 106 patients newly diagnosed and treated since the first survey, 63% had a normal IGF1. Seventy-five % had pituitary surgery and the overall remission rate (including low or suppressible GH) was 43%, but reached 50% and 68% in two centres responsible for over half of surgeries. Surgical procedures elsewhere (38 patients in 18 hospitals) allowed remission in 24% (range 0–100%). Radiotherapy was used in 3 cases only. Primary medical therapy (mostly SSA,  $n=24$ ) normalized IGF1 in 42%; adjuvant medical therapy (SSA or DA,  $n=24$ ) in 50%, and combined treatment with SSA and pegvisomant ( $n=11$ ) in 64%.

\* A normal IGF1 was present in 74% of the remaining 34 patients from AcroBel-2, with diagnosis before 2003 (33 operated; 13 irradiated; 10 on medical therapy).  
Conclusion

In the recent survey, a more frequent disease remission or control was observed, due to a greater use of surgery with improved outcome and to intensification of medical treatment including introduction of pegvisomant.

**P247****A multicenter study on acromegalic patients treated with pegvisomant monotherapy or with pegvisomant plus somatostatin analogues: role of exon 3 deleted GH receptor polymorphism**

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**Introduction**

To date, two pharmacogenetic studies investigated the effect of the common exon 3 deletion of GH receptor (d3-GHR) variant in small series of acromegalic patients treated with Pegvisomant (Peg), suggesting an association of d3-GHR allele with better response to Peg.

**Aim**

To assess the influence of d3-GHR variant in a large cohort of acromegalics with active disease and resistance to somatostatin analogues (SSA) treated with either Peg monotherapy or Peg plus SSA.

**Patients and methods**

A multicenter cross-sectional pharmacogenetic study was conducted in 16 Italian Endocrinology Centers. Data of 127 Peg treated patients were recorded with online case report form and DNA was genotyped by the coordinating center.

**Results**

Eighteen patients (13.9%) were d3-GHR homozygotes, 41 (34.3%) heterozygotes, and 68 (51.9%) were homozygotes for full-length GHR (fl-GHR) without any significant differences in allele frequencies in respect of both normal subjects and non-Peg treated acromegalics. However, the distribution of GHR genotypes among Peg patients did not follow the Hardy-Weinberg equilibrium. A similar frequency of d3-GHR allele was observed in 64 patients receiving Peg + SSA therapy. Pegvisomant dosage was similar in patients having or not d3-GHR both in Peg ( $1.3 \pm 0.42$  vs  $1.5 \pm 0.6$ ,  $P=0.396$ ) and in combined treatment ( $1.2 \pm 0.4$  vs  $1.3 \pm 0.7$ ,  $P=0.482$ ). Fourteen patients experienced adverse effects (lipohypertrophy, increased hepatic enzymes, pain) that were not associated with GHR genotype. Nine patient had tumour residue growth (5 fl-GHR homozygotes, 4 d3-GHR).

**Conclusions**

This study did not confirm the association of d3-GHR with better response to Peg treatment both in monotherapy and combined with SSA. Further studies are needed to assess whether the unfitted Hardy-Weinberg equilibrium exclusively found in Peg treated patients may be attributed to a random genetic drift or may reflect a role of d3-GHR in the severity of disease activity and outcome.

**P248****Detection of acromegaly by automatic face classification software**

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Acromegaly is accompanied by increased morbidity and mortality. The delay between onset of first symptoms and diagnosis of the disease is 6 to 10 years. Acromegaly causes typical changes of the face. We hypothesized that face classification software might help distinguishing between subjects with and without acromegaly on regular photographs and, thus, might help improving early recognition of acromegaly.

**Methods**

We took frontal and side photographs of the faces of 57 patients with acromegaly (29 women, 28 men) and of 60 sex- and age-matched controls. We grouped patients into subjects with mild, moderate, and severe facial features of acromegaly by overall impression. We then analyzed all picture by using two principles of similarity analysis: analysis of texture using Gabor jets and analysis of geometry using facial landmarks. We used the leave-one-out cross-validation method to classify subjects by the software. Additionally we asked three acromegaly experts and three general internists to classify all pictures by visual impression.

**Findings**

The software correctly classified 71.9% of patients and 91.5% of controls, using a combination of texture and geometry analysis. Correct classification rates for patients by visual analysis were 63.2 and 42.1% by experts and general internists, respectively. Correct classification rates for controls were 80.8 and 87.0% by experts and internists, respectively.

Patients with mild facial features of acromegaly ( $n=24$ ) were correctly classified at 58.3, 38.9 and 20.8% by software, experts, and internists, respectively. Similar classification rates were achieved by the software and experts for patients with moderate or severe features of acromegaly.

**Interpretation**

Acromegaly can be detected by computer software using photographs of the face. Classification accuracy is higher than by medical experts or general internists. This is particularly the case for patients with mild features of acromegaly.

with combination of DA/CB or MLT/CB did not significantly changed compared to the wells treated with CB alone. All tested substances stimulated cell proliferation.

**Conclusion**

Results confirm the *in vivo* anti-secretory effect of cabergoline on prolactin without an anti-proliferative effect on tumor cells. Prolactinoma cell culture from transsphenoidal surgery proved to be a good experimental model to test substances that may inhibit tumor growth and to search for specific factors of aggressiveness in the context of personalized medicine.

Note: The study was funded by PNII 41014 research grant.

**P250****Evolution of invasive treatment-resistant prolactin (PRL) adenoma**

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Giant prolactinomas are rare and their treatment and outcome has been addressed only in isolated case reports. Some patients develop treatment-resistant tumors. We describe a patient with an invasive PRLoma resistant to conventional therapy. A 40-year-old female was diagnosed at age 22 a PRLoma who, although having received all available formulations of dopamine agonists over a period of 18 years, responded neither clinically, nor hormonally (PRL 9560.0 ng/ml,  $nv < 20$ ). At 42 years she underwent trans-sphenoidal tumor resection, but PRL levels remained elevated (1050 ng/ml) even under high cabergoline dosage (0.75 mg/die). Empirical somatostatin analog treatment (octreotide 120 mg s.c./4 weeks) was also tested with with no result. After nearly 20 years from diagnosis suddenly the tumor started growing in size with severe headache, impairment of cognitive function and PRL level increase ( $> 10\,000$  ng/ml). The polyethylene glycol (PEG) precipitation test recovered elevated serum prolactin indicating that no macroprolactin was present. MRI showed the further growth of the large pituitary mass, invading the left cavernous sinus, with partial internal carotid artery occlusion and compression of the hippocampus and the third ventricle. Despite the additional trans-sphenoidal surgical procedure followed by gamma-knife radiosurgery, the patient is still with size tumor progression (diameter close to 6 cm). Because the patient has been judged unsuitable for re-intervention or gamma-knife treatment, and external radiotherapy has been without efficacy, she is now waiting for treatment with receptor-mediated radiotherapy with Yttrium-90 DOTATOC. This case emphasizes the importance of giant prolactinomas monitoring, the possible sudden change of their behaviour with invasive and compression complications and the need to secure to all available treatments.

**P249****Prolactinoma cell culture: experimental model for tumor growth**

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We present an experimental study on human prolactinoma in cell culture from a patient that partially responded to cabergoline treatment and had to have surgical treatment.

**Aim**

The aim of the study was to assess the effect of cabergoline, dopamine and melatonin on prolactin secretion and cell proliferation in human prolactinoma cell culture from a patient that had transsphenoidal surgery.

**Material and methods**

Tissue specimen derived from pituitary adenoma fragments removed in routine transsphenoidal surgery. Cells were plated at  $10^5$  cells/well into 24-well plate in a final volume of 1 ml. The test substances, cabergoline (CB) in doses between  $10^{-6}$ - $10^{-9}$  M, dopamine (DA)  $10^{-9}$  M, melatonin  $10^{-6}$  and  $10^{-9}$  M or in combination were added and incubated for 4 days. The supernatant was centrifuged and kept frozen until prolactin (PRL) assay. Another 96-well plate was seeded with  $10^4$  cells/well for MTT proliferation assay and treated following the same schedule in 0.1 ml/well. Proliferation was assayed by adding 20  $\mu$ l of 5 mg/ml MTT, incubation 3.5 h in cell incubator, removing media and adding 150  $\mu$ l MTT solvent (4 mM HCl, 0.1% Nonidet P-40 in isopropanol) and reading absorbance at 590 nm. Prolactin was assayed by electrochemiluminescence with Roche reagents.

**Results**

Cabergoline significantly inhibited PRL secretion in a dose dependent manner. At  $10^{-6}$  M the inhibition was of 59.45%. MLT slightly increased prolactin secretion; dopamine had no effect on PRL secretion. The PRL levels in the samples treated

**P251****Lost to follow-up in acromegaly, results of ACROSPECT observational study**

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Lost to follow-up (LTFU) is a frequent problem in chronic diseases not evaluated yet in acromegaly despite the long-term need of medical treatment and complications survey in this disease.

**Objectives**

Evaluation of the prevalence of LTFU patients in a multi-center cohort, characteristics of defaulters and description of their evolution after they have been searched and found.

**Méthods**

Observational multicentric (25 centers study). Each center was asked for the number of acromegalic patients followed up between 1997 and 2007 and for the number of the LTFU patients in the same period. Patients LTFU were defined as the ones who missed follow-up appointments in at least 2 years. These patients

and their GP were recalled by phone and mail and asked about reasons to non-attendance. They were invited either to come to the former center for evaluation of acromegaly or to fill up a medical questionnaire.

#### Results

A total of 482 patients under 2252 were identified as LTFU, mean 21.4% of the cohort with a variation between the centers from 7.7 to 49%. At the last evaluation before LTFU, IGF1 was normal without treatment in 54%, normal with a medical treatment in 16% and not controlled in 30% of the patients. Recent news have been collected in 362 patients with 62 dead. Most frequent reason of non-attendance was follow-up by another physician in 69%. Percentage of patients with medical treatment decreased from 28 to 15%. Among the 87 patients who came back in the center, acromegaly was not controlled in 20% and the treatment had to be modified in 17%, lack of medical survey was observed in 16%.

#### Conclusion

In acromegaly LTFU concerns 21.4% of the patients. Treatments are stopped in these patients in half of the cases. When the patients are reevaluated 20% are not controlled. As acromegaly can now be controlled quality of follow-up is a real clinical challenge.

## P252

### Initial management and complications of Cushing disease (CD): a French multicenter study of 437 patients

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#### Introduction

CD is a rare disease. Our aim was to describe demographic and clinical characteristics together with initial management of patients with CD.

#### Patients and methods

A retrospective analysis of 437 CD patients diagnosed between 1996 and 2009, followed in 5 tertiary centers (Bordeaux, Grenoble, Lyon, Marseille, Paris) was performed in the frame of the national program for rare diseases.

#### Results

347 (80%) were women. Mean age at diagnosis was  $40.5 \pm 15.8$  years, and mean time between first symptoms and diagnosis was  $34.4 \pm 38.4$  months. A bilateral inferior petrosal sinus sampling was performed in pretreatment evaluation in 32% of patients. Pretreatment pituitary magnetic resonance (MR) imaging was normal in 20% of patients, allowed adenoma visualisation in 66.7% and was inconclusive in 13.3%. Initial clinical and biological evaluation before treatment showed: body mass index above 25 and  $30 \text{ kg/m}^2$  in respectively 31 and 35%, hypertension (53%), elevated fasting or post-prandial glycemia (26%), psychiatric disorder (32%), clinical hypogonadism (38%), and fractures (21%). Pretreatment bone mineral density was only available in 110 patients: lombar T-score and femoral T-score were under 2.5 DS in respectively 16 and 8% of these patients. 338 patients (77%) had transsphenoidal surgery as first line treatment, leading to positive histology of corticotroph adenoma in 81%. In the 299 patients with immediate postoperative data available, remission was obtained in 225 (75%) cases. Remission rate was higher in patient with positive pre-treatment MR (89 vs 71%,  $P=0.004$ ).

#### Conclusion

This large multicenter cohort of patients with CD allowed to characterize initial demographics and clinical manifestations of the disease, together with immediate pituitary surgery outcomes. The longitudinal study of this cohort should enable to determine long term evolution of CD manifestation after treatment.

## P253

### The value of T2 weighted MR-imaging for stratification to medical therapy in newly diagnosed acromegaly

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#### Background

Signal intensity in T2 weighted (w) MRI in somatotroph pituitary adenomas has been described to correlate with histological granulation pattern. Long-term somato-statin analogue (SSA)-response is associated with both histological subtype and T2wMRI.

#### Objective

To describe secretory baseline characteristics in newly diagnosed acromegalic patients in relation to T2 signal intensity. Moreover, to test whether T2 w images could predict preoperative SSA-response.

#### Design/patients/methods

Retrospective observational cohort study. Forty-seven patients with newly diagnosed acromegaly were included and classified in three categories according to signal intensity in T2 w MRI; hypointense, isointense and hyperintense. Baseline IGF1 and GH, and GH response to SSA test were recorded. Long-term response of SSA on tumour volume and invasiveness (SIPAP-score) were analyzed in a subgroup of 25 patients pretreated medically prior to surgery. Histological granulation pattern was evaluated.

#### Results

Of 14 (30%) adenomas were hypointense, 12 (26%) isointense, and 21 (45%) hyperintense. Median IGF1 (ratio IGF1/ULN) was 3.3, 3.4 and 2.1 in the hypointense, isointense and hyperintense group respectively ( $P<0.05$  for difference between groups). Corresponding values for GH were 43.8, 40.4 and  $13.6 \text{ mU/l}$  ( $P<0.05$ ). The response to SSA test dose (% reduction in GH) was 86%, 83%, 69% ( $P=0.05$ ). There was no significant difference between groups in tumour volume ( $n=47$ ) or tumour volume reduction in patients pretreated with SSA ( $n=25$ ). Median reduction in SIPAP-score after SSA-treatment was 0.5, 2 and 0 ( $P<0.05$ ).

All eight sparsely granulated adenomas were hyperintense on T2 w images.

#### Conclusions

T2 hyperintense tumours had lower GH and IGF1 values at baseline and a reduced SSA responsiveness in an acute test. The effect of SSA treatment on tumour invasiveness in hyperintense adenomas is small.

T2 w MRI signal intensity in newly diagnosed acromegaly might improve stratification in relation to medical therapy.

## P254

### Lack of efficacy of 24-month treatment with the GH receptor antagonist pegvisomant in patient with active acromegaly resistant to long-term, high-dose somatostatin analog treatment: effects on IGF1 and GH levels

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In acromegaly symptom control can be achieved by lowering insulin-like growth factor 1 (IGF1) concentrations to the age-adjusted normal range. However, even with optimal surgery and current medical therapies (dopamine agonists, somatostatin analogues), 30–50% of patients do not achieve target concentrations of IGF1 and GH. Pegvisomant (PEGV), a new GH-receptor antagonist, given as s.c. injections at variable dosages, could normalises IGF1 concentrations in ~70% of acromegalic patients. We report the peculiar case of a 26 years acromegalic male with a GH producing adenoma first treated with transfenoidal surgery; not cured and then resistant to octreotide therapy. He received daily PEGV injections of dosage determined by IGF1 levels titration from 10 to 40 mg/day. PEGV at a maximum dosage of 40 mg/day failed to suppress IGF1 levels into the normal range (1159 ng/ml, nv 215–628). However, owing to its high structural homology to GH, PEGV may interfere in the assay resulting in inaccurate GH measurement (AN Paysley *et al. E J Endocrin* 2007). To ascertain the reason of PEGV resistance we tried to measure patient GH levels. Under pegvisomant treatment GH serum levels were in the low range in our patient. We then performed a specific assay for PEGV (Clinical Pharmacology, Pfizer): values were 1000 and 8544 ng/ml (at the same time GH 1.0 ng/ml; IGF1 830.0 ng/ml). Such pegvisomant concentrations are somewhat lower than would be expected for a subject under PEGV 40 mg/day (typical values would be 14.000–20.000 ng/ml). One possible explanation for this data is that PEGV would prevent sandwich

formation in the test, resulting in decreased GH measured in serum. At the same time pegvisomant could act as an antigen and the patient may have developed a significant immunogenic neutralizing response to PEGV.

## P255

### Quality of life and cortisol diurnal rhythm after 3 months of medical treatment for Cushing's disease

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#### Introduction

Cushing's disease (CD) is characterized by various symptoms, amongst which fatigue, muscle weakness and depression. The chronic state of hypercortisolism severely impairs quality of life (QoL). In addition, the physiological cortisol diurnal rhythm (CDR) is disturbed in CD. Transsphenoidal surgery is the primary treatment for CD, but long-term remission rates are disappointing. We performed a prospective trial in which stepwise medical treatment was applied with the somatostatin-analog pasireotide, the dopamine agonist cabergoline and the adrenal-blocking agent ketoconazole. We now report the effect of this regimen on QoL and CDR.

#### Methods

Seventeen patients with CD were treated for 80 days with pasireotide monotherapy or combination therapy with cabergoline and, if necessary, ketoconazole. Hereafter, patients continued medical therapy or underwent surgery. Using five questionnaires, QoL was assessed at baseline, day 80 and in the extension period and compared to literature-derived healthy controls.

#### Results

At baseline, QoL was significantly reduced compared to healthy controls according to 8/20 subscales. After 80 days, 15/17(88%) had normalized urinary free cortisol excretion. However, emotional reaction was the only QoL-related parameter that improved according to the Nottingham health profile ( $P < 0.05$ ). According to the RAND-36 questionnaire, patients reported more pain at day 80 ( $P < 0.05$ ), which might be a sign of steroid-withdrawal. QoL significantly improved in 8 patients after 764 days (mean) of biochemical remission after medical ( $n=3$ ) or medical and surgical ( $n=5$ ) treatment. In 7/17 patients, we observed a trend towards recovery of CDR, without any correlation with QoL.

#### Discussion

QoL in patients with untreated CD is significantly impaired compared to healthy controls from literature references. While no effect on QoL was found after 80 days of successful medical treatment, it seems to improve after a sustained period of biochemical remission. Finally, medical therapy for CD that blocks tumor secretory activity may initiate recovery of cortisol diurnal rhythm.

## P256

### Low incidence of adrenal insufficiency after transsphenoidal surgery in patients with acromegaly: a long-term follow-up study

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#### Background

The long-term prevalence of adrenal insufficiency after transsphenoidal surgery for GH secreting pituitary adenomas was unknown. However, recently a single study reported a high prevalence of adrenal insufficiency after surgical and/or medical treatment without postoperative radiotherapy in acromegalic patients.

#### Aim

To assess the prevalence and incidence rate of adrenal insufficiency in consecutive patients during long-term follow-up after successful transsphenoidal surgery for acromegaly.

#### Methods

In 91 consecutive patients in remission after transsphenoidal surgery, we retrospectively reviewed insulin tolerance tests, CRH stimulation tests, metyrapone tests and ACTH stimulation tests used to assess corticotrope function.

#### Results

Early postoperatively, insufficient adrenal function was observed in 18% of patient ( $n=16$ ), which was transient in 8 and irreversible in 8 other patients in the first year of postoperative follow-up. Therefore, after the first year, the prevalence of adrenal insufficiency was 9%. Late, new-onset adrenal insufficiency developed

in only 3 patients, 13, 18 and 24 years postoperatively, resp. The incidence rate of late adrenal insufficiency after successful surgery was 2/1000 person years.

After long-term follow-up, with a mean duration of  $17.7 \pm 8.1$  years, the prevalence of secondary adrenal insufficiency was 12% in patients in remission after surgery for acromegaly.

#### Conclusion

The prevalence of adrenal insufficiency 1 year after surgery was 9%, whereas during prolonged follow-up the incidence rate of adrenal insufficiency was only 2/1000 person years in patients in remission after surgery. Therefore, development of late onset adrenal insufficiency is a very infrequent complication in patients with acromegaly in remission after transsphenoidal surgery only.

## P257

### Fertility rate in acromegalic women: a single center experience on 70 patients before and after treatment

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Women with acromegaly present often menstrual irregularity, anovularity and infertility. A direct role of GH and IGF1 excess on the hypothalamus-pituitary-gonadal axis, hyperprolactinemia and an impaired gonadotrophin secretion related to a tumor mass effect, or polycystic ovary syndrome have been suggested as possible mechanisms related to infertility. However, no systematic data are available on fertility in these patients. The aim of this retrospective study was to evaluate the fertility rate and characteristics in 120 women with acromegaly attending the outpatient service of our Department between 1995 and 2010, where 70 women with diagnosis of acromegaly were within the reproductive age range (21–43 years). Gonadal function was assessed on the basis of menstrual status and hormone profiles before and after treatment of acromegaly. Information on the desire of pregnancy, nature of conception (spontaneous or after ovarian stimulation) and pregnancy, was based on medical records and an *ad hoc* designed questionnaire distributed to these patients. At the diagnosis, 42 women (60%) had menstrual disturbances, mainly characterized by oligo-amenorrhea. Of these 25 (60%) had hypogonadotrophic hypogonadism, 12 (30%) had hyperprolactinemia, whereas 5 (10%) patients had a clear-cut polycystic ovary syndrome. During the active disease, 25 women (35%) had desire of pregnancy but had difficulties in conception and starting pregnancy. In the last 15 years, nine women became pregnant only after the normalization of GH and IGF1 levels. Three patients became pregnant after surgical treatment, whereas six women were receiving medical treatment with somatostatin analogs when they conceived, and treatment was continued during the pregnancy in two of them. Only one patient underwent ovarian stimulation and intrauterine insemination. In conclusion, fertility is commonly impaired in women with acromegaly, 60% of our patients had gonadal dysfunction, 35% of them had desire of pregnancy and 36% of these latter patients become pregnant but only after normalization of GH/IGF1 levels. Effective treatment for acromegaly, represented by surgery or medical therapy seem to improve fertility, since 30% of patients conceived after surgery and 70% become pregnant during treatment with somatostatin analogs.

## P258

### Prevalence of germline mutations of AIP gene in sporadic aggressive somatotropinomas

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Most kindreds of familial isolated pituitary adenomas (FIPA) with mutated AIP develop somatotropinomas, characterized by an aggressive clinical phenotype including early age at diagnosis, large tumours and frequent invasiveness. In non-family cases, the prevalence of AIP mutations in pituitary adenomas is lower than 10%. There is no information of AIP prevalence in isolated somatotropinomas characterized by poor response to conventional treatment.

#### Aim

To investigate the prevalence of AIP mutations in non-family cases of somatotropinomas with poor response to conventional treatment.



Patients and methods

A total of 50 patients (22 males/28 females, age  $52 \pm 15$ ) and 16 controls were included in this study performed at six University Hospitals in Spain. None of them had family history of pituitary adenomas or other endocrine tumours. All patients failed to respond to conventional treatment including surgery and somatostatin analogs. Some of them received adjuvant radiotherapy and most cases required pegvisomant treatment to control residual GH hypersecretion. AIP gene analysis was performed by using standardized PCR protocol (*Science* 312 1228–1230), in which the coding regions of exons 1, 2, 3, 4, 5 y 6 were amplified. Possible deletions/duplications were studied using MLPA.

Results

One out of 50 cases showed a not previously described mutation for the AIP gene, consisting in c.26G>A (p.Arg9Gln). The patient was a female with clinical diagnosis of acromegaly performed at the age of 20 years, and bearing a macroadenoma.

Conclusion

AIP germline mutations show a low prevalence in non-family acromegalic patients with tumours resistant to conventional treatment.

**P259**

**Predictive markers of recurrence in radically resected pituitary macroadenomas**

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Introduction

Pituitary adenomas are benign neoplasms which grow slowly, but that can recur even after apparent radical resection. Until now, markers predicting regrowth and invasiveness have not been completely characterized.

Design

We investigated cell proliferation and apoptosis parameters in 20 radically resected pituitary macroadenomas (six GH, two PRL, two ACTH, one FSH and nine non-secreting), in order to predict recurrence risk. Tumour regrowth was demonstrated by MRI in a 5-year follow-up. Proliferative activity and DNA ploidy were analyzed by flow cytometry (FCM) performed on fresh surgical specimens. Immunohistochemical expression of proliferative index MIB-1 and of anti-apoptotic protein Bcl-2 was analyzed on paraffin-embedded specimens.

Results

Six adenomas recurred after surgery, regardless of hormonal hypersecretion. Pre-surgical tumour size was not significantly higher in recurrent than in non-recurrent adenomas ( $P=0.08$ ). Pre-surgical MRI demonstrated cavernous sinus (CS) invasiveness in all recurrent tumours and in 6 non-recurrent ones ( $P=0.042$ , by Fisher's exact test). FCM showed aneuploid DNA content in 5/20 adenomas, one of which recurred. Cell percentages in S (%SPF) and G2+M (%G2-M) phase and proliferative index ( $PI = \%SPF + \%G2-M$ ) were significantly higher in aneuploid than in diploid adenomas ( $P<0.05$ ), but tumour recurrence was independent of all FCM parameters. MIB-1 expression and mean percentage of MIB-1+ve cells were higher in recurrent than in non-recurrent tumours, although not significantly ( $P=0.094$  and  $P=0.061$ , respectively). Bcl-2 expression was detected in 12/15 pituitary adenomas, involving  $63 \pm 35\%$  of tumor cells, regardless of tumour recurrence.

Conclusion

In this group of radically resected pituitary macroadenomas, neuroradiological finding of CS invasiveness, but neither FCM parameters nor MIB-1 and Bcl-2 expressions, is useful for predicting tumour recurrence.

**P260**

**Mechanism and management of hyperglycemia associated with pasireotide: results from studies in healthy volunteers**

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Introduction

The multi-receptor targeted somatostatin analogue pasireotide has high affinity for sst<sub>5</sub>; this receptor may play an important role in glucose metabolism.

Two studies in healthy male volunteers evaluated the mechanism and management of hyperglycemia associated with pasireotide.

Methods

A randomized, Phase II study assessed insulin secretion and glucose metabolism during 8 days of pasireotide 600 or 900 µg sc bid ( $n=19$  for both) with oral glucose tolerance test (OGTT), hyperglycemic clamp (HC) with arginine stimulation, and hyperinsulinemic-euglycemic clamp (HIEC). A randomized, 1-week, five-arm Phase I study evaluated glucose metabolism following OGTT with pasireotide 600 µg sc bid co-administered with four classes of antihyperglycemic drugs (metformin, nateglinide, vildagliptin or liraglutide) or pasireotide alone ( $n=18$  each).

Results

Following OGTT (Phase II study), significant decreases in mean plasma insulin AUC ( $P<0.001$ ) and increases in mean plasma glucose AUC ( $P<0.001$ ) were observed during treatment in both dose groups. Following HC, final plasma insulin levels were ~50% those at baseline; decreases in AUC were significant ( $P<0.001$ ) in both dose groups. Significant decreases ( $P<0.01$ ) in glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) AUC were observed. Following HIEC there were no statistically significant changes in basal endogenous glucose production or glucose disposal rate (i.e. insulin sensitivity). The plasma glucose  $AUC_{0-4h}$  on day 7 (Phase I study) decreased by a mean of 2, 10, 15, and 29%, respectively, when pasireotide was co-administered with metformin, nateglinide, vildagliptin and liraglutide, compared with pasireotide alone. Treatment was generally well tolerated in these groups.

Conclusions

Hyperglycemia associated with pasireotide is related to decreases in insulin secretion, with no changes in insulin sensitivity. Pasireotide also significantly decreased incretin hormone response. The mechanism of action of GLP-1 analogues (e.g. liraglutide), DPP-4 inhibitors (e.g. vildagliptin) and insulin secretagogues (e.g. nateglinide) can effectively address the hyperglycemia on pasireotide therapy.

**P261**

**The efficacy of octreotide LAR in acromegalic patients**

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Aim

To investigate the efficacy of octreotide LAR therapy in acromegalic patients as primary or secondary therapy.

Methods

Ten acromegalic patients diagnosed at Clinic of Endocrinology in Sarajevo (seven females and three males, mean age  $55.2 \pm 7.2$  years, age range 40–65 years, five patients with microadenoma and five patients with macroadenoma) were treated with octreotideLAR. Operated were 60% of patients. Concentration of hGH and IGF1 was evaluated at 0, 6 and 12 months, while MRI images were taken at 0 and 12 months. All patients had active acromegaly. Somatostatin suppression tests were done before octreotide were given. At our center, this is first time to use OctreotideLAR in acromegaly treatment. Eight patients received SandostatinLAR 30 mg/28 days, one patient received 20 mg and one 60 mg/28 days. Normal IGF1 concentrations for age and sex and a GH  $<2.5$  ng/ml were defined as biochemical control. Basic statistics were applied.

Results

Before treatment GH were  $50.13 \pm 22.44$  ng/ml (range: 19–105), IGF1 were  $753.66 \pm 103.48$  ng/ml (range: 542–896). Four patients (40%) were followed with primary octreotideLAR treatment. After 6 and 12 months the hGH decreased to  $2.74 \pm 1.70$  ng/ml (range: 1.3–7.0) and  $1.86 \pm 0.56$  ng/ml (range: 1.0–3.0) respectively, while the IGF1 became  $345.33 \pm 73.33$  ng/ml (range: 265–489) and  $254.10 \pm 23.08$  ng/ml (range: 210–289) respectively. Prior to treatment the size of pituitary adenomas was 9.57 mm (min 3.0; max 20), while after 12 months of treatment, the size decreases to 8.0 mm (min 1.0; max 18.0). After therapy, decrease of GH below 2.5 ng/ml were achieved in 90% patients, decrease of the tumor size were achieved in 60% while normalization of IGF1 were achieved in 100% of the patients respectively. One of patients with macroadenoma was used 60 mg octreotideLAR as secondary therapy after surgical treatment, because a dose of 30 mg was not enough for decrease of GH and IGF1. All differences were statistically significant ( $P<0.05$ ), but in group of acromegalic patients treated with octreotideLAR as primary therapy that difference was more significant.

Conclusions

OctreotideLAR treatment of acromegalic patients not only decreases hGH and IGF1 concentrations, but also appears to diminish a size of the tumour, in about 60% of patients. The somatostatin analogues are more efficient in primary treatment of acromegalic patients.

## P262

**A novel AIP mutation related to familial isolated pituitary adenomas (FIPA)**

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**Introduction**

It has been estimated that 15–20% of FIPA families harbor an AIP gene mutation (AIPmut). To our knowledge ~50 sequence variants -pathological and otherwise- have been described to date. We report a new FIPA family with an extensive genealogy, in which 4 members have pituitary adenomas in the setting of a novel AIPmut.

**Patients and methods**

The index patient is a 37-year-old man, who presented with childhood onset of somatotropinoma and underwent surgery and radiotherapy at the age of 17. His uncle (now aged 70 years), was diagnosed with a somatotropinoma at the age of 16, and also treated with surgery and radiotherapy. The sister of this latter man (the 72-year-old aunt of the index case) presented with secondary amenorrhea at age 18, and was later diagnosed with a macroadenoma at the age of 48 after she complained of visual disturbances. She also underwent surgery and radiotherapy. The fourth affected member is the 65-year-old cousin of the latter two patients. He is 1.97 cm tall, with a long standing hypogonadal phenotype; he had hypopituitarism affecting the gonadal, thyroid and somatotrope axes. His MRI shows a wide sella with an eroded floor, and there is a clinical suggestion of potential apoplexy in the past. A genealogic tree was drawn and due to the FIPA presentation, germline AIP sequencing was performed and showed all four affected subjects to have a novel c.543delT AIPmut, which would predict a truncated AIP protein.

**Conclusions**

This 4-member AIPmut positive FIPA family with a presentation that typifies the range of clinical scenarios encountered in AIPmut carriers with pituitary adenomas. As the disease presentation was quite aggressive in these cases and as early intervention has appeared to control disease in some of the individuals, we are currently screening for AIPmut carriers among the family.

## P263

**Octreotide pharmacokinetics and biochemical control of acromegaly using a subcutaneous octreotide hydrogel implant**

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Two randomized open-label, phase II studies evaluated an octreotide hydrogel implant to suppress serum GH and IGF1 levels in adult acromegaly patients. Implants were inserted subcutaneously in the upper arm for 6 months. Patients in study 1 received 1 (*n*=5) or 2 (*n*=6) 52-mg octreotide (60 mg octreotide acetate) hydrated implants; patients in study 2 received a hydrated (H) or nonhydrated (NH) 84-mg octreotide acetate implant (*n*=34, 1 excluded from efficacy and pharmacokinetic population). Efficacy data are shown (table).

**Table 1**

	52 mg ( <i>n</i> =11)		84 mg ( <i>n</i> =33)
	1 implant	2 implants	1 implant (NH or H)
Mean ± s.d. <i>C</i> <sub>max</sub> (pg/ml)	1705 ± 589	4182 ± 2288	1291 ± 628 (NH) 2399 ± 883 (H)
Mean ± s.d. AUC <sub>0-24</sub> (pg·mo/ml)	6702 ± 1944	14 427 ± 6189	4182 ± 1239 (NH) 5922 ± 1708 (H)
% reduction in mean IGF1 at mo 1/mo 6 (versus baseline)	45/44	59/52	29/18
% reduction in mean GH at mo 1 / mo 6 (versus baseline)	91/86	66/74	74/63
Normalized IGF1, <i>n</i> (%)	1 (20)	2 (33)	17 (52)
Mean GH < 5 ng/ml, <i>n</i> (%)	5 (100)	5 (83)	23 (70)
Mean GH < 2.5 ng/ml, <i>n</i> (%)	4 (80)	4 (67)	13 (39)
Mean GH < 1 ng/ml, <i>n</i> (%)	1 (20)	2 (33)	2 (6)

The most frequent treatment-related adverse event was diarrhea (64%, study 1; 18%, study 2). The octreotide hydrogel implants provided consistent and sustained octreotide release, resulting in IGF1 and GH suppression over 6 months; the 84-mg NH octreotide hydrogel implant is currently being tested in phase III studies.

## P264

**Transition to endoscopic transsphenoidal pituitary surgery: a single-center experience**

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**Background**

Surgery of the pituitary gland is increasingly being performed through an endoscopic approach. We report the results of the first 26 patients after transition from microsurgery to endoscopic transsphenoidal surgery at our institution.

**Methods**

Medical records of 26 consecutive patients (13 females) with a median age of 59 years who underwent endoscopic transsphenoidal surgery by a single surgeon from 2008 to 2010 and had a follow-up of at least 3 months were reviewed retrospectively.

**Results**

Fifteen patients (58%) presented with clinically non-functioning macroadenomas, 7 (27%) with hormone secreting tumors and 4 (15%) intra- or suprasellar cysts. Complete tumor resection was achieved in 10 patients (39%), near total resection (residual tumor volume < 1 cm<sup>3</sup>) in 8 (31%) and partial resection in 6 (23%, 2 missing). Visual fields remained intact in 46%, returned to normal in 12%, improved in 23% and showed no change in 15% (1 missing). Pituitary function was not affected by surgery in 18 patients (69%). Complete resolution of partial hypopituitarism was noted in 3 patients and partial recovery in 2. Three patients developed partial hypopituitarism (12%) and one a permanent diabetes insipidus. In patients with hormone secreting tumors (7) remission was noted in 3 (2 with Cushing's disease, one with acromegaly), whereas further therapy was necessary in 4. Perioperative complications occurred in 16 patients (62%). Disturbances of salt and water metabolism were noted in 12 patients. CSF leaks occurred in 5 patients and required repeat surgery in 2. Three patients had an infection in their postoperative course.

**Conclusions**

Significant tumor reduction can be achieved by the endoscopic approach while endocrine function is maintained or improved in the majority of patients. The high incidence of perioperative complications was mainly due to transient salt and water disorders.

## P265

**Pasireotide (SOM230) demonstrates efficacy in patients with Cushing's disease: results from a large, randomized-dose, double-blind, Phase III study**

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**Introduction**

Pasireotide is a multi-receptor targeted somatostatin analogue with high affinity for sst<sub>5</sub>, which is commonly expressed in corticotroph adenomas, thus having potential as therapy for Cushing's disease.

**Methods**

One hundred and sixty-two patients with persistent/recurrent or *de novo* (if not surgical candidates) Cushing's disease were randomized (double-blind) to pasireotide 600 µg (*n*=82) or 900 µg (*n*=80) sc bid. After 3mo, patients with UFC > 2 × ULN (ULN: 145 nmol/24 h) or UFC > baseline were unblinded and the dose increased by 300 µg bid. All others continued on the same double-blind

dose to 6mo. Months 6–12 were open-label with dose titration performed when needed. Primary endpoint: UFC  $\leq$  ULN at 6mo without dose up-titration from the randomized dose.

#### Results

The median percent decrease in UFC from baseline to month 2 was  $\sim$ 50% in both treatment arms and remained stable throughout the study. At 6mo, 14.6% (600  $\mu$ g) and 26.3% (900  $\mu$ g) of patients met the primary endpoint; at 12mo, 13.4% (600  $\mu$ g) and 25.0% (900  $\mu$ g) of patients had UFC  $\leq$  ULN. Patients with baseline UFC  $\leq$  5  $\times$  ULN were more likely to achieve normalized UFC. Most uncontrolled patients could be identified within 2mo, based on UFC. Serum and salivary cortisol and plasma ACTH were also reduced. As mean UFC decreased, clinical signs and symptoms, and QoL improved. The safety profile of pasireotide was similar to that of other somatostatin analogues (mostly transient GI discomfort), except for hyperglycemia; 70% of patients had a hyperglycemia-related AE. Elevated fasting blood glucose and HbA1c were seen soon after pasireotide initiation. Patients without diabetes at baseline had a lower degree of hyperglycemia. Thirteen (8.0%) patients had an AE of hypocortisolism, responsive to dose reduction.

#### Conclusion

Results from this Phase III study show that pasireotide significantly reduces elevated cortisol levels and provides clinical benefit in patients with Cushing's disease, supporting its potential for use as the first specific pituitary-targeted treatment in this disorder.

## P266

### Clonality analysis of pituitary adenomas: a pilot study

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#### Introduction

Mono-clonality of pituitary adenomas is an established fact. Still, there are exceptions and polyclonality may correlate with aggressive tumor behavior. In order to test association of clonality with pituitary adenoma characteristics in a series from Romania, we implemented a protocol for X-chromosome inactivation analysis at the androgen receptor (AR) locus (HUMARA) and validated it in a number of tumor samples.

#### Objective

To establish and validate a protocol for pituitary tumor clonality assessment using HUMARA.

#### Subjects and methods

We tested blood DNA samples from 77 pituitary adenoma female patients and 72 healthy female controls, for assessment of average heterozygosity and identification of AR alleles in our population. DNA was extracted from RNAlater-conserved tissue, for 4 somatotroph tumors (ACM) and 3 nonfunctioning adenomas (NFA). For HUMARA, DNA was incubated with HpaII or water (control) and fluorescent-labeled PCR products of AR exon1 (including the CAG repeat and 2 HpaII sites), were sized with the CEQ8000 Analyzer (Beckman) and converted to CAG-repeat numbers. The clonality ratio was calculated from the areas-under-curve of heterozygous alleles, comparing HpaII-digested versus non-digested DNA. Values  $<$ 0.4 indicate mono-clonality.

#### Results

For blood DNA, average heterozygosity was 84.7%; median (range) CAG repeats was 23 (15–30), in controls ( $n=72$ ), and 92.21%; 22 (11–29) in pituitary adenoma patients ( $n=77$ ), respectively. HUMARA revealed that 4 out of 7 adenomas were monoclonal, 2 somatotropinomas appeared polyclonal and clonality of 1 sample could not be assessed (HpaII-digested DNA did not amplify).

#### Conclusions

The HUMARA protocol proved reliable for RNA-later conserved pituitary adenoma tissue and will be used in a larger study. The 2 polyclonal adenomas need confirmation by HUMARA of micro-dissected tissue, in order to exclude contamination with normal cells.

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## P267

### Effect of gender and on the invasiveness of prolactinomas

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#### Introduction

Prolactinomas, although considered benign, show various intensity of aggressiveness. Some remain constant for whole life period while some harbour initially with invasive extension to the parasellar area. We aimed to evaluate the effect of age and gender on the invasiveness of prolactinomas.

#### Material-methods

One hundred and thirteen patients with prolactinoma were retrospectively evaluated. Magnetic resonance images were carefully examined and patients were classified as having invasive or non-invasive adenomas. Presence of cavernous sinus invasion, suprasellar extension or dimensions larger than two centimeters at the initial presentation judged as an invasive adenoma. We compared age at the diagnosis and gender in patients with and without invasive adenoma.

#### Results

Twenty-seven (23.9%) patients were male with  $40.3 \pm 12.6$  years of age, while female patients' age was  $32.6 \pm 8.3$  years. Within the 113 patients 45 (39.8%) had evidence of invasive tumour. Invasive group and non-invasive group were  $37.6 \pm 12.0$  years and  $32.3 \pm 7.9$  years old respectively ( $P < 0.01$ ). When compared patient age separately in male and female groups, age difference was not statistically significant in patients with and without invasive adenoma. Considering male patients 81.5% was classified as having invasive adenoma while in female patients invasive adenoma ratio was only 26.7% ( $P < 0.01$ ).

#### Conclusion

We conclude that male gender is an important risk factor for the invasiveness of prolactin secreting adenomas. Although patients with invasive prolactinoma are slightly older than non-invasive adenomas, the difference disappears when we compare ages in gender subgroups.

## P268

### Frequency of acute-onset symptoms and other features of craniopharyngioma presentation in children and adults

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#### Introduction

Clinical symptoms in craniopharyngioma have been repeatedly described in the literature, but few studies have addressed the issue of acute-onset symptoms in children or adults. Refinements of diagnostic procedures and altered scanning routines over the last two decades may in theory have affected patterns of clinical presentation.

#### Objective

To study the clinical phenotype of patients with newly diagnosed craniopharyngioma, with focus on variations according to age, gender and calendar year period, and to estimate the frequency of acute-onset symptoms.

#### Material and methods

In a retrospective cohort of all Danish craniopharyngioma patients ( $n=189$ ) diagnosed during the period 1985–2004, initial symptoms, clinical findings (including pituitary function) and results of neuroimaging procedures were systematically collected from medical records of each patient. Comparisons were made between subgroups based on age, gender and calendar year period, using Pearson's  $\chi^2$  test or Fisher's exact test, as appropriate.

#### Results

Acute-onset symptoms related to vision, cranial nerve function or intracranial pressure were reported in 24 patients (13%); most frequently among children, who also more often presented with hydrocephalus. Cold intolerance and depression were more frequent among adults. Frequencies of common endocrine and non-endocrine characteristics were similar among men and women.

Hypopituitarism was observed in 26–56% of children and 26–82% of adults, respectively, depending on the axis in question. The use of CT and MRI differed between the two periods. Apart from radiologic calcification and 3rd ventricle involvement being more frequently reported during the first half of the study period, clinical symptoms and signs did not differ between the two periods.

## Conclusions

Acute-onset symptoms were seen in 13% of patients with newly diagnosed craniopharyngioma and, like hydrocephalus, more commonly among children than adults. Presenting symptoms and the extent of hypopituitarism were largely similar in men and women and across the study period.

## P269

**Macroprolactinomas and pregnancy: analysis of a 38-women cohort treated by dopamine agonists**

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Pregnancy is associated with an increased risk of tumor growth in macroprolactinoma leading to discuss surgery in such cases.

## Objectives

Impact of pregnancy on prolactin secretion and tumor growth in a cohort of women with macroprolactinoma, including large tumors >20 mm, and medically treated.

## Methods

Retrospective study of all the women with macroprolactinomas diagnosed in two university hospitals, treated by dopamine agonists, with at least one pregnancy after diagnosis.

## Results

A total of 38 women, 82 spontaneous pregnancies, 64 children for 35 women. On diagnosis: mean age 23 years, median prolactin level 305 µg/l (90–7804), prolactin level ≥500 µg/l in 15 women, median tumor size 15 mm (size ≥20 mm in 12 cases), abnormal visual field in 7 cases.

Before pregnancy, 16 patients received cabergoline, 20 bromocriptine, 2 quinagolide. Prolactin level was controlled in 37 women. Visual troubles disappeared totally in 6 patients, partially in one.

Residual tumor was not visible on RMI in 10 patients, ≥10 mm in 11 (maximum 23 mm). During pregnancy, medical treatment was stopped in 20 patients (5 adenomas ≥20 mm). 8 patients had symptomatic (5 headache, 3 visual troubles) tumor evolution which was controlled by dopamine agonists either reintroduction or dose increase in 7 patients. Surgery was mandatory in one case of apoplexy during pregnancy (second trimester) and one immediately *post-partum*.

Prolactinoma size at diagnosis, residual size after medical treatment and treatment stop during pregnancy were not statistically significant risk factors of tumor enlargement.

## Conclusion

This study, performed in a large cohort, confirms that pregnancy is a risk period of symptomatic macroprolactinoma enlargement (21%) and apoplexy but no specific risk factor for adenoma growth was found. Medical treatment is able to control tumor enlargement in almost all the cases except apoplexy which requires surgery. Macroprolactinomas, even large ones, do not seem to require debulking before pregnancy.

## P270

**Primary empty sella is associated with increased cardiovascular risk, regardless the occurrence of pituitary dysfunction**

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## Introduction

Primary empty sella (PES) is a frequent 'incidental' finding reported in 5–25% of general population and is associated with endocrine abnormalities in 8–60% of cases. In this collaborative multicentric study we evaluated cardiovascular risk by assessing clinical characteristics, glucidic profile and lipid parameters.

## Design

A total of 94 PES patients (39M-55F; mean age 50.3±0.9 years; mean BMI 28.9±0.5 kg/m<sup>2</sup>) and 126 normal subjects (C) matched for sex, age and BMI were studied. Hypothalamic-pituitary function was evaluated at baseline and following conventional dynamic tests (GHRH plus arginine, ACTH test) in all patients. Cardiovascular risk parameters were assessed in all subjects. Statistical analysis was carried out by non parametric combination test and partial correlation test.

## Results

Sixty-four patients (68%) had a single or multiple pituitary hormone deficiency: growth hormone deficiency was diagnosed in 56 (87.5%), central hypothyroidism in 35 (54.6%), hypogonadotropic hypogonadism in 32 (50%) and central hypoadrenalism in 24 (37.5%).

Mean systolic blood pressure (SBP), HOMA index, total and LDL cholesterol, triglycerides, LDL/HDL and total/HDL cholesterol ratios were significantly higher and HDL cholesterol levels were significantly lower in patients, regardless the occurrence of endocrine dysfunction, than in C group ( $P < 0.02$ ). These cardiovascular risk parameters were not significantly different between patients with or without hypopituitarism. Partial correlation test showed that occurrence of central hypothyroidism was associated with high total and LDL cholesterol levels, LDL/HDL, total/HDL cholesterol ratios and HOMA index ( $P < 0.02$ ), whereas hypogonadotropic hypogonadism was associated to low HDL cholesterol, high LDL/HDL and total/HDL cholesterol ratios ( $P < 0.02$ ). On the contrary, central hypoadrenalism was associated to low systolic and diastolic blood pressure ( $P < 0.02$ ).

## Conclusions

Patients with empty sella show increased cardiovascular risk parameters regardless of endocrine dysfunction. In particular, occurrence of central hypothyroidism and/or hypogonadotropic hypogonadism was associated with glucidic and lipid abnormalities.

## P271

**Evaluation of health-related quality of life in patients with Cushing's disease and patients with non-functioning pituitary adenomas**

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## Objectives

Our aim was to assess health-related quality of life in patients with Cushing's disease (CD) and patients with non-functioning pituitary adenomas (NFPA).

## Methods

We assessed patients with CD and with NFPA treated at our outpatient units. Health-related quality of life (HRQoL) was assessed with the SF-36, a widely used generic measure of health status and the CushingQoL, a newly developed disease generated questionnaire to evaluate HRQoL in patients with Cushing's syndrome.

## Results

Fifty patients with CD (82% female, mean age 46.4±11.6) and 60 patients with NFPA (35% female, 60.2±10.6 years) participated in the study. There was a higher prevalence of macroadenomas in NFPA versus CD (83.3 vs 20.0%). 98.0% of the CD patients had been operated, 27.7% irradiated and 10% received medical treatment. CD was biochemically controlled in 37 patients (74.0%).

According to the SF-36 questionnaire, self-perceived health was very good in 8 (16%), good in 22 (44%), fair in 13 (26%) and poor in 7 (14%) patients with CD, whereas in the NFPA group, self-perceived health was excellent in 1 (1.7%), very good in 9 (15%), good in 33 (55%), fair in 13 (21.7%) and poor in 3 (5%) patients respectively. There was no significant difference between the two groups.

With the CushingQoL questionnaire, mean (s.d.) global CushingQoL score was 21.12 (10.82). None of the CD patients obtained the highest or lowest possible score. The linear regression analysis using the global CushingQoL score as the dependent variable identified a significant correlation between age ( $P = 0.044$ ), biochemical remission of the disease ( $P = 0.002$ ) and HRQoL.

## Conclusions

HRQoL is impaired in both patients with CD and patients with NFPA. Age as well as biochemical remission of the disease might play an important role in HRQoL.

**P272**

**Topoisomerase II  $\alpha$  expression in human pituitary adenomas as the prognostic factor**

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Pituitary adenomas represent more than 80% of all sella turcica tumors and 10–15% of all intracranial tumors in adults. Topoisomerase II  $\alpha$  (TopoII $\alpha$ ) is recognized as a reliable tumor proliferative activity marker. Evaluation of its expression facilitates the identification of more aggressive neoplasms.

**Aim**

To assess the topoII $\alpha$  expression in pituitary adenomas as the marker of the tumor aggressiveness, recurrence and progression.

**Material and methods**

Retrospective study included 60 patients (23 males, 37 females; aged  $46.7 \pm 17.6$  years) operated due to pituitary adenoma, followed-up for 2 years after the surgery. TopoII $\alpha$  nuclear expression was assessed immunohistochemically in tissue samples in 'hot spot' areas and indexed as % of all nuclei. The tumor recurrence, seen in 36.7% of patients, was assessed on repeated pituitary MRIs.

**Results**

73% of samples stained for topoII $\alpha$  (median index of 0.71%). The statistically significant difference in topoII $\alpha$  indices was noted between non-secreting and secreting adenomas (0.8 vs 0.41%). The topoII $\alpha$  indices were also statistically higher in macroadenomas, in tumors causing visual impairment, invading the suprasellar space and cavernous sinuses. TopoII $\alpha$  index  $> 1\%$  was independent risk factor of tumor recurrence or progression (RR – 4.65, 95% CI – 1.80–11.51); visual chiasm involvement (RR – 5.04, 95% CI 1.11–22.90), cavernous sinuses invasion (RR – 5.22, 95% CI 1.24–21.89), but not of suprasellar growth (RR – 4.01, 95% CI 0.94–17.10). The sensitivity and specificity of the topoII $\alpha$  index  $> 1\%$  in predicting pituitary adenoma recurrence was 63.6 and 86.8 respectively (area under ROC curve 0.76).

**Conclusions**

The topoII $\alpha$  expression in pituitary adenomas is related to the more aggressive tumor behavior. The index of topoII $\alpha$  higher than 1% is independent risk factor of early pituitary adenoma recurrence and progression. The topoII $\alpha$  expression should be routinely assessed in pituitary adenomas, as it gives important prognostic data.

**P273**

**Cyclooxygenase 2 expression in human pituitary adenomas**

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In many neoplasms, excessive angiogenesis is prognostic factor, independent from proliferation. Previous studies confirmed correlation between cyclooxygenase-2 (COX-2) expression and micro-vascularization density in pituitary tumors. Moreover, nonsteroidal anti-inflammatory drugs have strong inhibiting effect on COX-2 expression and possible anti-tumor activity.

**Aim**

To assess COX-2 expression in pituitary adenomas, its relation to adenoma type as well as to determine the role of COX-2 as prognostic factor of patients with pituitary tumors.

**Methods**

The retrospective study included 60 patients aged 18–85 years, after pituitary tumor surgery. COX-2 expression, determined by immunohistochemical methods, was analyzed in relation to the radiological and histopathological tumor features, clinical symptoms caused by adenomas and postoperative course of disease.

**Results**

COX-2 expression was present in 87% of adenomas with median index of 57.5%. The highest COX-2 indices characterized hormonally inactive adenomas and gonadotropinomas, the lowest - prolactinomas. There were no statistically significant differences in COX-2 expression regardless patients age and sex, tumor size, its invasiveness, visual impairment, recurrence/progression during postoperative course, the need to reoperate or irradiate.

**Conclusions**

COX-2 expression is of little prognostic value in patients with pituitary adenomas. However high COX-2 expression in pituitary adenomas, especially those hormonally inactive, makes it possible target for treatment of those tumors.

**P274**

**Recent changes in clinical presentation and therapeutic approach in MEN1-related pituitary adenomas**

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**Background and objective**

Pituitary adenomas (PAs) affect about half of the patients with multiple endocrine neoplasia type 1 (MEN1); in the most of cases PA is a PRL-oma and generally more aggressive than the sporadic form. The aim of this study is to evaluate the recent changes in clinical presentation and therapeutic approach of MEN1-related PAs.

**Patients and methods**

The study population included 17 patients with MEN1-related PA followed-up between 1990 and 2010. The whole study population included 8 PRL-omas, 2 GH-omas (associated with PRL secretion in one), 7 non-functioning adenomas. Patients were divided in two groups: group A (PA diagnosed before 2008, 9 patients) and group B (PA diagnosed in 2008–2010, 8 patients).

**Results**

The prevalence of macroadenoma was 67% in group A and 37% in group B. PA-related symptoms were present in 67% of patients of group A and 50% of patients of group B. PA was treated in 78% of patients group A (2 transphenoidal surgery, 5 medical therapy) and 50% of patients group B (4 medical therapy). Medical therapy included cabergoline for PRL-omas, somatostatin analogues for GH-oma and both for GH-PRL adenoma. Both group A patients treated with transphenoidal surgery had postoperative tumor persistence. A significant hormone response was observed in all cases of medically-treated patients of group A and in 75% of medically-treated patients of group B. At the pituitary MRI, tumor shrinkage was observed in 33% of cases in group A and 50% in group B.

**Conclusions**

Clinical presentation of MEN1-related PA is changed in the last years, resulting in early diagnosed and less aggressive tumors. For these reasons and for the availability of effective medical compounds, transphenoidal surgery is now less necessary and medical therapy with cabergoline and/or somatostatin analogues is the main therapeutic approach.

**P275**

**Prolactinoma and thyroid function**

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It has been hypothesized that prolactin may act on the immune system. It has been suggested that prolactin may act on the immune system and may be implicated in the pathogenesis of autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, as mild hyperprolactinemia has been found in patients with systemic lupus erythematosus.

We tested the hypothesis that prolactin may act on the immune system and induce the development of autoimmune thyroiditis.

We followed up for 2–8 years, 18 patients with prolactinoma and 2 patients with hyperprolactinemia. Within the patient population studied 10 patients were men and 10 were women. The patients were aged 37–68 years. Within the patient population 10 patients had a macroprolactinoma, 8 had a microprolactinoma. Surgery was used for the management of 2 patients, while all others were treated by the administration of dopamine agonists.

Within the patient population studied 2 patients had pituitary deficiency and were taking thyroxine, 2 had primary hypothyroidism due to Hashimoto thyroiditis and 2 had autoimmune Hashimoto thyroiditis, being euthyroid. The rest of the patients were euthyroid at diagnosis and remained euthyroid during follow up.

Long-term hyperprolactinemia observed in patients with prolactinoma does not appear to be related to the development of autoimmune thyroid disease, specifically autoimmune thyroiditis or thyroiditis Hashimoto.

**P276****Late development of resistance to cabergoline in a giant macroprolactinoma with aggressive carvenous sinus and sphenoidal bone invasion**G Mlawa, S Deshmukh, A Nasruddin & D Sandeman  
Southampton General Hospital, Southampton, UK.**Background**

Dopamine agonists (DA) are used as the first-line treatment in patients with macroprolactinomas. Late resistant to DA(cabergoline) therapy may occur. Patient with resistant macroprolactinoma may end up receiving a relative high dose of DA. These patients should be advised to take up radiotherapy option as earlier as possible to avoid the need of high dose dopamine agonist. We present a 63-year-old man who presented in 1999 with visual loss, due to giant macroprolactinoma compressing the optic chiasma (image1), his initial prolactin level was > 130 000 mIU/L. He responded well to cabergoline, with full recovery of his vision and prolactin maintained in the normal range (<500) on 1 mg/week of cabergoline. There was significant reduction in tumour size with small residual tumour extending into the right carvenous sinus. Prolactin levels gradually rose to > 3000 over 2 years (2005–2007) despite moderate increase in cabergoline, but there was no change in tumour size. Few months later there was dramatic increase of prolactin to > 20 000 associated with development of right 6th nerve palsy. MRI demonstrated significant tumour enlargement within the right carvenous sinus with replacement of sphenoid bone. The patient was offered radiotherapy at this stage but he declined. There was minimal response despite increasing cabergoline doses to 10 mg/week, and subsequently he underwent external beam radiotherapy in May 2008. Due to concerns of possible valvulopathy on long term high dose cabergoline (cumulative dose of 1.352 g up to June 2009), quinagolide was gradually introduced and cabergoline dose tapered down over 6 months. His ECHO revealed mild aortic regurgitation, with left ventricular dysfunction, and evidence of LBBB. Currently is 18 months post radiotherapy and remains on quinagolide 300mg daily recent prolactin of around 3000 mIU/L. His 6th nerve palsy remains but with evidence of substantial tumour regression on MRI.

**Discussion**

Late-onset resistance to cabergoline can occur as demonstrated in our patient and in this case associated with aggressive tumour growth. Early radiotherapy should be considered, and switching to different dopamine agonist which is not 'ergot-derive' may be beneficial both in terms of treatment response and avoiding risk of valvulopathy.

**P277****Minimal active acromegaly may be effectively treated by prolonged injections of octreotide LAR**

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In acromegaly, an excessive GH secretion by pituitary adenoma, minimal active disease may be defined as slow progression of characteristic signs and symptoms at presence of slightly elevated or normal IGF1 levels and lack of GH suppression after glucose load. Nadir post-glucose GH level defining active disease is under permanent discussion and depends on assessment methods. IGF1 reference range is wide, must be age-adjusted and often is sex-dependent. From mid-00's excess over upper limit reference range (ULN) for IGF1 is used to define disease activity. Acromegaly contributes to decrease in life expectancy, coexisting disease progression and social disclosure. Somatostatin analogues (SA) are effective in normalization of GH and IGF1, reducing symptoms and tumor shrinkage. Sporadically published data shows prolonged effectiveness of octreotide LAR.

**Aim**

To assess prospectively if octreotide LAR is effective if given every 6 weeks in group of minimal active acromegaly. Group: 11 patients (9F/2M) mean age 62 s.d. 14.8 y, with acromegaly diagnosed mean 9 years earlier, treated with somatostatin analogues for mean 7 years (s.d. 2.9). Out of 11 patient 7 received octreotide after uncurative surgery. In 6 of 7 surgery was preceded by SA pretreatment. In all cases minimal active acromegaly was confirmed and therapy effectiveness during standard octreotide 10–20 mg every 4 weeks (GH 1.1 ng/ml s.d. 0.9; IGF1 155 ng/ml s.d. 176=0.6 ULN s.d. 0.7) proven. No patients underwent radiotherapy. Intervention: octreotide LAR 20 mg given every 6 weeks (stable dose, injections intervals titrated accordingly to IGF1 level, target – lower than ULN).

**Results**

After 6 months of octreotide LAR 20 mg titration patients received injection every 5 weeks (1); 6 weeks (7); 7 weeks (1); 8 (1); 9(1). Mean GH levels during

stabilized treatment did not differ significantly (1.9 s.d. 1.9 NS v. previous therapy). IGF1 show trend to increase (mean 217 ng/ml s.d. 104  $P=0.3$ ; 0.9 ULN s.d. 0.4). No clinical worsening were observed. Clinical symptoms score did not differ in any of cases. We did not observed any changes in concomitant diseases intensity.

**Conclusions**

In patients with minimal active acromegaly, biochemical control and symptoms release may be controlled by titration of intervals between injections of octreotide LAR. During 2 years of observation we proven effectiveness and safety of this approach.

**P278****Radiological features of breast benign lesions in patients with hyperprolactinemia**D Radojkovic, M Pesic, S Antic, S Radenkovic, S Kostic & V Ciric  
Endocrinology Clinic, Clinical Center, Nis, Nis, Serbia.**Background**

This study was conducted to determine whether prolactin (PRL) influences radiological findings of benign breast lesions.

**Patients and methods**

We observed 50 patients with nipple discharge: 23 patients with normal serum prolactin levels (group 1;  $n=23$ ) and 27 patients with hyperprolactinemia (group 2a;  $n=27$ ). Patients observed during this study underwent breast ultrasonography (US) and mammography. Patients with hyperprolactinemia underwent US follow-up examination in period from 6 until 12 months, after serum prolactin levels were normalized (group 2b). We compared radiological findings in patients with normal serum prolactin levels to patients with hyperprolactinemia (before and after hyperprolactinemia treatment).

**Results**

The most frequent radiological findings in I group patients were dilated lactiform ductuli and cystic lesions with statistical significances  $P<0.001$  and  $P<0.01$  in comparison to other radiological findings. In II group patients significantly less presence of dilated lactiform ductuli was observed ( $P<0.05$ ) and proportional lower frequency of solid lumps compared to I group. Our results showed significantly lower incidence of dilated lactiform ductuli, after hyperprolactinemia treatment ( $P<0.001$ ). After serum PRL level normalization less presence of solid lumps and enlarged axillary's lymph nodes is also observed in comparison to I group. Cystic lesions were significantly decreased ( $P<0.05$ ) and pseudocystic lesions were completely withdrawn in patients with normalized serum prolactin levels.

**Conclusion**

Radiological presentation of benign breast diseases is under prolactin influence. Hyperprolactinemia treatment results in withdrawal of mammary duct ectasia, pseudocystic formations, cystic lesions and solid lumps. Breast ultrasonography and mammography should be considered as routine diagnostic procedures in patients with hyperprolactinemia.

**P279****A patient with ACTH-dependent Cushing's syndrome and an unexpected neuropathological diagnosis**I Kreitschmann-Andermahr, F J Hans, M Mull, W Karges & K Nolte  
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Ganglion cells in the sella turcica are rarely associated with endocrinological disease. We report the case of a 72-year-old woman who presented with typical signs and symptoms of hypercortisolism. Endocrinological work-up confirmed ACTH-dependent Cushing's syndrome with loss of circadian cortisol rhythmicity and a pathological dexamethasone suppression test. MRI revealed right-sided sellar floor excavation but no clear-cut adenoma. Via a right-sided transnasal approach, after dissection with the microdissector, greyish, diffuent tissue with a softer consistency than normal pituitary tissue was removed. Histologically, the tissue was composed of pituitary epithelial cells admixed with bipolar ganglion cells in a glial background. The ganglion cells were weakly chromogranin-A positive, whereas the endocrine cells exhibited a stronger chromogranin-A positivity and ACTH positivity. The overall picture was characteristic for 'pituitary adenoma with neuronal choristoma' – PANCH- syndrome. Such tumours are very rare entities. Most patients exhibiting PANCH present with acromegaly and <10 cases associated with Cushing's syndrome have been described in the literature. This implies that the epidemiological importance of these tumours is probably negligible. However, it cannot be excluded that they

occur more frequently but are not diagnosed in the often times small amount of tissue sent in for neuropathological examination. Moreover, the entity gives rise to speculations about its tumorigenesis. Here, a transdifferentiation of adenohypophyseal cells into neuronal cells is discussed. Alternatively, a primary ganglion cell tumour could induce adenohypophyseal hypoplasia through hormonal stimulation. A third hypothesis involves the transformation into two cell types under the influence of a common tumorigenesis factor. Clinically, the patient described above exhibits no residual disease and is kept on clinical surveillance.

## P280

### Immunoglobulin G4-related infundibulo-hypophysitis: report of 4 cases and review of the literature

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Inflammatory lesions of the pituitary gland are rarely encountered. Recently, the concept of IgG4-related systemic disease was proposed and several cases associated with infundibulo-hypophysitis were reported. We report 4 additional cases and review the cases in the literature. Case 1: 75-year-old female had diabetes insipidus due to infundibulo-neurohypophysitis for 25 years and chronic thyroiditis. She developed obstructive jaundice and surgery showed autoimmune pancreatitis. Case 2: 78-year-old female developed hyponatremia, headache and visual field defect. MRI showed thickened pituitary stalk and enlarged gland. Transsphenoidal surgery was performed to decompress and pathology showed marked inflammatory fibrosis. Chronic sialoadenitis and retroperitoneal fibrosis was diagnosed with elevated serum IgG4 levels. Case 3: 70-year-old male had sialoadenitis and diabetes insipidus with thickened pituitary stalk. He had chronic thyroiditis and hypogonadism. Serum IgG4 was elevated. He developed hydronephrosis caused by retroperitoneal fibrosis. Case 4: 72-year-old male had syncope, and MRI showed marked enlargement of stalk and pituitary gland. Surgery showed inflammatory pseudotumor with marked infiltration of IgG4-positive plasma cells. Serum IgG4 was elevated. Glucocorticoid therapy rapidly reduced pituitary mass. Fifteen cases (male 12, female 3, median age: 70 years) have been reported in the literature. Associated IgG4-related diseases included retroperitoneal fibrosis (7), pancreatitis (6) and Mikulicz disease/salivary gland (4). Pituitary pathology was investigated in 4 cases, demonstrating the inflammatory pseudotumor with fibrosis and infiltration of IgG4-positive plasma cells.

IgG4-related infundibulo-hypophysitis is a new member of hypophysitis. Determination of serum IgG4 and search for systemic IgG4-related lesions are mandatory to avoid invasive pituitary surgery.

## P281

### Assessment of retinopathy in acromegaly

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#### Introduction

Diabetic retinopathy (DR) is a vision threatening chronic microvascular complication of diabetes mellitus (DM). The potential roles of GH and insulin-like growth factor 1 (IGF1) in DR have been evaluated *in vitro* and *in vivo*. The present study aimed to assess the frequency and severity of retinopathy in acromegalic patients with various disease activity and glucose tolerance states (GTS).

#### Methods

All acromegalic patients followed in the outpatient Clinic of Endocrinology Departments of Baskent University Hospitals were included. Each patient's fundoscopic examination was carried out by an experienced ophthalmologist in each center with a standard method. Acromegaly disease states were evaluated with basal GH and IGF1 measurements and GH measurement during an oral glucose tolerance test (OGTT) where appropriate. Glucose tolerance states were assessed with fasting and postprandial glucose, glycohemoglobin measurements and OGTT where appropriate. The relations between retina findings, disease activity, and GTS were examined.

#### Results

The study group comprised 41 acromegalic cases (20 females) with a mean age of  $45.6 \pm 12.1$  years. 25 patients had active disease with median GH level of  $3.4$  ( $0.7-29.6$ ) ng/ml, IGF1 level of  $475$  ( $203-1970$ ) ng/ml, and median disease duration of  $29$  ( $3-104$ ) months. 16 patients had controlled disease with median GH level of  $1.0$  ( $0.17-2.5$ ) ng/ml, IGF1 level of  $162$  ( $68.9-348$ ) ng/ml, and median disease duration of  $40$  ( $7-420$ ) months. 5 patients in the active disease group had DM of which two were uncontrolled. The biochemically controlled acromegaly group included 5 patients with controlled DM. Overall; one patient in the entire group had background retinopathy (2.4%).

#### Conclusion

Rarity of retinopathy including those with active acromegaly and uncontrolled DM may suggest that disease activity in acromegaly might not contribute to retinopathy.

## P282

### Success rate of surgery in patients with macroprolactinoma

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In prolactin secreting pituitary adenomas, surgery markedly reduced with the efficacy of dopamine agonists in controlling prolactin hypersecretion and reducing tumor mass. Some surgeons, instead of necessity of almost life-long medical therapy, prefer and recommend surgery as a first line treatment with potentially high success rates especially in microprolactinomas. We evaluated our patients with prolactinoma operated mostly without any evidence of resistance or intolerance to dopamine agonist therapy.

Retrospective analyses of 64 patients with prolactinoma treated with surgery were included in the study. Many of the patients were operated with the aim to diminish constraint effect of the adenoma on visual area or pituitary function without any evidence of resistance or intolerance to the dopamine agonists. Fifty-seven (89%) patients had got macroadenoma while only 7 (11%) had got microadenoma. Magnetic resonance imaging of sella turcica obtained 3-6 months after operation revealed apparent remnant in 29 patients (45%). Biochemical cure was obtained in only 18 (28%) patients. Cure probability was calculated 57% (4/7) in microadenomas and 25% (14/57) in macroadenomas. Success rate for visual field deficit was 38% (10/28) and 14 patients (50%) inform only slight improvement. Four (12%) patient did not inform any improvement in their visual acuity. In 46 patients without cure after surgery, long term treatment with dopamine agonists attained apparent improvement of hyperprolactinemia and related symptoms in 45 of 46 patients (98%).

We conclude that surgery for macroadenomas do not achieve good success rate even for cure of prolactinoma but also for improvement of visual field deficit or hypopituitarism. Medical therapy should be the only first line treatment in all patients with prolactinoma unless in patients with urgent indication of surgical intervention.

## P283

### GH deficiency in cured acromegalic patients: metabolic effects of recombinant hGH replacement

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#### Background

GH deficiency (GHD) may occur in about 60% of acromegalics treated and cured by surgery or radiotherapy. Effects of GH replacement have not yet been extensively studied in such a patients.

#### Aim

To investigate whether rhGH replacement improve metabolic parameters in acromegalic patients who become GHD.

#### Patients and methods

Forty GHD patients (mean age (s.d.):  $48 \pm 10$ , BMI  $27 \pm 3$  kg/m<sup>2</sup>) were evaluated: 8 were acromegalics treated with rhGH (Group A: 5F&3M), whereas 12

acromegalics who refused treatment (Group B: 8F&4M) and 20 subjects with non-functioning pituitary adenoma on rhGH (Group C: 10F&10M) served as controls. BMI, body fat (BF%), waist circumference, IGF1 levels, glucose metabolism and lipid profile were assessed at the time of GHD diagnosis, after 12 and 36 months of rhGH treatment (mean rhGH dose  $0.28 \pm 0.02$  mg/day).

#### Results

At baseline Group B showed higher IGF1 levels than Groups A and C ( $153 \pm 38$  vs  $90 \pm 27$  and  $80 \pm 41$  ng/ml respectively,  $P=0.001$ ), and higher post-OGTT glucose levels than group A ( $127 \pm 34$  vs  $90 \pm 32$  mg/dl,  $P=0.05$ ), while no difference among Groups was recorded for the other parameters. After 12 months, IGF1 levels significantly increase in group A and C, remaining in the middle-upper part of the normal range. A decrease in BF% (from  $36.3 \pm 5.2$  to  $31.7 \pm 2.4$  and from  $33 \pm 9$  to  $30 \pm 9$  in Group A and C respectively,  $P<0.01$ ) and total-cholesterol (from  $252 \pm 50$  to  $203 \pm 57$  and from  $261 \pm 56$  to  $209 \pm 51$  mg/dl in Group A and C respectively,  $P=0.04$ ) was observed. In Group A fasting and post-OGTT glucose levels, HbA1c and HOMA did not change on rhGH. No side effects were recorded.

#### Conclusions

In GHD acromegalics, rhGH improved body composition and lipid profile, without deterioration of glucose tolerance. GH replacement should be considered in these patients, as in patients with GHD from other causes.

## P284

### Improvement in clinical signs and symptoms of Cushing's disease following 12 months' pasireotide therapy

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#### Introduction

The multi-receptor targeted somatostatin analogue pasireotide has demonstrated efficacy in reducing cortisol in patients with Cushing's disease in a large, randomized, double-blind, Phase III study. The effects of pasireotide on the signs and symptoms of Cushing's disease were also investigated as secondary objectives in this trial.

#### Methods

Adult patients with persistent/recurrent or *de novo* Cushing's disease were randomized to receive pasireotide 600 ( $n=82$ ) or 900 ( $n=80$ ) µg sc bid for 12 months. Dose increases of 300 µg bid were permitted after month 3 depending on 24-h urinary-free cortisol (UFC) levels. Clinical signs and symptoms of Cushing's disease were evaluated at regular intervals. Health-related quality of life (HRQoL) was assessed using the CushingQoL questionnaire at baseline, month 3, 6 and 12.

#### Results

Reductions in mean UFC were accompanied by a rapid and substantial reduction in both mean ( $\pm$  s.d.) systolic and diastolic blood pressure (from  $133.5 \pm 19.1$  to  $126.1 \pm 14.1$  mmHg and  $86.4 \pm 12.7$  to  $82.8 \pm 9.7$  mmHg respectively) over the course of the 12-month study period. Mean weight decreased from  $81.6 \pm 21.6$  to  $74.4 \pm 18.7$  kg, accompanied by a reduction in BMI. Mean LDL-cholesterol and triglyceride levels were reduced from  $3.52 \pm 1.02$  to  $3.04 \pm 1.16$  mmol/l and  $1.83 \pm 1.02$  to  $1.55 \pm 0.80$  mmol/l respectively. Mean HRQoL scores increased from values of  $41.1 \pm 20.4$  at baseline to  $50.4 \pm 20.1$  at month 6 and  $52.5 \pm 19.2$  at month 12. Improvements in facial rubor, supraclavicular and dorsal fat pads were also observed. These clinical changes were achieved irrespective of patients achieving normalized UFC levels. Adverse effects were fairly typical of somatostatin analogues, except for hyperglycemia.

#### Conclusion

Rapid and substantial improvements in the signs and symptoms of Cushing's disease were seen following pasireotide treatment, and were maintained during 12 months' therapy. Improvements in these parameters were not dependent on UFC normalization, suggesting a reduction in UFC can be associated with long-term clinical benefit.

## P285

### Pituitary calf transplantation into the human, 50 years after

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#### Introduction

Hundred years ago Harvey Cushing attempt first human pituitary transplantation. This operation, in different modifications, was performed in the next 50 years, but now is used rather only in animal experiment. The problem of effective therapy of hypopituitarism, other than substitution, is however still open. That encouraged us to present a patient 50 years after pituitary xenotransplantation.

#### Case

A 75-year-old woman was admitted to hospital because of weakness and malaise. The symptoms occurred when her doctor reduced the dose of levothyroxine (because of the low TSH). The result of physical examination was appropriate for age, blood pressure 130/70 mmHg, pulse 75 b/min. Daily rhythm of cortisol was preserved ( $16.0$  µg/dl morning and  $8.3$  µg/dl afternoon) by normal morning ACTH ( $31$  pg/ml). Serum levels of thyroid hormones were in lower limit of normal range (FT3\_3 pg/ml, FT4\_0.8 ng/dl), similar as thyroid antibody. TSH, however, was very low ( $0.04$  µIU/ml), correspondingly with low prolactin ( $0.4$  ng/ml), FSH ( $1.5$  mU/ml) and LH ( $0.75$  mU/ml). Other laboratory parameters were normal. MRI showed at the bottom of the Turkish saddle, a small, asymmetrical pituitary, bruised by the arachnoids hernia. According to medical documentation, in 1959 calf pituitary was transplanted into her peritoneal cavity(?), because of Sheehan syndrome. Patient claimed that the graft was 'not accepted' contrary to other patients. The menstruation did not return. After that she was treated by different physicians, but complete reconstruction of treatment was impossible.

#### Conclusion

The *postpartum* pituitary failure with a preserved adrenal axis function is not so frequent, but not as rare as ectopic xenotransplantation. Was this attempt irrelevant for patient's health? The answer would be easier if we could observe other patient after successful transplantation. Unfortunately, despite of poor HLA expression in the pituitary, we have never met such person.

## P286

### The impact of transsphenoidal surgery on glucose homeostasis and insulin resistance in acromegaly

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#### Introduction

Impaired glucose tolerance and overt diabetes mellitus are frequently associated with acromegaly. The aim of this study was to find whether these alterations could be reversed after transsphenoidal surgery.

#### Patients and methods

Two hundred and thirty-nine acromegalic patients were studied before and 6–12 months after transsphenoidal surgery. 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). To estimate insulin resistance we used homeostasis model assessment (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI).

#### Results

At the moment of diagnosis, diabetes mellitus was present in 25% of the acromegalic patients. After surgery, the prevalence of diabetes mellitus normalized to the level present in the general Polish population. We found a statistically significant reduction after surgery in plasma glucose levels both fasting ( $89.45 \pm 13.92$  vs  $99.12 \pm 17.33$  mg/dl,  $P<0.001$ ) and during OGTT. Similarly, a prominent reduction in insulin secretion was found after surgery compared to the moment of diagnosis ( $15.44 \pm 8.80$  vs  $23.40 \pm 10.24$  mIU/ml,  $P<0.001$ ). After transsphenoidal surgery, there was a significant reduction in HOMA-IR ( $3.08$  vs  $6.76$ ,  $P<0.0001$ ) and a significant increase in QUICKI ( $0.32$  vs  $0.29$ ,  $P<0.001$ ). There were no statistically significant differences after surgery in fasting glucose and insulin levels between patients with controlled and inadequately controlled disease.

#### Conclusions

We concluded that in acromegalic patients glucose homeostasis alterations and insulin sensitivity can be normalized after transsphenoidal surgery, even if strict biochemical cure criteria are not fulfilled.



**P287****Tumor size and parasellar extension influence on macroprolactinomas' outcome**

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**Introduction**

Parasellar extension of macroprolactinomas defined on imaging criteria was reported to be an independent predictor of hormonal resistance to dopamine agonists (DA).

**Methods**

Two hundred and twelve patients with macroprolactinomas (87 M/125 F), treated with DA for 5 years median period; prolactin (fluoroimmunoassay or chemiluminescence), CT scan and/or MRI with contrast agents were performed; maximum diameter evolution was reported.

**Results**

Parasellar extension or giant tumors ( $\geq 4$  cm) were encountered in 71 patients (33.5%) – group A, while 141 tumors formed group B. According to prolactin normalization, patients were classified into cured patients ( $n=16$ ), pseudo-cured patients (recurrence of hyperprolactinemia after DA withdrawal) ( $n=11$ ), patients responsive to DA, but not fulfilling the criteria for DA withdrawal ( $n=157$ ), patients initially resistant to DA therapy ( $n=28$ ). Patients responsive to DA (cured, pseudo-cured and responsive) had significant lower prevalence of parasellar extension (57/184, 30.9%) versus patients resistant to DA (15/28, 53.6%),  $P=0.02$ . Within responsive group, prevalence of parasellar extension and giant tumors was similar between cured patients (4/16, 25%), pseudo-cured patients (3/11, 27.3%), and responsive patients (50/157, 31.8%),  $P=NS$ . Regarding tumor volume outcome, tumor shrinkage  $\geq 50\%$  or small residual necrotic tumor was noticed significantly more frequent in group A (40/71, 56.3%) versus group B (49/141, 34.8%),  $P=0.003$ ; complete disappearance of tumor, tumor shrinkage  $<50\%$ , stationary tumor and tumor increase were similar in both A, B groups.

**Conclusion**

Parasellar extension and/or tumor size over 4 cm are associated in macroprolactinomas with resistance of prolactin normalization on dopamine agonist therapy, but not with resistance to tumor shrinkage.

**P288****Pegvisomant therapy in acromegaly: a multicenter observational study**

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Patients with acromegaly resistant to conventional drug treatment currently can advantage with GH-receptor antagonist pegvisomant. To date, at doses up to 40 mg/day, it is capable of normalizing circulating IGF1 in until 97% of patients. Here we present the multicenter experience in Rome with Pegvisomant as a therapeutic option in acromegaly. This is an observational study including a total of 61 patients (21 males and 40 females) treated with pegvisomant for up to 7 years. Of all patients, 59 were reported to have had surgery and 17 to have received radiation therapy; 57 had received somatostatin analogues. Before starting Pegvisomant, all patients had IGF1 values above the upper limit of age and sex-adjusted normal range. A total of 53 patients (87%) normalized the IGF1 value on pegvisomant therapy. A transitory increase of liver enzymes was observed in 7 (11.5%) patients and completely resolved after temporary stopping of pegvisomant. In 2 patients we observed adverse reaction at the injection site. No patient had an increase of the tumor volume at pituitary magnetic resonance imaging and, in 1 case, control MRI documented a gradual decrease of pituitary adenoma which disappeared after 6 years of treatment with pegvisomant, without other pharmacological association. In 2 cases, the treatment with at 10 mg daily dose induced an excessive reduction of IGF1 with onset of severe fatigue and diffuse myalgia, suddenly and significantly improved in both patients after alternate-day administration. Our experience indicates that pegvisomant is a safe and effective treatment in acromegaly and that it is well-tolerated. However, caution should be taken in starting treatment at the lowest dose and accurately

monitoring IGF1 levels 4/6 weeks after the initiation of pegvisomant. Moreover, it is necessary to carefully consider all new symptoms manifested by patients treated with pegvisomant.

**P289****ACTH-secreting pituitary adenomas express higher SSTR5 than SSTR2 protein levels by immunohistochemistry using new monoclonal antibodies against SST-binding domains**

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Pasireotide (SOM230), a somatostatin (SST) analogue targeting multiple SST receptors, is effective in reducing urinary free cortisol in patients with Cushing disease (CD), due to inoperable or recurrent ACTH secreting pituitary adenomas. The efficacy of SOM230 seems to be dependent on the presence of appropriate SSTRs, mainly SSTR2 and 5, on the membrane of tumor cells. Polyclonal antibodies available up to now recognizing cellular intracellular sites of receptors and their recycling products are of limited performance in paraffin-embedded tissues. Aim of this study was to evaluate by immunohistochemistry the expression of SSTR 2 and 5 on ACTH-secreting cells using new MoAbs against the sst-binding domain. We used paraffin tissue sections from 45 ACTH-secreting pituitary adenomas. 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**

SSTR2 and SSTR5 were found respectively, in 80% and in 100% of adenoma samples. The immunostaining intensity score was higher for SSTR5 (high/medium-high) than for SSTR2 (low/medium-low). Negative control reactions were performed by incubation with a pre-immune serum. The specificity of the reactions was tested by pre-adsorption of antibodies with their immunospecific peptides.

**Conclusion**

SSTR5 was present in all of ACTH-secreting pituitary adenomas examined, and was associated with SSTR2 positivity in a majority of them. An higher immunostaining score was found for SSTR5 than for SSTR2. These data demonstrate that Y-SSTR2 and 5 MoAbs are able to detect SSTRs on paraffin embedded histological sections. Finally, our findings support the use of SOM230 in CD due to resistant/recurrent ACTH-secreting pituitary tumors.

**P290****Cardiac risk in patients with treatment naïve, first-line medically controlled and first-line surgically cured acromegaly in comparison to matched data from the general population**

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The influence of first-line transphenoidal surgery (TSS) or first-line somatostatin analogue (SSA) treatment on coronary risk factors in patients with acromegaly has rarely been examined. Aim of this study was to evaluate risk factors for coronary heart disease in 3 different patient groups with treatment naïve, active (ACT), first-line medically controlled (MED) and first-line surgically treated (SUR) acromegaly and to calculate the Framingham Risk Score (FS).

**Design**

Retrospective-comparative-nonrandomized-case-control study.

**Patients and methods**

Forty acromegalic patients (28 men, 15 treatment-naïve, 13 SSA treated, 12 operated by TSS, aged 45–74 years) were matched for age and gender to 200 controls from the general population. Coronary risk factors were assessed by interviews and direct laboratory measurements. For purposes of the study, only controlled patients in MED and SUR group were included.

**Results**

Compared to matched controls ACT patients had increased prevalence of left ventricular hypertrophy (LVH) (30.8 vs 3.2% ( $P < 0.001$ )), HbA1c ( $6.9 \pm 1.4$  vs  $5.4 \pm 0.7\%$  ( $P < 0.0001$ )), a trend of higher prevalence of hypertension (38.5 vs 36.9% ( $P = 0.08$ )) and a FS in the high-risk group ( $22.0 \pm 22.7$  vs  $16.1 \pm 8.1\%$  ( $P = 0.81$ )). Odd's ratio (OR) by multivariate conditional logistic regression for FS was 1.06 (0.99–1.13) ( $P = 0.08$ ). MED and SUR groups were similar for gender, age, disease duration and IGF1 levels. MED patients had a significantly increased diastolic blood pressure ( $89 \pm 9$  vs  $79 \pm 10$  mmHg ( $P = 0.001$ )), prevalence of LVH (41.7 vs 1.7% ( $P < 0.0001$ )), BMI ( $31.3 \pm 5.2$  vs  $27.5 \pm 4.4$  kg/m<sup>2</sup> ( $P = 0.02$ )), prevalence of diabetes mellitus (33.3 vs 10.0% ( $P = 0.03$ )), HbA1c ( $6.8 \pm 1.3$  vs  $5.5 \pm 0.7\%$  ( $P = 0.0005$ )) and FS ( $21.2 \pm 9.7$  vs  $12.4 \pm 7.7\%$  ( $P = 0.002$ ), OR 1.11 (1.01–1.21) ( $P = 0.03$ )) while in SUR group only prevalence of LVH (40.0 vs 4.1% ( $P < 0.001$ )) and HbA1c ( $6.4 \pm 1.2$  vs  $5.5 \pm 0.8\%$  ( $P = 0.006$ )) were significantly elevated but not FS ( $14.8 \pm 8.2$  vs  $12.6 \pm 8.1\%$  ( $P = 0.21$ ), OR 1.07 (0.97–1.18) ( $P = 0.19$ )).

**Conclusion**

When comparing treatment naïve, medically treated and surgically cured patients with acromegaly to age- and gender-matched cohorts of the general population, we have found a significantly increased CV risk in patients 12 months after first-line SSA treatment but not in patients after first-line surgery.

**P291****GH receptor mRNA expression and the effect of pegvisomant on GH secretion by somatotroph pituitary adenomas**

S J C M M Neggers<sup>1</sup>, M Bidlingmaier<sup>3</sup>, P M van Koetsveld<sup>2</sup>, R A Feelders<sup>1</sup>, D M Spruij-Mooij<sup>2</sup>, W W de Herder<sup>1</sup>, A J van der Lely<sup>1</sup> & L J Hofland<sup>2</sup>

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**Background**

Of the currently available treatment regimes for acromegaly, pegvisomant (PEG-V) has the highest efficacy. During PEG-V treatment, GH serum levels increase. The exact mechanism behind this is remains unclear. It could be explained by an ultra-short feedback loop via GH receptors in the anterior pituitary gland.

**Objective**

To assess the level of GH receptor (GHR) mRNA expression in somatotroph adenomas and to evaluate whether GHR blockade by PEG-V can lead to an increase in GH levels.

**Design**

In 32 somatotroph adenomas and 4 samples of human liver tissue after RNA extraction, mRNA expression of the full length GHR and d3GHR variant were assessed by real time PCR. After 24 and 72 h incubation with PEG-V, in different concentration ( $10^{-6}$ – $10^{-9}$  M), GH secretion was assessed in three primary GH-secreting cultures. With a two-site immunofluorometric assay having no cross-reaction with PEG-V GH concentrations were determined.

**Results**

In somatotroph adenomas, detectable levels of full length GHR and the d3GHR variant were found in 27 of 32 (84%) and in 23 of 32 (72%) cases respectively. GHR mRNA levels, full length and d3GHR, in the somatotroph adenomas amounted 0.8 and 4.8% of the expression level in human liver. The addition of PEG-V did not increase GH secretion by the cultured GH-secreting pituitary adenoma cells.

**Conclusion**

In human somatotroph pituitary adenomas the expression of GHR mRNA is low and PEG-V does not increase GH secretion *in vitro*. There was no relationship between expression of GHR in the adenoma and the effect of PEG-V. Therefore, the increase in GH level during PEG-V treatment in acromegaly patients seems not mediated by interference with an ultra-short feedback loop via GHR in the somatotroph adenoma, but rather via reduced feedback due to the lowered circulating IGF1 levels.

**P292****Carotenoids inhibits cell proliferation, arrest cell cycle and induces apoptosis in pituitary tumor cells**

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Pituitary adenomas account for ~10% 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 preventive strategies, and in this context, the use of functional foods with chemopreventive compounds appears to contribute much in this process. Among these substances, we highlight the consumption of carotenoids is associated with reduced risk of chronic diseases including cancer and vascular diseases. This study aimed to evaluate the influence of  $\beta$ -carotene and lycopene in proliferation, cell cycle and apoptosis of mouse ACTH-secreting pituitary adenoma cells (AtT-20). AtT-20 cells were incubated with DMEM supplemented with 10% fetal bovine serum under an atmosphere of 5% CO<sub>2</sub> at 37 °C. The cells were incubated with different concentrations of carotenoids (0.5–40  $\mu$ M) for 48 and 96 h. Assessment of cell proliferation was assessed using the colorimetric MTT. The analysis by flow cytometry was used to assess the distribution of the phases of the cell cycle. Apoptosis was assessed using the annexin V-fluorescein isothiocyanate apoptosis detection kit I. The results of cell viability showed that both lycopene and  $\beta$ -carotene decreased the proliferation of AtT-20 after 48 and 96 h. The cell cycle analysis revealed that lycopene increased proportion of cell line AtT-20 in the G0/G1 phase and decreased cells in S and G2/M phases after 96 h. Furthermore,  $\beta$ -carotene induced an accumulation in S and G2/M phases in the line AtT-20 after 96 h. The process of apoptosis was induced by  $\beta$ -carotene (10  $\mu$ M) after 96 h, and lycopene in a dose-dependent manner induced apoptosis after 48 and 96 h. Lycopene and  $\beta$ -carotene showed as potent inhibitors of cell growth, suggesting a modulation of the cell cycle of pituitary tumor cells, with an increase in apoptosis and the effect was dose and time dependent.

**P291****GH receptor mRNA expression and the effect of pegvisomant on GH secretion by somatotroph pituitary adenomas**

S J C M M Neggers<sup>1</sup>, M Bidlingmaier<sup>3</sup>, P M van Koetsveld<sup>2</sup>, R A Feelders<sup>1</sup>, D M Spruij-Mooij<sup>2</sup>, W W de Herder<sup>1</sup>, A J van der Lely<sup>1</sup> & L J Hofland<sup>2</sup>

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**Background**

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**Objective**

To assess the level of GH receptor (GHR) mRNA expression in somatotroph adenomas and to evaluate whether GHR blockade by PEG-V can lead to an increase in GH levels.

**Design**

In 32 somatotroph adenomas and 4 samples of human liver tissue after RNA extraction, mRNA expression of the full length GHR and d3GHR variant were assessed by real time PCR. After 24 and 72 h incubation with PEG-V, in different concentration ( $10^{-6}$ – $10^{-9}$  M), GH secretion was assessed in three primary GH-secreting cultures. With a two-site immunofluorometric assay having no cross-reaction with PEG-V GH concentrations were determined.

**Results**

In somatotroph adenomas, detectable levels of full length GHR and the d3GHR variant were found in 27 of 32 (84%) and in 23 of 32 (72%) cases respectively. GHR mRNA levels, full length and d3GHR, in the somatotroph adenomas amounted 0.8 and 4.8% of the expression level in human liver. The addition of PEG-V did not increase GH secretion by the cultured GH-secreting pituitary adenoma cells.

**Conclusion**

In human somatotroph pituitary adenomas the expression of GHR mRNA is low and PEG-V does not increase GH secretion *in vitro*. There was no relationship between expression of GHR in the adenoma and the effect of PEG-V. Therefore, the increase in GH level during PEG-V treatment in acromegaly patients seems not mediated by interference with an ultra-short feedback loop via GHR in the somatotroph adenoma, but rather via reduced feedback due to the lowered circulating IGF1 levels.

**P293****Prevalence of sleep apnea syndrome (SAS) in patients with acromegaly**

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Sleep apnea syndrome is highly prevalent in acromegaly and only partially reversible after biochemical control of the disease. Consequences of SAS are serious and are associated with increased morbidity and mortality.

The present study evaluates clinical aspects in acromegalic patients with sleep apnea syndrome (SAS), diagnosed and followed-up in the Clinic of Endocrinology, Timisoara, in the period 2007–2010. All the cases were confirmed and investigated as present guidelines recommend.

We collected general data, medical history, physical evaluation, sleep questionnaires, anthropometric measurements, polysomnography for apnea-hypopnea index (AHI normal 0–4, mild 5–14, moderate 15–29, severe over 30), oxygen desaturation, co morbidities and measured mean values, standard deviation, 95% confidence interval (CI).

The polysomnographic investigated study group comprised 13 patients with GH secreting pituitary adenomas.

AHI normal 1 of 13, mild 2 of 13, moderate 4 of 13, severe 6 of 13, 10 females, 3 males, age  $48 \pm 11.6$  years (31–62) 95% CI 40.99–55.01, 33.7% smokers, obesity 10 of 13 with BMI  $34.4 \pm 5.98$  kg/m<sup>2</sup> (25–45) 95% CI 30.77–38, neck circumference  $40.46 \pm 3.48$  cm 95% CI, 38.36–42.56, abdominal circumference  $104.23 \pm 11.8$  cm 95% CI 97.11–111.3. Epworth Sleepiness Scale  $8.16 \pm 5.35$  (2–20), 95% CI 4.92–11.39. Oxygen desaturation  $94.308 \pm 1.55$ , 95% CI 93.37–95.24, lowest desaturation  $82.69 \pm 11.7\%$  (51–96), 95% CI 75.6–89.78. Hypertension 7 of 13, most in stage 2 and 3, duration  $13.6 \pm 8.02$  years (7–27) 95% CI 3.64–23.55, 5 of 13 with resistant hypertension.

OSAS is frequent in acromegaly. Most patients are obese female with few symptoms and less somnolence, decreased oxygen desaturation, high prevalence of systemic hypertension, mostly resistant.

## P294

### Patients previously treated for nonfunctioning pituitary macroadenomas have disturbed sleep characteristics, circadian movement rhythm, and subjective sleep quality

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#### Context and objective

Fatigue and excessive sleepiness have been reported after treatment of nonfunctioning pituitary macroadenomas (NFMA). Because these complaints may be caused by disturbed nocturnal sleep, we evaluated objective sleep characteristics in patients treated for NFMA.

#### Design

Controlled cross-sectional study.

#### Subjects and methods

We studied 17 patients (8 women, mean age 54 years), in remission of NFMA during long-term follow-up (8 years, range 1–18 years) after surgery ( $n=17$ ) and additional radiotherapy ( $n=5$ ) without co-morbidity except for hypopituitarism and 17 controls matched for age, gender, and BMI. Sleep was assessed by nocturnal polysomnography, sleep and diurnal movement patterns by actigraphy, and quality of life and subjective sleep characteristics by questionnaires.

#### Results

Compared to controls, patients had reduced sleep efficiency ( $P=0.008$ ), less REM-sleep (17.1 vs 25.4%,  $P<0.001$ ), 10% more N1 sleep ( $P=0.001$ ) and more awakenings, in the absence of excessive apnea or periodic limb movements. Actigraphy revealed a longer sleep duration and profound disturbances in diurnal movement patterns, with more awakenings at night and less activity during the day. Patients scored higher on fatigue, and reported impaired quality of life.

#### Conclusion

Patients previously treated for NFMA suffer from decreased subjective sleep quality, disturbed distribution of sleep stages and disturbed circadian movement rhythm. These observations indicate that altered sleep characteristics may be a factor contributing to impaired quality of life and increased fatigue in patients treated for non-functioning macroadenomas.

## P295

### The dual PI3K/mTOR inhibitor NVP-BEZ235 has a potent antiproliferative action in human nonfunctioning pituitary adenomas

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NVP-BEZ235 is an orally available ATP competitive inhibitor of class I PI3K and mTOR. As such it suppresses Akt phosphorylation downstreams to PI3K and to mTOR complex 2 in addition to p70/S6K1 phosphorylation downstreams to mTOR complex 1 and displays potent antiproliferative activity in tumors with dysregulated the PI3K/Akt pathway. Administration of NVP-BEZ235 in human nonfunctioning pituitary tumors in primary cell culture suppressed cell viability by more than 50% in 37 out of 40 cases. The effect was cytostatic causing cell cycle arrest at the G1 phase. Transition through the G1 phase is governed by cyclin-cyclin dependent kinase (Cdk) complexes which phosphorylate retinoblastoma, triggering E2F transcription and the expression of proteins that are needed to drive the cell further into mitosis. NVP-BEZ235 treatment in immortalized pituitary tumor cells suppressed E2F transcriptional activity and subsequently cyclin E levels. NVP-BEZ235 decreased cyclin D1 and D3 levels, but only at high concentrations (100 nM) and did not affect Cdk4/6 levels. In contrast it increased the cell cycle inhibitor p27/Kip1 levels (even at lower concentrations; 10 nM), but not its transcription indicating regulation at posttranscriptional level. Altogether these data indicate a potential for the dual PI3K/mTOR inhibitor NVP-BEZ235 for the pharmacological treatment of non-functioning pituitary adenomas.

## P296

### Primary pituitary tumors: 10-year single center experience

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Primary pituitary tumors are relatively rare and mainly benign.

The aim was to study the primary pituitary tumors presenting during a 10-year period in a single center.

The archives of all patients with primary pituitary tumors having been hospitalized from 01.01.2000 to 30.06.2010 in the Department of Endocrinology and Metabolism of Metaxa Hospital were studied. Within this cohort of patients 128 were female (63%) and 75 (37%) were male (ratio 1:1.7). All patients had benign neoplasms. The majority of the patients had acromegaly (78 patients, 38%) and non secreting pituitary adenomas (58 patients, 28%). Patients with prolactinoma represented 17% (35 patients), while patients with Cushing's disease 11% (23 patients). In 1 patient (<1%) a TSH secreting adenoma was diagnosed. Multiple hormone secreting adenomas had 4 patients (2%). Within this cohort 3 patients (<2%) had adenomas secreting GH and prolactin and 1 had an adenoma secreting GH and TSH. In 3 (<2%) patients cysts were found in the pituitary and in 1 of them it proved to be Rathke's cyst. Finally, in 1 patient (<1%) a craniopharyngioma was diagnosed.

In conclusion, primary pituitary tumors are rare and mainly benign. In our cohort prolactinomas appear not to be as many as expected, as currently patients with prolactinoma are treated mainly as outpatients.

## P297

### Pituitary apoplexy: clinical characteristics, circumstances of diagnosis and evolution in a Romanian patient series

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Pituitary apoplexy is a rare syndrome due to hemorrhagic infarction of a pituitary adenoma. We analyze the circumstances of diagnosis, clinical features and evolution in a retrospective review of patients with pituitary apoplexy admitted between 2000 and 2010, diagnosed by pathological exam and/or suggestive imaging for pituitary haemorrhage.

#### Results

From 31 patients with pituitary apoplexy (11 women, 20 men) with a mean age of 48 years at diagnosis (20–75 years), 24 patients had clinically non-functioning pituitary adenomas, 3 acromegaly, 3 prolactinomas and 1 Cushing's disease – all macroadenomas. In 90% of patients (28/31) the pituitary adenoma has not been previously diagnosed. In all patients apoplexy was clinically overt, with headaches in 96.5%, visual disturbances in 75.8%, vomiting in 51.8%, diabetes insipidus (DI) in 6.4% of patients. Previous or current pregnancy was noted in 2 patients, dopamine agonist treatment in 3, trauma in 2, thrombolysis for coronary event in 1 patient. Pituitary surgery was done in 27 patients (7 by frontal, 20 by transphenoidal approach). In 7 patients with hypopituitarism identified at diagnosis, the pituitary function recovered in 2 of the 4 operated patients. Cure of a hypersecreting tumor was recorded in 1 operated and in 2 non-operated patients. After surgery, hypopituitarism was diagnosed in 21/26 patients (80.8%), transient DI in 6/26 (23%). However surgery induced complete recovery of the visual disturbances in 9/22 (41%) and partial recovery in 8/22 (36%) of the affected patients. After a median follow-up of 31.5 months (6–300 months), hypopituitarism improved in 5/24 patients (20%), none of the tumors showed re-growth, in 6/18 patients (33%) the tumors decreased spontaneously by more than 50% and 5/20 patients (25%) were tumor-free.

#### Conclusion

Pituitary apoplexy may result in spontaneous tumor remission (6% in our series), but is frequently associated with hypopituitarism.

## P298

### Effects of short (6 months) and long (18 months) term treatment with GH-receptor antagonist pegvisomant (PEG) on rhythm disturbances in acromegaly

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#### Aim

To evaluate the effects of short and long-term treatment with PEG on rhythm disturbances in a cohort of acromegalic patients resistant to long-term high-dose therapy with SA.

**Patients and methods**

Thirteen patients (4 M, 9 F, aged  $44 \pm 9.25$  years) entered the study. All patients started PEG at initial dose of 10 mg daily, then increased of 5 mg every 6 weeks on the basis of IGF1 levels, until IGF1 normalisation or until the achievement of the maximum dose of 40 mg daily. A standard 24-h ECG registration was performed in all patients at study entry and after 6 and 18 months of PEG to evaluate: mean (HR), maximum (MHR) and minimum (mHR) heart rate, pauses number (P) and duration (PD), supraventricular episodes number (SE) and duration (SED), ectopic beats number (EB) and duration (EBD).

**Results**

Compared to study entry, a slight but not significant decrease in HR, MHR and mHR was observed after 6-month PEG. Moreover, compared to study entry a significant decrease in HR ( $P=0.03$ ), MHR ( $P=0.05$ ) and mHR ( $P=0.05$ ) was found after 18-month PEG. At study entry, one patient showed 36 P with PD of 2200 ms, 6 SE with SED of 400 ms and 1 EB with EBD of 200 ms; after long-term PEG, no P, no SE and no EB were recorded. Another patient, the youngest man, at study entry showed no P and no SE; after 6-month PEG 21 SE with SED of 920 ms were recorded, whereas after 18-month PEG 4 P with PD of 200 ms and 2 SE with SED of 600 ms were recorded.

**Conclusions**

In acromegalic patients resistant to long-term high-dose SA therapy long-term treatment with PEG reduces HR, MHR and mHR so that to improve hyperkinetic syndrome.

**P299****Pituitary dysfunction types and clinical presentation of pituitary microadenomas in childhood and adolescence**

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Magnetic resonance imaging has dramatically improved the diagnostic accuracy in the evaluation of the hypothalamo-pituitary region and investigation of morphological and structural pituitary abnormalities.

**Aim**

To estimate pituitary dysfunction types and clinical presentation of pituitary lesions on MRI investigation, assigned as hypointense intrasellar mass (microadenoma-MA).

**Patients and methods**

We examined 19 children and adolescents aged from 5 to 17.5 years (mean 12.24) with microadenomas. Assessment of growth, pubertal staging and complete evaluation of basal pituitary function were carried out in all patients. Dynamic tests of somatotrophic, corticotrophic and gonadotrophic functions were done where appropriate.

**Results**

At admission 4 patients with microprolactinomas presented short stature, obesity gynecomastia and puberty disorders and additional 6 with non functioning microadenomas (NFMA) presented short stature and delayed puberty. Four patient had precocious puberty, one male with beta subunit secreting microadenoma, and 3 females with non functioning pituitary micro adenomas. Among two adolescents with tall stature and NFMA one have delayed puberty. Adolescent with prolonged gynecomastia was diagnosed, due to dynamic tests, as primary hypothyroid and with hyperprolactinaemia. Also interesting results were found in 4 obese adolescent girls with secondary amenorrhea. Although with normal basal hormonal status, they underwent meticulous dynamic investigation, because of presence of pituitary lesions, and we discovered primary hypothyroidism and hyperprolactinaemia in all of them. Appropriate treatment led to shrinkage or complete radiological resolution of MAs.

**Conclusion**

Children and adolescents with pituitary MRI lesions, assigned as MA presented a wide variety of clinical characteristics. MRI finding of MA obligated us to perform a careful dynamic examination in patients with normal basal hormonal status and allowed accurate diagnosis and treatment. Clinical presentation even in NFMA is evidence of hypothalamic dysfunction, not always detectable even in dynamic tests.

**P300****Secondary erythrocytosis due to acromegaly**

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A 53-year-old man with acromegaly was referred for neurosurgical treatment. At admission: GH 240 ME/l at all time points of glucose tolerance test, IGF1 – 415.1 ng/ml, brain MRI showed pituitary adenoma  $34 \times 32 \times 27$  mm extending suprasellar, deforming and displacing optic chiasm, parasellar to both cavernous sinuses and surrounding left internal carotid artery. Clinical blood tests revealed marked erythrocytosis –  $6.1 \times 10^{12}$ , Hb – 200 g/l, HCT – 62%; urobilinogen 50 mmol/l was found in urine; otherwise the tests were normal. For exclusion of erythraemia the patient underwent trepanobiopsy of bone marrow which showed normal haemopoiesis and suggested a secondary nature of erythrocytosis. In preoperative period 1100 ml of blood were evacuated by phlebotomy in 6 sessions, which resulted in reduction of erythrocyte count to  $5.1 \times 10^{12}$ , Hb 160 g/l, HCT 50% and disappearance of urobilinogen. The patient was then operated by transnasal approach with debulking of most adenomatous tissue but without biochemical remission of acromegaly (GH 92–99 at glucose tolerance test). A week after surgery – erythrocytes  $4.5 \times 10^{12}$ , Hb – 141 g/l, HCT – 44.1%. The patient was prescribed a octreotide depo injections 20 mg/mth and at 6 months from operation was feeling well, with clinical remission and normalization of IGF1 and all blood count parameters.

This is the first clinical observation of a secondary erythrocytosis in an acromegalic patient to the best of knowledge of authors.

**P301****Patients with active acromegaly (AA) have less hepatic triglyceride than normal subjects using proton magnetic resonance spectroscopy (1H MRS)**

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A decrease in insulin sensitivity (IS) is associated with increasing visceral adiposity. An inverse correlation has been demonstrated between Intramyocellular (IMTG) and intrahepatocellular triglyceride (HCTG) concentrations and IS. Patients with AA have reduced IS but IMTG and HCTG content has not been studied.

Eight patients with AA (IGF-I > ULN, median  $1.83 \times$  ULN (range 1.16–4.38)) (6 M), median age 39.5 (range 23–66) years, median BMI  $30.6(23.5–38.6)$  kg/m<sup>2</sup> and 5 healthy volunteers (HV) ((4M) median age 32(29–39) years, median BMI  $27.4(24.9–29)$  kg/m<sup>2</sup>) were recruited.

On a Philips 3 Tesla Achieva MR scanner, full-body T1-weighted multi-slice imaging was performed and MRIcro was used to calculate fat mass (FM) in body compartments. The 1H MRS spectra obtained from liver, anterior tibialis (AT) and gastrocnemius (Gc) muscles (R leg) were analysed using jMRUI to de-convolute peaks and provide a ratio of triglyceride content/water (as % water content).

AA (fasting glucose median 5.3 mmol/l, range 4.7–6.2) patients had significantly less HCTG content c.f. HV (median 0.1%, range 0–5.5 vs 12%, 2.1–22,  $P=0.01$ ) despite no overall differences in weight or FM distribution.

There was no difference in IMTG content in either muscle (CH2 moiety) as % water; (Gc), AA median 0.81(0.61–4.9)% vs HV 2.73(0.31–9.12)%,  $P=1$ , (CH3 moiety) AA 0.32(0–0.58)% vs HV 0.33(0–0.45)%,  $P=1$ , (TA) (CH2 moiety) AA 0.16(0–2.07)% vs HV 0.73(0.33–9.12)%,  $P=0.21$ , (CH3 moiety) AA 0(0–0.29)% vs HV 0.12(0–9.12)%,  $P=0.33$ ).

HCTG content is significantly reduced in AA compared to healthy volunteers. Patients with known AA have reduced IS and acromegaly appears to be unique in having impaired IS associated with low hepatic triglyceride. The mechanisms involved require further exploration, but may relate to the lipolytic actions of GH.

**Obesity****P302****Obesity in GDM**Luisa Ruas<sup>1</sup>, Marcia Alves<sup>1</sup>, Sandra Paiva<sup>1</sup>, Elvira Marta<sup>1,2</sup>, António Lobo<sup>1,2</sup>, Paulo Moura<sup>1,2</sup> & Manuela Carvalheiro<sup>1</sup><sup>1</sup>University Hospital Endocrinology Department, Coimbra, Portugal;<sup>2</sup>University Hospital Obstetric Department, Coimbra, Portugal.**Introduction**

Retrospective study, 2003–07, of 619 GDM women.

**Patients and methods**

Two groups according to pre-pregnancy BMI: Go  $\geq 30$ , Gno  $< 30$  kg/m<sup>2</sup>. Influence of BMI in: family history of DM, previous GDM or macrosomia, gestational age at time of GDM diagnosis, weight gain, blood pressure, A1c, need and doses of Insulin, time and type of delivery, new-born weight, complications and re-evaluation post partum.

**Results**

Mean age 33.03 years old, mean BMI: 26.39, Go: 79.9% = BMI: 34.19 and Gno: 20.1% = BMI: 24.43. Family history Go: 65.2%, Gno: 58.5%  $P=0.4$ . Previous GDM Go: 14.1%, Gno: 10.2%  $P=0.26$ . Previous macrosomia Go: 10.5%, Gno: 7.3%  $P=0.3$ . GA. Ws at GDM diagnosis Go: 26.72 and Gno: 28.75  $P=0.002$ . Weight gain was adequate in 70.7% Gno: 39.6% of Go; excessive in 29.3% Gno: 60.4% Go:  $P<0.05$ , OR = 3.684. Normal BP in 85.5% Go: 96.0% Gno; chronic hypertension in 6.7% Go: 1.4% in Gno; hypertension induced by pregnancy 3.8% Go and 2.6% Gno. A1c: 5.36% Go: 5.15% Gno:  $P<0.05$ . Insulin 42% Go: 28.1% Gno:  $P<0.05$ ; OR = 1.851, beginning of insulin (ws) Go: 29.11, Gno: 30.27 ( $P=0.28$ ) and total dose Go: 22.4 U/d and Gno: 16.85 U/d  $P=0.025$ . Caesarean section Go: 37%, Gno: 33.3%. Macrosomic 9.9% Go: 6.7% in Gno:  $P=0.24$ . Fetal morbidity 8.5% Go: 5.9% Gno:  $P=0.33$ . Re-evaluation: Normal 82% Go: 91.5% in Gno: IFT 11.5% Go: 2.4% Gno, IGT 6.6% Go: 4.9% Gno and DM in 1.2% Gno.

**Conclusions**

In GDM obesity was found to be an increased risk factor for maternal and fetal morbidity. Earlier GDM diagnose, excessive weight gain, hypertension, earlier insulin need and higher doses, earlier delivery, caesarian delivery, high baby weight and macrosomic babies and fetal morbidity. Obesity has a positive correlation with the development of carbohydrates intolerance and is a risk factor for maternal and fetal outcomes.

**P303****Obesity, metabolic syndrome, hepatic steatosis, fibrosis and viral load as negative factors affecting early (EVR) and sustained (SVR) virological response in patients with chronic hepatitis c treated with peginterferon and ribavirin**M Tataru Abagiu<sup>1</sup>, E F Georgescu<sup>1,2</sup>, R Teodorescu<sup>2</sup> & L Stanescu<sup>1,2</sup><sup>1</sup>Filantropia University Hospital, Craiova, Romania; <sup>2</sup>University of Medicine and Pharmacy, Craiova, Romania.

Our aim was to evaluate the impact of some clinical (obesity, metabolic syndrome), biochemical (HOMA-IR), histological (fibrosis, necroinflammation, steatosis) and viral factors (HCV viraemia) on both EVR and SVR in patients with genotype 1 chronic hepatitis C (CHC) treated with peginterferon plus ribavirin.

**Patients and methods**

We evaluated retrospectively 188 naïve patients with CHC treated with peginterferon plus ribavirin at standard weight-based doses for 48 weeks (96M/92F; mean age  $42.75 \pm 0.84$ ; mean weight  $69.63 \pm 1.11$  Kg; mean BMI  $24.03 \pm 0.4$ ). Biopsies were assessed for inflammatory activity and fibrosis, as quantified by the modified Knodell histological activity index. Steatosis was categorized by the proportion of hepatocytes per low-power field with fatty changes:  $>5\%$ ,  $>5-33\%$ ,  $34-66\%$ ,  $>66\%$ . All patients were evaluated for metabolic syndrome (MS) using the NCEP-ATP III criteria. All parameters were introduced in multivariate analysis in order to evaluate their contribution to EVR and SVR.

**Results**

EVR was achieved in 115/188 pts (61.17%) while SVR occurred in 82/115 (71.3%). After adjusting for sex and age, independent factors that negatively interfered with both EVR and SVR were: fibrosis score (OR: 0.478; 95% CI: 0.140–0.912;  $P=0.031$ ), steatosis (OR: 0.138; 95% CI: 0.035–0.537;  $P=0.004$ ),

HOMA-IR index (OR: 0.478; 95% CI: 0.266–0.857;  $P=0.013$ ) and viral load (OR: 0.424; 95% CI: 0.240–0.746;  $P=0.003$ ). After excluding the patients with MS criteria ( $n=32$ ), EVR was observed in 102/156 (65.4%) and SVR in 82/102 (80.4%). Factors that independently influenced both EVR and SVR were: fibrosis score (OR: 0.468; 95% CI: 0.295–0.743;  $P=0.001$ ), steatosis (OR: 0.535; 95% CI: 0.342–0.834;  $P=0.006$ ), obesity (OR: 0.779; 95% CI: 0.650–0.935;  $P=0.007$ ) and viral load (OR: 0.784; 95% CI: 0.650–0.945;  $P=0.011$ ).

**Conclusion**

Fibrosis, steatosis and obesity, as well as viral load are independent parameters that can affect both EVR and SVR in genotype 1 CHC patients treated with peginterferon and ribavirin at standard doses for 48 weeks, regardless the presence of MS. If MS is present, HOMA-IR index can also additionally impair viral response.

**P304**

Abstract withdrawn.

**P305****Testosterone therapy increased muscle mass and lipid oxidation in ageing men**L Frederiksen<sup>1,2</sup>, K Højlund<sup>1,2</sup>, D M Hougaard<sup>3</sup>, K Brixen<sup>1,2</sup> & M Andersen<sup>1,2</sup><sup>1</sup>Department of Endocrinology, Odense University Hospital, Odense, Denmark; <sup>2</sup>Institute of Clinical Research, University of Southern Denmark, Odense, Denmark; <sup>3</sup>Statens Serum Institute, Copenhagen, Denmark.**Background**

The indication for testosterone therapy in ageing hypogonadal men without hypothalamic, pituitary or testicular disease remains to be elucidated. The aim of this study was to investigate the effect of testosterone therapy on insulin sensitivity, substrate metabolism, body composition and lipids in ageing men with relative hypogonadism using a predefined cut-off level for bioavailable testosterone.

**Methods**

A randomized, double-blinded, placebo-controlled study of testosterone treatment (gel) on 38 men, aged 60–78 years, with bioavailable testosterone  $< 7.3$  mmol/l and a waist circumference  $> 94$  cm. Insulin-stimulated glucose disposal (Rd) and substrate oxidation were assessed by euglycemic hyperinsulinemic clamps combined with indirect calorimetry. Lean body mass (LBM) and total fat mass were measured by DXA, and serum total testosterone was measured by tandem mass spectrometry. Bioavailable testosterone was calculated. Coefficients (b) represent the placebo-controlled mean effect of intervention.

**Results**

Lean body mass ( $b=1.9$  kg,  $P=0.003$ ) increased while HDL-cholesterol ( $b=-0.12$  mmol/l,  $P=0.043$ ) and total fat mass (TFM) decreased ( $b=-1.2$  kg,  $P=0.038$ ) in the testosterone group compared to placebo. Basal lipid oxidation ( $b=5.65$  mg/min per m<sup>2</sup>,  $P=0.045$ ) increased and basal glucose oxidation ( $b=-9.71$  mg/min per m<sup>2</sup>,  $P=0.046$ ) decreased in response to testosterone therapy even when corrected for changes in LBM. No change in insulin-stimulated Rd was observed ( $b=-0.01$  mg/min per m<sup>2</sup>,  $P=0.92$ ).

**Conclusion**

Testosterone therapy increased muscle mass and lipid oxidation in the absence of an effect on insulin-stimulated glucose uptake. These data suggest the existence of opposite-directed effects of testosterone on factors related to insulin sensitivity in ageing men with relative hypogonadism.

**P306****Development and characterization of high-affinity leptins and leptin antagonists**M Shpilman<sup>1</sup>, L Niv-Spector<sup>1</sup>, M Katz<sup>2</sup>, C Varol<sup>2</sup>, G Solomon<sup>1</sup>, E Elinav<sup>2</sup> & A Gertler<sup>1</sup><sup>1</sup>The Hebrew University, The Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot, Israel; <sup>2</sup>Institute for Gastroenterology and Liver Disease, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.

Leptin is a pleiotropic hormone acting both centrally and peripherally. It participates in variety of biological processes including energy metabolism, reproduction and modulation of the immune response. So far structural elements affecting leptin binding to its receptor remain unknown. We employed random mutagenesis of leptin, followed by selection of high-affinity mutants by yeast-surface display and discovered that replacing residue D23 with a non-negatively charged amino acid leads to dramatically enhanced affinity of leptin for its soluble receptor. Rational mutagenesis of D23 revealed the D23L substitution to be most effective. Coupling the D23 mutation with alanine mutagenesis of three amino acids (L39A/D40A/F41A) previously reported to convert leptin into antagonist resulted in potent antagonistic activity. These novel superactive mouse and human leptin antagonists (D23L/L39A/D40A/F41A), termed respectively SMLA and SHLA, exhibited over 60-fold increased binding to leptin receptor and 14-fold higher antagonistic activity *in vitro* relative to the L39A/D40A/F41A mutants. To prolong and enhance *in vivo* activity, SMLA and SHLA were monopegylated mainly at the N-terminus. Administration of the pegylated SMLA to mice resulted in a remarkably rapid, significant and reversible 27-fold more potent increase in body weight (as compared to pegylated MLA), due to increased food consumption. Thus, recognition and mutagenesis of D23 enabled construction of novel compounds that induce potent and reversible central and peripheral leptin deficiency. In addition to enhancing our understanding of leptin interactions with its receptor, these antagonists enable *in vivo* study of leptin's role in metabolic and immune processes, and hold potential for future therapeutic use in disease pathologies involving leptin.

**P307****Ghrelin effects on nutrition and digestion in pest insects**

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The gypsy moth, *Lymantria dispar* L., is one of the most destructive phytophagous pests of the northern hemisphere. It shows a tremendous capacity to increase in numbers, and feeds on a wide range of trees and shrubs. However, several different systems driving feeding behavior and digestion of *Lymantria dispar* L. have not yet been elucidated.

Ghrelin along with several other hormones has significant effects on appetite and growth in humans and animals. The aim of our study was to examine changes in nutritional indices (relative growth rate-RGR, relative consumption rate-RCR, efficiency of conversion of ingested food-ECI, efficiency of conversion of digested food-ECD), relative midgut mass, total proteases, leucine aminopeptidase and trypsin activities in the midgut and fat body mass of 4th instar caterpillars of the pest insect, *Lymantria dispar* L. after ghrelin treatment. Four subpicomolar injections of ghrelin (0.3 pmol) or physiological saline (control) were applied every 24 h to two separate groups of fifteen caterpillars. The nutritional indices, RGR, RCR, ECD and ECI were higher in the ghrelin treated than in the control group. Repeated administration of ghrelin in subpicomolar doses also elevated relative midgut mass, induced digestive enzyme activities and increased fat body mass. Such information provides more evidence for the application of these relatively simple model systems in future studies of the mechanisms underlying digestion in complex organisms. Understanding of the processes of feeding regulation should also have practical applications in biological control of its population number.

**P308****TNF $\alpha$  plays an essential role in the downregulation of sex hormone-binding globulin production by decreasing hepatic HNF-4 $\alpha$  through NF- $\kappa$ B activation**A Barbosa-Desongles, R Simo, A Lecube, C Hernandez & D Selva  
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The reason why obesity (a chronic low-grade inflammatory disease) is associated with low levels of sex hormone-binding globulin (SHBG) remains to be elucidated. In the present study we provide evidence that TNF $\alpha$  (a proinflammatory cytokine that increases with obesity) reduces SHBG production by human HepG2 hepatoblastoma cells. The effect of TNF $\alpha$  on human SHBG expression was mediated by NF- $\kappa$ B which causes the downregulation of HNF4 $\alpha$ : a key SHBG transcriptional regulator. Moreover, daily TNF $\alpha$  treatment of human SHBG transgenic mice reduced plasma SHBG levels as well as liver HNF4 $\alpha$  levels. Finally, a negative and independent correlation was found between plasma levels of TNF $\alpha$  receptor 1 and SHBG in obese patients. We conclude that sustained exposition to elevated levels of TNF $\alpha$  decreases plasma SHBG production by reducing hepatic HNF4 $\alpha$  levels via NF- $\kappa$ B activation. These findings open up a new mechanism linking obesity with the deleterious consequences arising from lower levels of SHBG.

**P309****Benzofuran derivatives inhibit 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in rodent adipose tissue**Y Kobayashi, K Daisuke, Y Yamaguchi, M Ueda, O Miyata & N Tagawa  
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Excess glucocorticoids (GC) promote visceral obesity, hyperlipidemia and insulin resistance. The main regulators of intracellular GC levels are 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1), which converts inactive GC into bioactive forms such as cortisol in humans and corticosterone in rodents. Therefore, the inhibition of 11 $\beta$ -HSD1 has considerable therapeutic potential for metabolic diseases including obesity and diabetes. Benzofuran is a key structure in many biologically active compounds such as benzobromarone, Malibatol A and (+)-liphagal. The aim of this study is to investigate the inhibitory effect of benzofuran derivatives on 11 $\beta$ -HSD1. Microsomes were prepared from mesenteric adipose tissue from male Wistar rats. 11 $\beta$ -HSD1 activity was determined by incubation under an NADPH-regenerating system where the incubation medium, MOPS buffer, contained G-6-P (6 mM), NADP+ (1 mM), G-6-PDH (0.35 units/ml), and 11-dehydrocorticosterone (1  $\mu$ M) with and without benzofuran derivatives (Compound 1–14, 50  $\mu$ M). The reaction was started by the addition of 15  $\mu$ l of microsomes (1.6 mg protein/ml) and the reactants were incubated at 37°C up to 40 min. Two milliliters of CH<sub>2</sub>Cl<sub>2</sub> solution was added to stop the reaction. Corticosterone produced was measured by HPLC. Significant inhibition of 11 $\beta$ -HSD1 activity was observed in compound 1 (57%), 5 (36%), 7 (37%) and 8 (50%) compared with control. Further, we investigated the effect of these compounds on 11 $\beta$ -HSD2 (conversion of corticosterone to 11-dehydrocorticosterone). Compound 7 and 8 did not suppress 11 $\beta$ -HSD2, whereas compound 1 and 5 inhibited 11 $\beta$ -HSD2 by 18.7 and 56.3%, respectively. Compound 7 and 8 inhibited 11 $\beta$ -HSD1 in time- and dose-dependent manner. Kinetic study revealed that compound 7 (the most potent and selective inhibitor) acted as a non-competitive inhibitor of 11 $\beta$ -HSD1 and the apparent Km value for 11-dehydrocorticosterone was 0.25  $\mu$ M. These results suggest that compound 7 may exert its inhibitory effect by interacting with the enzyme 11 $\beta$ -HSD1.

**P310****Effect of dehydroepiandrosterone on 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in rodent adipocytes**N Tagawa, E Minamitani, D Kiyonaga & Y Kobayashi  
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Dehydroepiandrosterone (DHEA) has been suggested to have an anti-obesity effect; however, the mechanism underlying this effect remains unclear. The effect

of DHEA on adipocytes opposes that of glucocorticoids, which potentiate adipogenesis. The key to the intracellular activation of glucocorticoids in adipocytes is 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1), which catalyses the production of active glucocorticoids (cortisol in humans and corticosterone in rodents) from an inactive 11-keto form (cortisone in humans and 11-dehydrocorticosterone in rodents). In humans and rodents, intracellular glucocorticoid reactivation is exaggerated in obese adipose tissue. Using differentiated 3T3-L1 adipocytes, we demonstrated that DHEA inhibited 11 $\beta$ -HSD1 activity at a concentration of 1  $\mu$ M within 10 min. Inhibition was also observed in a cell-free system comprised of microsomes prepared from rat adipose tissue and NADPH, a coenzyme of 11 $\beta$ -HSD1. A kinetic study revealed that DHEA acted as a non-competitive inhibitor of 11 $\beta$ -HSD1. Further, DHEA did not inhibit 11 $\beta$ -HSD type 2, which inactivates cortisol or corticosterone in tissues involved in water and electrolyte metabolism, in rat kidney microsomes at a concentration <25  $\mu$ M. Moreover, no conversion from DHEA to other sex steroid hormones or their precursors was observed under the present experimental conditions. These results indicate that the inhibition of 11 $\beta$ -HSD1 by DHEA depends on neither the transcriptional pathway nor the nonspecific manner. This is the first demonstration that DHEA inhibits 11 $\beta$ -HSD1 by non-transcriptional pathway, thereby providing a novel insight into the anti-obesity mechanism of DHEA and serving as an anti-glucocorticoid steroid.

### P311

#### Waist circumference is a better predictor of cardiovascular risk than body mass index in patients with type 2 diabetes mellitus

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There is a clear relationship between degree of obesity and abdominal obesity with the development of cardiovascular disease.

#### Objective

To assess body mass index (BMI) and waist circumference (WC) as predictors of cardiovascular risk (CVR) in type 2 diabetes mellitus (T2DM) patients.

#### Material and Methods

Cross-sectional study in 452 consecutive out-patients with T2DM who visited our clinic in Madrid, Spain for a routine follow-up. All patients weight and height were measured to the nearest 0.1 kg and 0.1 cm respectively. BMI was calculated as weight divided by square height ( $\text{kg}/\text{m}^2$ ). The WC was recorded as the smallest girth between the rib cage and the iliac crest. Ten-year cardiovascular risk was calculated using the risk tables (Framingham). According to the ADA recommendations for adults with T2DM and WHO we applied a BMI <25  $\text{kg}/\text{m}^2$  and a WC <102 cm in  $\delta$  and <88 cm in  $\eta$  as targets for good control. Comparisons of mean levels were performed with the Student's *t*-test, and comparison of proportions with the chi-square test. Multiple regression analyses adjusted for age and sex were performed with CVR as dependent variable, and BMI and WC as independent variables, using the 'stepwise' method to assess the contribution of each variable to the CVR. A level of  $P < 0.05$  was considered significant (SPSS, v. 13.0).

#### Results

The mean age was  $65.2 \pm 11.2$  years, 200  $\delta$  (44%). The mean BMI was  $30.9 \pm 6.1$   $\text{kg}/\text{m}^2$ , being higher in  $\eta$  than  $\delta$  ( $32.3 \pm 6.77$  vs  $29.2 \pm 4.7$   $\text{kg}/\text{m}^2$ ,  $P = 0.0000$ ). Fifty-one percent of patients were obese (BMI  $\geq 30$ ), and only 60 patients (13%) achieved a BMI <25  $\text{kg}/\text{m}^2$ . The percentage of patients who had a BMI  $\geq 30$   $\text{kg}/\text{m}^2$  was greater in  $\eta$  than  $\delta$  (64 vs 35%,  $P = 0.0002$ ), and the patients who achieved a BMI <25  $\text{kg}/\text{m}^2$  was higher in  $\delta$  than  $\eta$  (17 vs 11%,  $P = 0.05$ ). The WC average was  $102.2 \pm 12.8$  cm, without significant differences between  $\delta$  and  $\eta$ . The percentage of  $\eta$  who had a WC >88 cm was much higher than those with a WC  $\leq 88$  cm (83 vs 17%,  $P = 0.01$ ). The mean CVR was  $14.5 \pm 7.7\%$ , being higher in  $\eta$  than  $\delta$  ( $16.4 \pm 9.2\%$  vs  $13.0 \pm 6.2\%$ ,  $P = 0.0000$ ). Multiple regression analysis showed that only the WC contributed to the variance of CVR (20%,  $P = 0.0001$ ).

#### Conclusions

The results demonstrate the high prevalence of obesity and abdominal obesity, and its poor control in the diabetic population studied, especially in  $\eta$ , and support the need to recommend strict adherence to the diet of these patients to achieve recommended targets to reduce the CVR. The measurement of WC is a better predictor of CVR and easier to obtain than BMI.

### P312

#### Effects of natural dietary antioxidants on insulin and IGF1 in obese patients with insulin resistance

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Oxidative stress (OS) could play an important role in metabolic syndrome (MS)-related manifestations contributing to insulin resistance (IR). The reciprocal influences between OS and IR are not clear. We investigated the effects of dietary antioxidants on IR, studying 24 obese (7 males and 17 females, 27–66 years), with IR evaluated by HOMA index, during treatment with metformin, comparing 2 dietary treatments: hypocaloric diet (group A,  $n = 16$ ) or a diet enriched with natural antioxidants (group B,  $n = 8$ ). A personalized program, with mean caloric intake of 1500 Kcal, 25% proteins, low glycemic index CHO and a calculated antioxidant intake of 800–1000 mg/daily, derived from fruit and vegetables, was administered to group B. OGTT and evaluation of total, LDL and HDL-cholesterol, triglycerides, uric acid, albumin and IGF1 were performed before and after a 3-months treatment. Total antioxidant capacity was determined by H<sub>2</sub>O<sub>2</sub>-metmyoglobin system which, interacting with the chromogen ABTS, generates a radical with a latency time (LAG) proportional to antioxidant content. Despite a similar significant BMI decrease, we found a significant decrease of insulin AUC and a significant increase in IGF1 in group B, together with a trend in increasing LAG values in the same group.

**Table 1**

	BMI (kg/mq)	Insulin AUC (mcU/ml per 120')	IGF1 (ng/ml)	LAG (s)
Group A pre	37.6 $\pm$ 1.3	14912.6 $\pm$ 2008.2	155.1 $\pm$ 14.2	63.2 $\pm$ 3.1
Group A post	36.1 $\pm$ 1.3*	13789.5 $\pm$ 1594.9	163.1 $\pm$ 10.4	66.3 $\pm$ 5.3
Group B pre	35.2 $\pm$ 2.0	19179.0 $\pm$ 3848.4	109.7 $\pm$ 12.7	56.2 $\pm$ 5.1
Group B post	32.2 $\pm$ 1.4*	11362.9 $\pm$ 705.8*	140.7 $\pm$ 23.0*	60.6 $\pm$ 5.5

Mean  $\pm$  S.E.M.,  $P < 0.05$ , versus pretreatment values.

These data suggest that dietary antioxidants ameliorate insulin-sensitivity in obese insulin-resistant subjects enhancing the effect of insulin-sensitizing drugs, although the molecular mechanisms remain still to be elucidated. The reciprocal pattern of hormone response (insulin and IGF1) is similar to that observed after bariatric surgery-induced weight loss, suggesting an increase in GH sensitivity linked with an insulin decrease.

### P313

#### Environmetal and metabolic risk factors of obesity development in preschool children

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#### Materials and methods

We investigated 79 normal (1st group) and 99 overweight (2nd group) preschool children. Deficient sport and average physical activity (APA) duration, risen appetite (point-assessment), prolonged television viewing and computer using time in children were considered as risk-factors of juvenile obesity manifestation. Height, weight, waist circumflex (WC) was measured; body mass index (BMI) was calculated. Serum leptin levels were detected by immunoenzyme method. Groups also were divided according sex. Statistical analysis was performed by using ANOVA and Hi-square criteria, Pearson's correlation coefficient ( $r$ ) ( $P = 0.05$ ).

#### Results

Deficient sport ( $P = 0.02$ ) and APA ( $P = 0.01$ ) duration, increase appetite ( $2.8 \pm 1.8$  points in girls and  $3.3 \pm 1.1$  – in boys from the 2nd group as compared to the 1st one –  $0.2 \pm 0.7$  and  $0.2 \pm 0.5$  respectively ( $P = 0.001$ );  $r = 0.7$  between risen appetite and BMI ( $P = 0.0001$ ),  $r = 0.8$  between risen appetite and WC ( $P = 0.0001$ ) were noted in children from 2nd group. Prolonged television viewing and computer using were found in overweight girls ( $34.7 \pm 22.5$  and  $62.1 \pm 47.9$  min per day in 1st and 2nd groups appropriately ( $P = 0.006$ )). We ascertained correlations between leptin level and BMI  $r = 0.77$  ( $P = 0.0001$ ), leptin concentration and WC  $r = 0.59$  ( $P = 0.001$ ). Leptinemia also correlated

with low intensive physical activity duration ( $r=0.74$ ;  $P=0.04$ ), including TV viewing and computer using time  $r=0.59$  ( $P=0.004$ ).

#### Conclusions

Deficient sport, APA duration, risen appetite are environmental risk-factors of early obesity manifestation in preschool children. We revealed positive correlations between leptin concentration and BMI, WC, low intensive physical activity duration, including TV viewing and computer using time in preschoolers.

### P314

#### Gender differences of leptin receptor gene genotypes and leptin levels in obese children

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#### Aim

To compare gender frequency of leptin receptor gene (LEPR) genotype rates and leptinemia levels in overweight children.

#### Materials and methods

Three hundred children with adiposity were investigated (divided into groups: prepubertal (1st) m/f  $n=51/50$ , early pubertal (2nd)  $n=49/51$ , late pubertal (3d)  $n=50/49$ ), 80 girls and 89 boys with obesity and 90 lean girls and 48 lean boys (control) were genotyped in 6 exon of LEPR gene (Q223R polymorphism). Serum leptin was detected by immunoenzyme method; insulin, testosterone estradiol levels – by radioimmunoassay technique. Statistical analysis was performed using SPSS 16.0 ( $P=0.05$ ).

#### Results

There were no sex differences between leptin levels in 1st and 2nd groups ( $P=0.7$ ) ( $P=0.1$ ) in contrast to 3d group ( $P=0.0001$ ). Leptinemia was increased in pubertal start ( $P=0.021$ ) and subsequent leptin diminution with puberty development ( $P=0.024$ ) in overweight boys. However we ascertained leptin levels constant rising in obese girl with pubertal progression ( $P=0.001$ ). BMI and leptin ( $r=0.355$ ,  $P=0.0001$ ), insulin and leptin ( $r=0.371$ ,  $P=0.0001$ ) correlations were revealed. Leptinemia correlated with age ( $r=0.326$ ,  $P=0.009$ ) and serum testosterone concentration in pubertal boys ( $r=-0.444$ ,  $P=0.0001$ ), as compared to pubertal girls – only with estradiol concentration ( $r=0.356$ ,  $P=0.031$ ).

There were gender differences ( $\chi^2_{7.91}$ ;  $P<0.05$ ) between genotypes rates occurrence in obese children: 14.6% boys and 2.5% girls had RR-genotype, 21.3% girls and 15.7% boys – QQ. As compared to lean children: RR-genotype was found in 19.1% girls and 14.6% boys, QQ-genotype – in 25.8% girls and 20.8% boys ( $P>0.05$ ).

#### Conclusion

There was 6 time lower incidence of RR-genotype among obese girls than normal peers. No differences were found between overweight and normal boys in Q223R LEPR genotype rates occurrence. Q223R LEPR genotypes in girls with adiposity were significant differ from normal children irrespective of gender.

### P315

#### Testosterone and metabolic syndrome: a meta-analysis study

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#### Introduction

Metabolic syndrome (MetS) is often associated with male hypogonadism. The role of testosterone replacement therapy (TRT) in MetS has not been completely clarified.

To systematically analyse the relationship between androgen levels and MetS we performed a review and meta-analyses of available prospective and cross-sectional studies. A specific meta-analysis on the metabolic effects of TRT in available randomised clinical trials (RCTs) was also performed.

#### Methods

An extensive Medline search was performed including the following words 'testosterone', 'metabolic syndrome' and 'males'. Out of 323 retrieved articles, 302 articles were excluded for different reasons. Among the 20 published studies included, 13, 3, and 4 were cross-sectional, longitudinal, and RCTs, respectively. Another unpublished RCTs was retrieved on [www.clinicaltrials.gov](http://www.clinicaltrials.gov).

#### Results

MetS patients showed significantly lower T plasma levels, as compared to healthy individuals. Similar results were obtained when MetS subjects with and without erectile dysfunction were analyzed separately or when NCEP-ATPIII MetS criteria were compared to other definitions. Meta-regression analysis demonstrated that type 2 diabetes (T2DM) increased the MetS-associated T fall. In a multiple regression model, after adjusting for age and BMI, both T2DM and MetS independently predicted low testosterone (adj.  $r=-0.752$ ;  $P<0.001$  and  $-0.271$ ;  $P<0.05$  respectively). Analysis of longitudinal studies demonstrated that baseline testosterone was significantly lower among patients with incident MetS in comparison to controls (2.17(-2.41;-1.94) nmol/l;  $P<0.0001$ ). Combining the results of RCTs, TRT was associated with a significant reduction of fasting plasma glucose, HOMA index, triglycerides and waist circumference. In addition, an increase of HDL-cholesterol was also observed.

#### Conclusions

The meta-analysis of the available cross-sectional data suggests that MetS can be considered an independent association of male hypogonadism. Although only few RCTs have been reported, TRT seems to improve metabolic control, as well as central obesity.

### P316

#### Identification of a reliable biomarker for metabolic syndrome

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#### Introduction

Over the last decade there has been a steady rise in obesity and co-morbidity, but little is known about the rate of metabolic dysfunction among young adults in the United Arab Emirates. Various factors have been implicated as biomarkers of metabolic syndrome (MetS). The objective of this study was to analyze the relationships of various biomarkers (leptin, C-reactive protein (CRP), adiponectin, insulin, and uric acid) to the MetS components in lean, overweight, and obese young females.

#### Methods

This was a cross-sectional study of 69 apparently healthy young females, who were classified according to their body mass index (BMI) ( $\text{kg/m}^2$ ) into three groups: lean ( $\leq 25$ ), overweight ( $> 25$  and  $< 30$ ), and obese ( $\geq 30$ ). Estimated biomarkers were: leptin, insulin, adiponectin, high-sensitivity (hs)-CRP, uric acid, blood sugar, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, and triglycerides (TG).

Anthropometric measures, blood pressure, and homeostasis model assessment-insulin resistance (HOMA-IR) were also measured.

#### Results

Serum leptin, hs-CRP, insulin, and uric acid increased significantly ( $P<0.01$ ) with increased BMI. Only one significant correlation ( $P<0.05$ ) between the biomarkers and the MetS components was found in lean subjects (leptin versus waist circumference  $r=0.48$ ) as opposed to six in the obese group (hs-CRP versus waist circumference and systolic blood pressure,  $r=0.45$  and  $r=-0.41$ , respectively; insulin versus diastolic blood pressure,  $r=0.47$ ; adiponectin versus blood sugar,  $r=-0.44$ ; and uric acid versus waist circumference and TG,  $r=0.5$  and  $r=0.51$ , respectively).

#### Conclusion

Estimation of the levels of studied biomarkers could be an important tool for early detection of MetS before the appearance of its frank components. Uric acid seems to be the most reliable biomarker to identify obese subjects with MetS.



**P317****Serum Vitamin D and IGF1 levels in abdominal obesity women**O Belyaeva<sup>1</sup>, E Bazhenova<sup>1</sup>, D Azzi<sup>1</sup>, A Berezina<sup>1,2</sup>, N Katysheva<sup>1</sup>, O Galkina<sup>1</sup>, E Mischeva<sup>1</sup> & E Grineva<sup>1,2</sup><sup>1</sup>St Petersburg State Medical University, St Petersburg, Russian Federation; <sup>2</sup>Federal Centre of Heart, Blood and Endocrinology, St Petersburg, Russian Federation.**Context**

Decreased levels of both vitamin D and insulin-like growth factor 1 (IGF1) may be associated with obesity, however this had not been well established. We have examined the relationship of vitamin D status and circulating IGF1 with metabolic parameters in abdominal obesity women.

**Methods**

Serum 25(OH)D and IGF1 levels were measured by means of ELISA in 90 women (mean age 45.6±4.3) with abdominal obesity (according to IDF criteria, 2005). Anthropometric, metabolic measurements and standard oral glucose tolerance test were performed. The amount of adipose tissue was determined by dual-energy X-ray absorptiometry (DEXA).

**Results**

The prevalence of 25(OH)D level deficiency (<50 nMol/l) and insufficiency (<75 nMol/l) was 79.6% in women with abdominal obesity. Mean 25(OH)D level was 65.1±28.1 nMol/l. 25(OH)D level was inversely associated with the total body %fat and android %fat determined by DEXA ( $r = -0.2$ ,  $P = 0.005$  and  $r = -0.27$ ,  $P = 0.002$  respectively). We did not find correlations of 25(OH)D levels with body mass index (BMI), waist-to-hip ratio, blood pressure and insulin level. However we found correlation of 25(OH)D level with fasting plasma glucose level ( $r = -0.36$ ,  $P = 0.003$ ) and high-density lipoprotein (HDL) ( $r = -0.3$ ,  $P = 0.05$ ).

Mean IGF1 level was 125.4±34.0 ng/ml. Correlation analysis revealed negative correlations between IGF1 concentration and body weight ( $r = -0.23$ ,  $P = 0.03$ ), waist circumference ( $r = -0.3$ ,  $P = 0.007$ ), BMI ( $r = -0.28$ ,  $P = 0.01$ ), android %fat ( $r = -0.23$ ,  $P = 0.04$ ), A/G ratio ( $r = -0.21$ ,  $P = 0.05$ ) and total fat quantity ( $r = -0.27$ ,  $P = 0.03$ ). We did not find relationship between IGF1 level and glucose, insulin, lipids concentration as well as 25(OH)D status.

**Conclusion**

This study shows that both vitamin D and IGF1 concentrations are reduced in obese women and associated with the amount of adipose tissue in android region. Besides, low vitamin D levels were associated with impaired glucose and lipid metabolism.

Though no correlation between 25(OH)D and IGF1 levels were found, we are planning to continue our studies using a larger sample size for more reliable results.

**P318****Serum adiponectin levels in patients with type 1 gaucher disease**S Petakov<sup>1,2</sup>, N Suvajdzic<sup>1,3</sup>, S Ognjanovic<sup>2</sup>, T Isailovic<sup>2</sup>, D Macut<sup>1,2</sup>, M Djurovic<sup>1,2</sup>, I Bozic<sup>2</sup> & S Damjanovic<sup>1,2</sup><sup>1</sup>Medical school of Belgrade university, Belgrade, Serbia; <sup>2</sup>Clinic of Endocrinology, Belgrade, Serbia; <sup>3</sup>Clinic for Hematology, Belgrade, Serbia.

Gaucher disease (GD) is autosomal recessive lysosomal storage disorder caused by the deficiency of glucocerebrosidase with consequent massive accumulation of lipid-laden macrophages in various tissues. Estimated life expectancy is about 9 years shorter than for the reference population. Mortality due to macrovascular diseases is higher, and probably associated with metabolic abnormalities found in GD type 1 patients, including decreases in serum adiponectin levels. Because adiponectin is thought to have antiatherogenic and anti-inflammatory properties, we studied its serum concentration in a cohort of 15 patients with type 1 GD (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). We found no difference in serum adiponectin level in patients with GD type 1 as compared with healthy subjects (mean±s.e.m., 69.9±6.9 vs 73.0±10.8 ng/ml respectively). There was no difference in serum adiponectin levels between untreated and treated patients with type 1 GD. There was no correlation between body mass index and adiponectin levels even in group of GD patients on enzyme replacement treatment ( $n = 10$ ). In conclusion our results does not confirm previous results concerning the existence of hypo adiponectinemia in untreated and treated patients with GD type 1. Probably adiponectin has no role in decreased life expectancy due to macrovascular morbidity in these patients.

**P319****Undiagnosed thyroid dysfunction, leptin and body mass index**I Olaizola<sup>1,2</sup>, M L Granada<sup>3,4</sup>, M T Julián<sup>1,2</sup>, C Castell<sup>1</sup>, A Serra<sup>2,5</sup>, A Alastrué<sup>4,6</sup> & A Lucas<sup>1,2</sup><sup>1</sup>Endocrinology and Nutrition Service. Germans Trias i Pujol, Hospital, Badalona, Barcelona, Spain; <sup>2</sup>Department of Medicine. Autonomous University of Barcelona, Badalona, Barcelona, Spain; <sup>3</sup>Biochemistry Service. Germans Trias i Pujol, Hospital, Badalona, Barcelona, Spain; <sup>4</sup>Autonomous University of Barcelona, Badalona, Barcelona, Spain; <sup>5</sup>Nephrology Service. Germans Trias i Pujol, Hospital, Badalona, Barcelona, Spain; <sup>6</sup>General and Digestive Surgery Service. Germans Trias i Pujol, Hospital, Badalona, Barcelona, Spain; <sup>7</sup>General Direction of Public Health. Health Department. Generalitat of Catalonia, Barcelona, Spain.**Introduction**

Leptin seems to be a major link between obesity and thyroid dysfunction.

**Design**

To analyze the relationship between thyrotropin (TSH) and leptin (LP) plasma concentrations and body mass index (BMI), in 785 subjects (448 women) of 41.27±9.72 years and BMI 28.91±9.8 (18.04–93.98 kg/m<sup>2</sup>), without diagnosed thyroid disease or pregnancy, concentrations of TSH, free thyroxine (FT<sub>4</sub>), LP, glycemia and insulinemia were determined and HOMA index, calculated. The group of subjects was divided into three subgroups according to BMI: 18–30 ( $n = 594$ , 1), 30–40 ( $n = 97$ , 2) and >40 kg/m<sup>2</sup> (patients with morbid obesity,  $n = 94$ , 3). The statistical analysis was performed with the SPSS package using the logarithm of the variables without normal distribution.

**Results**

The undiagnosed hypothyroidism (TSH >4 mU/l) was 2.4, 4.1 and 9.9% for subgroups 1, 2 and 3 respectively ( $P = 0.002$ , 0.002). In the global group, the TSH correlated directly and significantly with BMI and the HOMA and inversely, with FT<sub>4</sub> ( $P = 0.007$ , 0.04, 0.006). In men, TSH only correlated directly and significantly with LP ( $P = 0.01$ ) and in women, directly, with BMI, LP and the HOMA ( $P = 0.015$ , 0.001, 0.014) and inversely with FT<sub>4</sub> ( $P = 0.001$ ). The multivariate analysis performed with TSH as the dependent variable showed that in men, the LP was the only predictor of the TSH concentration variations ( $P = 0.049$ ) while, in women, BMI and FT<sub>4</sub> were the predictors of these variations ( $P = 0.023$ ; <0.000).

**Conclusions**

Undiagnosed hypothyroidism is more prevalent in patients with obesity and even more in those with morbid obesity than in the non obese population.

The predictor factors of TSH concentration variations are different in men and women.

**P320****Low birth weight and obesity are associated with increased androgen levels among children with premature adrenarache**L Lekka, E Kousta, K Anyfandakis, S Leka & A Papanthassiou  
P and A Kyriakou Children's Hospital, Athens, Greece.**Introduction**

The aim of the study was to assess the impact of obesity and birth weight (BW) at diagnosis of premature adrenarache (PA) in a large cohort of greek children.

**Methods/design**

We assessed clinical and laboratory characteristics of 221 children (175 girls and 46 boys) at diagnosis of PA.

**Results**

At diagnosis of PA 149 children (67.4%) had pubic hair (Tanner stage ≥2), 10 (4.5%) had axillary hair (Tanner stage ≥2) and 62 (28.1%) had both axillary and pubic hair. Mean (±s.d.) bone age was significantly higher than chronological age (7.4±2.1 vs 6.6±1.8 years girls;  $P < 0.001$  and 8.6±2.3 vs 7.3±2.0 years boys;  $P < 0.001$ ). Among the 221 children with PA, 57 (26%) were overweight and 64 (29.0%) were obese, which is higher than the prevalence of obesity among prepubertal greek children. There was a positive correlation between BMI and testosterone levels ( $r = 0.17$ ,  $P = 0.02$ ), DHEAS levels ( $r = 0.27$ ,  $P < 0.001$ ) and bone age minus chronological age ( $r = 0.19$ ,  $P = 0.006$ ). Among the 187 full term pregnancy children there was a negative correlation between BW and testosterone ( $r = -0.21$ ,  $P = 0.005$ ), DHEAS ( $r = -0.15$ ,  $P = 0.038$ ), 17-OH-progesterone ( $r = -0.23$ ,  $P = 0.002$ ) and D<sub>4</sub>-Androstendione levels ( $r = -0.18$ ,  $P = 0.04$ ).

**Conclusions**

Obesity and lower birth weight are associated with increased androgen levels among children with PA.

**P321****Prevalence of metabolic syndrome in 850 obese children from infancy to adolescence**S Bellone<sup>1</sup>, F Prodam<sup>1,2</sup>, R Ricotti<sup>1</sup>, A Petri<sup>1</sup>, V Agarla<sup>1</sup>, C Guidi<sup>1</sup>, G Genoni<sup>1</sup> & G Bona<sup>1</sup><sup>1</sup>Division of Pediatrics, Department of Medical Sciences, University of Piemonte Orientale, Novara, Italy; <sup>2</sup>Endocrinology, Department of Clinical and Experimental Medicine, University of Piemonte Orientale, Novara, Italy.**Introduction**

Obesity and connected metabolic alterations are a growing problem in paediatric age, but few data are available in the youngest population. Poor evidences on metabolic syndrome (MS) definition are present in childhood and the most used definitions are those of the modified National Cholesterol Education Program and of the International Diabetes Federation (IDF). Aim of our study was to evaluate the presence of MS in obese children using these two definitions and the usefulness of clinical parameters.

**Subjects and methods**

We performed clinical and metabolic blood evaluations in 850 overweight and obese children ranging from 1.9 to 18.1 years of both sexes.

**Results**

Following NCEP criteria, some children showed glucose alterations: 4.9% showed IFG, 7.6% IGT and 0.9% type 2 diabetes mellitus. 49.6% showed low levels of HDL-cholesterol, 28.1% high levels of triglycerides; 81.7% high blood pressure and 16.1% acanthosis. Waist circumference (WC) was high in 96.7%. Waist/height ratio (W/Hr) was higher the cut-off of 0.5 in about all population. Prevalence of metabolic syndrome was 36.9% following NCEP ATP III and 55.1% following IDF definition. 28.0% of subjects demonstrated MS using both definitions. Percentage of disagreement between definitions (59.1% in the group under age of 10 years, 9.1 over 16 years) is lower increasing age of population and presence of metabolic complications.

**Conclusions**

In this large population of obese children WC was high in about all population demonstrating the presence of visceral obesity. W/Hr could be a simple and useful parameter to identify children at risk of metabolic syndrome. They showed in early ages high percentage of metabolic alterations like hypertension, dyslipidemia, disglycemia. IDF criteria could overestimate the presence of syndrome, above all in prepubertal children under 10 years of age. These data suggest that metabolic surveillance is needed in children with visceral obesity already in early age.

**P322****The effect of androgens on adipose tissue in PCOS**

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**Introduction**

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders of premenopausal women. It is also the most frequent cause of female infertility and affects 6.5–8% of women in their reproductive age. Two out of three criteria diagnoses PCOS: oligo- or anovulation, clinical or biochemical signs of hyperandrogenism and polycystic ovaries at ultrasound. In addition, PCOS women frequently display features of the metabolic syndrome, such as abdominal obesity and type 2 diabetes mellitus. To investigate the possible interaction between ovarian dysfunction and the metabolic syndrome we determined the effect of androgens on differentiation, insulin sensitivity and lipolysis in 3T3-L1 adipocytes.

**Material and methods**

3T3-L1 cells, a mouse white pre-adipocyte cell line, were stimulated with androgens during differentiation. Quantitative PCR was performed to measure expression levels of adipocyte marker genes. The effect of androgens on insulin sensitivity was analyzed by western blot analysis. Further, lipolysis was determined by measuring glycerol release.

**Results**

Our preliminary results showed that dihydrotestosterone (DHT) stimulated expression levels of early adipogenic markers (PPAR $\gamma$  and C/EPB $\alpha$ ), while the expression of important marker genes for end stage adipogenesis (AP2, adiponectin and leptin) were inhibited. Insulin-stimulated glucose metabolism is mediated through insulin-induced Akt phosphorylation. Our experiments showed that DHT suppressed insulin-induced Akt phosphorylation, suggesting that DHT-treatment results in reduced insulin sensitivity. Insulin insensitivity is

also reflected by an increased lipolytic activity. In contrast, basal levels of lipolysis were significantly decreased by 30% after 24 h stimulation with testosterone.

**Conclusion**

Our results show that DHT reduces insulin sensitivity, a crucial feature in the pathophysiology of PCOS and metabolic syndrome. Furthermore, our results suggest that androgens may program differences in adipocyte differentiation and/or function. Concluding, adipose tissue dysfunction, possible secondary to ovarian dysfunction, may be one of the causes of increased prevalence of metabolic syndrome in PCOS patients.

**P323****Functional differences between visceral and subcutaneous fat pads originate in the adipose stem cell**G Cantini<sup>1</sup>, S Baglioni<sup>1</sup>, M Francalanci<sup>1</sup>, G Poli<sup>1</sup>, E Borgogni<sup>1</sup>, A Di Franco<sup>1</sup>, R Squecco<sup>1</sup>, F Francini<sup>1</sup>, M Lucchese<sup>2</sup>, G Perigli<sup>1</sup>, M Serio<sup>1</sup> & M Luconi<sup>1</sup><sup>1</sup>University of Florence, Florence, Italy; <sup>2</sup>AOU Careggi, Florence, Italy.

Metabolic pathologies mainly originate from dysfunctions of the white adipose tissue (AT). Functional differences in AT and their impact on metabolism seem associated with the fat depot regional distribution, in particular the subcutaneous (SAT) and visceral (VAT) pads. Human adult adipose stem cell (ASC) populations have recently been characterized from SAT and VAT. In this study, using human ASC cultures obtained from paired biopsies of abdominal SAT, S-ASC, and VAT, V-ASC, we addressed the question whether the functional differences observed between the two compartments may be already present in the adipose stem cell instead of being restricted to mature adipocytes. V-ASC showed significantly greater dimensions than the corresponding S-ASC, as evaluated by Millipore Scepter (mean diameter:  $23.4 \pm 4.8 \mu\text{m}$ , V-ASC and  $20.5 \pm 3.9 \mu\text{m}$ , S-ASC,  $P < 0.005$ ) and by electrophysiology path clamping. Investigating the ability of ASC to proliferate *in vitro* a statistically significant difference in the proliferation rate between the two paired populations was observed, being S-ASC growth rate significantly higher than the one observed in V-ASC, as evaluated by cell counting, Ki67 immunostaining, MTS and bromodeoxyuridine incorporation (mean fold increase respectively of 2.55, 2.07, 2.33 and 1.63,  $P < 0.001$ ). Following 3 weeks of *in vitro*-induced adipogenic differentiation performed in parallel conditions on V-ASC and S-ASC, S-ASC demonstrated a significantly higher adipogenic potential, as evaluated by Adipored staining of intracellular lipids (mean fold increase absorbance S- versus V-adipocytes:  $1.83 \pm 0.09$ ,  $P < 0.001$ ), as also confirmed by a significantly higher expression in *in vitro* differentiated adipocytes of adipogenic genes and proteins such as PPAR $\gamma$ , adiponectin, FABP4. Our findings strongly suggest that VAT and SAT functional differences already originate in the adult adipose stem cell, which maintains the memory of the fat pad of origin.

**P324****Basal energy expenditure and fat mass changes in breast cancer patients**Nermin Tuncbilek<sup>1</sup>, Sibel Güldiken<sup>2</sup>, Neslihan Soysal Atile<sup>2</sup>, Betül Ekiz Bilir<sup>2</sup> & Deniz Köken<sup>1</sup><sup>1</sup>Trakya University Medical Faculty Radiology Department, Edirne, Turkey; <sup>2</sup>Trakya University Medical Faculty Internal Medicine Endocrinology Division, Edirne, Turkey.**Aim**

Basal energy expenditure (BEE), also called basal metabolic rate, increases in cancer patients and is thought to contribute to the weight loss observed in cancer cachexia. Cancer type, pathological stage and duration of disease influence BEE. The aim of this study was to evaluate BEE and body composition in breast cancer patients.

**Methods**

Forty-three women with breast cancer whose received adjuvant chemotherapy and 80 healthy age and body mass index (BMI)-matched control were enrolled in the study. Anthropometric measurements were recorded. BEE was measured by Harris-Benedict (HB) formula. Fat mass assessed by the TANITA. Preperitoneal, visceral and subcutaneous fat were evaluated by ultrasonography.

**Results**

Compared with the controls, patients with breast cancer showed significant increase in BEE ( $P < 0.001$ ), and visceral fat mass ( $P < 0.05$ ). Peritoneal fat mass was significantly decreased in breast cancer group ( $P < 0.01$ ). There was no

significant difference between groups. BEE showed positive correlation with total fat mass ( $r=0.8$ ,  $P<0.001$ ), subcutaneous fat mass ( $r=0.56$ ,  $P<0.001$ ) and visceral fat mass ( $r=0.4$ ,  $P<0.05$ ) in breast cancer patients. Regression analysis showed that total fat mass increase was an independent risk factor on BEE increase in breast cancer patients ( $P<0.001$ ,  $\beta=0.79$ , 95% CI=8.0–14.4).

#### Conclusion

Our data supports previous studies showed a greater BEE for breast cancer patients. Not only the local fat distribution but also the total fat mass increase affects on BEE in patients with breast cancer. When compared with healthy subjects, total fat mass increase didn't observed in breast cancer patients but increase in visceral fat mass which is accepted as a marker of metabolic disturbances was remarkable. These body fat mass changes may have important health implications for survivors.

### P325

#### Waist line radius to subcutaneous adipose tissue thickness- better insight to lipid stores centralisation

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#### Introduction

It is well known fact that central lipid stores are related to glucose metabolism impairment. Standard anthropologic methods for measuring lipid stores centralisation are waist, waist to hip ratio and waist to height ratio. Still, often these markers fail to relate to difference in glucose metabolism between equally obese patients.

#### Method

We analysed relation between waist line radius and subcutaneous adipose tissue thickness, determined with ultrasound 3 cm left of umbilicus on waist line, as a measure of lipid stores centralisation. Participants were randomly sampled males, overweight and obese divided by fasting glucose level as normoglycemic, impaired fasting glucose or diabetes mellitus type 2. Standard anthropologic measures were taken and compared. We analysed homeostatic model for insulin resistance and insulin secretion. Results were statistically analysed with analysis of variance followed by Tackey  $T$  test and analysis of correlation.

#### Results

We found statistically significant difference between three equally obese groups when subcutaneous tissue thickness was added compared to standard markers of lipid stores centralisation. We also notified statistically strong correlation between such new marker and HOMA IS particularly in IFG group.

#### Conclusion

Relation between waist line radius and subcutaneous adipose tissue thickness provides significant correlation with HOMA IS in equally obese patients when standard anthropometric markers of lipid stores centralisation failed. This could prove to be useful tool for early discovering and monitoring of deleterious lipid store centralisation particularly in IFG group.

### P326

#### Serum leptin level and its association with body composition in obese children

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Body composition using dual energy X-ray absorptiometry (DEXA) can be used as an specific method for measuring fat mass and identify individual risk for cardiovascular and metabolic complications in obese children.

#### Objective

To investigate serum leptin level and its relationship with fat distribution in obese children.

#### Materials and methods

Eighty-seven children (male/female=43/44, mean age  $12.81 \pm 0.5$  years), obese (BMI >95 percentile for age and sex). DEXA was used to body composition. Serum leptin level was measured using ELISA. Analysis was performed non-parametric (ANOVA, test of Mann-Whitney  $U$ ) and parametric ( $t$ -Student criterion) methods ( $P<0.05$ ).

#### Results

BMI in a group of girls  $28.3 \pm 0.52$  kg/m<sup>2</sup>, a group of boys  $29.7 \pm 0.68$  kg/m<sup>2</sup> ( $P=0.05$ ). DEXA data (m/f): Gynoid (%) –  $51.05 \pm 0.93/53.98 \pm 0.58$  ( $P>0.05$ ), Android (%) –  $46.93 \pm 0.68/51.29 \pm 0.52$  ( $P=0.05$ ), A/G –  $1.09 \pm 0.02/1.05$

$\pm 0.01$  ( $P>0.05$ ), total body –  $42.5 \pm 0.71/46.65 \pm 0.54$  ( $P=0.05$ ), fat –  $39.55 \pm 2.3/34.2 \pm 1.4$  kg ( $P=0.01$ ), free fat –  $50.5 \pm 2.4/41.3 \pm 1.5$  kg ( $P=0.05$ ), arms+legs/trunk –  $0.99 \pm 0.03/0.93 \pm 0.03$  ( $P>0.05$ ), legs/total –  $0.38 \pm 0.01/0.37 \pm 0.01$  ( $P>0.05$ ), trunk/total –  $0.49 \pm 0.01/0.51 \pm 0.01$  ( $P>0.01$ ), lean mass –  $47.8 \pm 2.4/38.8 \pm 1.5$  kg ( $P=0.05$ ).

Serum leptin level was positively correlated with body composition indicators: boys group: free fat ( $r=0.48-0.85$ ,  $P<0.01$ ), lean mass ( $r=0.52-0.69$ ,  $P<0.01$ ), Gynoid (%) ( $r=0.5-0.74$   $P<0.01$ ), Android (%) ( $r=0.65-0.85$   $P<0.05$ ), girls group: free fat ( $r=0.69-0.85$ ,  $P<0.01$ ), lean mass ( $r=0.4-0.52$ ,  $P<0.01$ ), Gynoid (%) ( $r=0.64-0.85$   $P<0.05$ ), Android (%) ( $r=0.65-0.78$   $P<0.01$ ). Correlation between OT/ON to the A/G (m/f –  $r=0.56/0.6$ ) without gender differences were established.

#### Conclusion

Gender differences in body composition were revealed in obese children. Serum leptin concentration is significantly correlated with body composition.

### P327

#### Inflammatory T-lymphocyte proliferation in morbid obesity

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Obesity is one of the leading causes of preventable death in the Western world, and its prevalence is dramatically increasing. Although obesity is primarily a metabolic disease, immunological aberrations also significantly contribute to obesity-related morbidity. The well-established state of chronic low-grade inflammation is considered to be crucial in the development of complications such as diabetes and atherosclerosis. Moreover, obesity is associated with increased susceptibility to infections, cancer and autoimmunity. Thus, disturbed immune regulation may exist in obese individuals. However, until now, a detailed description of systemic immunological changes in obesity is lacking.

We studied the effects of obesity on the T-cell compartment of morbid obese subjects. Blood was collected from eight morbid obese subjects and twenty lean control subjects. The T-cell compartment was defined phenotypically using flow cytometry and molecularly by T-cell receptor excision circle (TREC) analyses. We found a selective increase in T-lymphocyte number in morbid obese subjects, which was mainly caused by an increase in CD4<sup>+</sup> T-lymphocytes. Also, in morbid obese subjects TREC content was decreased in all T-lymphocyte subpopulations, demonstrating that the increase in T-lymphocytes is mainly caused by increased proliferation. Moreover, in morbid obese subjects we found increased plasma levels of IL-7 and CCL5, both potent enhancers of T-lymphocyte proliferation.

These data demonstrate that morbid obesity is associated with an increase in mainly CD4<sup>+</sup> T-lymphocytes. Most likely, this is related to increased plasma levels of cytokines that stimulate T-lymphocyte proliferation. This increase in T-lymphocytes may contribute to the increased risk of autoimmunity and cancer described in morbid obesity.

### P328

#### Letrozole normalizes serum testosterone but has no clinical effects in men with obesity-related hypogonadotropic hypogonadism

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#### Introduction

Hypogonadotropic hypogonadism is frequently observed in morbidly obese men, due to aromatase-dependent conversion of androgens to estrogens. The clinical impact of this sex hormone imbalance is unknown.

#### Aim

Evaluate the clinical effects of aromatase inhibition in obesity-related hypogonadism.

#### Methods

Double-blind, placebo-controlled, 6-month trial in severely obese men (BMI >35 kg/m<sup>2</sup>) with obesity-related hypogonadism (serum total testosterone <10 nmol/l). Starting dose (Letrozole or placebo) 1 tablet/week, subsequent

monthly dose escalation up to a maximum of 7 tablets/week or until a serum total testosterone of 20 nmol/l. The dose was reduced if serum estradiol decreased below 40 pmol/l.

#### Results

Eighteen patients on Letrozole and 21 receiving placebo completed the study. Mean age  $44.6 \pm 1.1$  years (mean  $\pm$  S.E.M.), BMI  $41.1 \pm 0.8$  kg/m<sup>2</sup>. At baseline, both groups were well matched for all study parameters. Placebo treatment did not affect serum hormone levels. Letrozole decreased serum estradiol from  $119 \pm 10$  to  $59.2 \pm 6.1$  pmol/l ( $P=0.0001$ , normal range (NR) 40–160 pmol/l), increased serum LH from  $3.3 \pm 0.3$  to  $8.8 \pm 0.9$  U/l ( $P<0.0001$ , NR:2–9 U/l) and free testosterone from  $244 \pm 19$  to  $691 \pm 39$  pmol/l ( $P<0.0001$ , NR:225–625 pmol/l). Both groups demonstrated a decrease in body weight of about 5 kg, and a decrease in abdominal circumference of about 4 cm. Changes in fat free mass, fat mass and bone density also did not differ between groups. Glucose metabolism, lipid profiles, physical exercise capacity and psychological characteristics did not change during treatment.

#### Conclusion

Despite a marked rise in serum free testosterone, low dose aromatase inhibition had no somatic or psychological effects in men with obesity-related HH. We hypothesize that, with respect to non-sexual somatic and psychological parameters, males primarily thrive on oestrogens, not testosterone.

### P329

#### Small animal PET/CT imaging reveals altered *in vivo* insulin function in brown adipose tissue of obese mice

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Brown adipose tissue (BAT) is an insulin sensitive organ having a very high uptake of glucose per gram of tissue. The role of insulin on glucose uptake in BAT is still poorly understood, moreover, it is not clear whether BAT insulin function is compromised with obesity. We used a small animal PET/CT imaging approach to analyse insulin function *in vivo* in lean and obese mice using a glucose analogue PET tracer (<sup>18</sup>F-FDG). Three groups of mice were analysed after administration of different diet regimens leading to progressive obesity levels: standard chow diet (SD), high fat diet (HFD, 40% fat content), super high fat diet (SHFD, 60% fat content). Each group of animals underwent two repeated PET/CT scans: the first in fasting state (basal state), the second after insulin administration (0.7 U/kg). Glucose levels, analysed during PET/CT procedures with a glucometer, revealed a slight and a severe compromise of insulin sensitivity in HFD and SHFD groups, respectively. Accordingly, PET/CT image analysis of <sup>18</sup>F-FDG uptake in the BAT revealed that insulin strongly increased PET signal in BAT of SD mice, whereas insulin induced <sup>18</sup>F-FDG uptake was lower in HFD group, and completely absent in SHFD group. To confirm the data, and to analyse whether counter-regulatory mechanisms originated by insulin-induced hypoglycemia influenced PET/CT analysis, we repeated the imaging procedure described above in a new cohort of SD, HFD and SHFD mice in which euglycemia was maintained by external glucose infusion through a jugular catheter. Image analysis of <sup>18</sup>F-FDG uptake in the BAT of mice analysed in euglycemic conditions was indistinguishable from the previous approach in all the three groups, confirming the presence of an altered insulin function in the BAT of obese mice. These findings, which reveal that obesity is associated with an altered insulin function of BAT, highlight small animal PET/CT as a powerful new tool for non-invasive analysis of insulin sensitivity.

### P330

#### Haematic circulating endocannabinoids (ECs) in lean, overweight and obese: the role of the gender

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A number of publications recently provided measurements of plasma ECs by liquid chromatography–tandem mass spectrometry (LC–MS/MS), however the reported circulating levels in normal subjects are scattered in a very wide range of concentrations. A quantitative LC–MS/MS method for anandamide (AEA),

palmitoylethanolamide (PEA), oleoylethanolamide (OEA) and 2-arachidonoyl-glycerol (2AG), chromatographically resolved by its inactive isomer 1AG, was validated in house. We firstly evaluated the stability of ECs during the early stages of the blood processing. Five blood aliquots were withdrawn in EDTA-containing tubes from 20 subjects, and blood was kept at 4°C until centrifugation after 0, 30, 60, 120 and 240 min, followed by storage of plasma at –80°C. Probably due to a blood cells spill-over, we found AEA, PEA and OEA dramatically increase to about 150% of the t0 concentration in the first 30 min, reaching 250% after 240 min, while 2AG level slowly decreases to 80% of t0 concentration after 240 min, maybe due to isomerisation. Once equipped for the proper storage of plasma within 15–20 min from the withdrawal, samples from 215 healthy and completely drug free volunteers were taken. Normal values (pmol/ml) in 112 normal weight subjects were: AEA:  $1.16 \pm 0.31$ ; PEA:  $12.25 \pm 2.71$ ; OEA:  $4.22 \pm 1.44$ ; 2AG:  $0.88 \pm 0.34$ ; no gender differences were observed. In females increased AEA and PEA levels were shown both in 34 overweight (22 and 11%, respectively) and 13 obese subjects (21 and 7%, respectively); in male obese group ( $n=13$ ) AEA, PEA and 2AG levels increased of 36, 31 and 42%, respectively, but no differences were observed in overweight group ( $n=43$ ). No changes were observed in OEA levels. A gender specific association between circulating ECs and overweight/obesity was revealed: AEA and PEA in females, AEA, PEA and, to a greater extent, 2AG, in male obesity.

### P331

#### Enhanced muscle mass by ALA treatment increase myostatin-mediated pathways in adipose tissue

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Myostatin is expressed predominantly in skeletal muscle and at significantly lower levels in adipose tissue. The loss of myostatin by gene disruption prevents an age-related increase in adipose tissue mass and partially attenuates the obese and diabetic phenotypes. Inhibition of myostatin signaling could be useful to prevent and/or treat obesity and diabetes. Male OLETF and LETO rats as non-diabetic controls were treated with or without ALA (100 mg/kg per day) and/or insulin (2 U/kg body weight per day) for 8 weeks from their age of 24 weeks. Using quantitative real-time polymerization chain reaction, insulin might increase ActRIIb and FSTL3 in skeletal muscle of diabetes rats partially through enhancing IGF1 and IGF-IR gene expression. Although oral intake of ALA might decrease body fat accumulation, larger amount of skeletal muscle mass from gastrocnemius relatively saved compared with insulin-alone treated group and control group. Myostatin, ActRII and FSTL3 gene expression with ALA-treated group might increase in epididymal fat tissue relation to decreasing IL15, which inhibits fat deposition via a direct action on adipose tissue in rodents and inhibit or oppose TNF- $\alpha$  mediated pathway in both skeletal muscle and adipose tissue. This present data suggest that ALA had better effect in adipose tissue as energy expenditure and metabolic actions, myostatin signaling may play a role in the response of adipocytes to diabetes.

### P332

#### Effects of food, hormones and autonomic nervous activity on rhythmic expression of clock genes and metabolic genes in rat white adipose tissue

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#### Background

Shift workers and rodents with clock gene mutations show increased risk of metabolic syndrome and type 2 diabetes (T2DM). Conversely, obesity and T2DM are related to circadian disturbances in humans and rodents. The circadian release of adipokines from white adipose tissue (WAT) plays a pivotal role in energy metabolism. Its regulatory mechanism is unclear at present.

#### Aim

To determine the daily expression patterns of genes involved in circadian and metabolic pathways in different compartments of rat WAT. Subsequently, to investigate effects of scheduled feeding on these expression patterns.

#### Methods

Experiment 1: 64♂ Wistar rats were killed at 8 timepoints during the 12 h light:12 h darkness cycle. Blood was collected, perirenal (prWAT),

subcutaneous-(sWAT), epididymal-(eWAT) and mesenteric WAT (mWAT) were dissected and RT-qPCR was performed.

Experiment 2: rats ( $n=9$  per ZT) were entrained to a six-meals-a-day feeding schedule. Each meal consisted of a 10 min feeding opportunity once every 4 h. Animals remained on this schedule for 4 weeks before they were killed at ZT2 and ZT14. Controls were fed *ad libitum* ( $n=3$  per ZT). Blood was collected, prWAT and sWAT were dissected and RT-qPCR was performed.

#### Results

Experiment 1: in intact rats, circadian genes as well as part of the metabolic genes, showed distinct day/night rhythms in prWAT and sWAT. Only minor differences between prWAT and sWAT were found.

Experiment 2: day/night rhythms of most circadian genes were attenuated in absence of the day/night rhythm in feeding activity. In metabolic genes no significant effect of feeding regimen was found. Overall, effects were more pronounced in prWAT as compared to sWAT.

#### Conclusion

The clock gene rhythm amplitude in prWAT is partly dependent on rhythmic food intake. Additionally, hormones and nervous input likely contribute to fine-tuning of the rhythmic expression of clock- and metabolic genes in WAT and are subject of our future studies.

### P333

#### Investigating the role of FTO in obesity: dysregulation of satiety signalling

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In 2007 a genome wide association study linked SNPs in intron 1 of FTO with an increased body mass index. Individuals with the 'risk' allele are on average 3 kg heavier than those with the 'protective' allele. Furthermore, those with the 'risk' allele have demonstrated greater energy intake. Mouse models of FTO have been generated including a conditional overexpression allele of FTO, which has 2 additional copies of FTO (FTO-4). These mice have increased body weight when globally expressed, which suggests that the FTO SNPs may lead to weight gain by increasing the activity and/or expression levels of FTO.

FTO-4 mice are hyperphagic. To investigate the cause, hormones, which are crucial for control of food intake and satiety, will be analysed.

Male 6 weeks old FTO-4 and wild-type mice fed on high fat diet were acclimatised to 16 h overnight fasting for 3 weeks. In subsequent weeks after fasting blood samples, food intake and body weight, fat and lean mass data and finally tissues were collected. Analysis of blood and tissue samples was then performed.

Significant dysregulation of specific hormones can be seen. Active ghrelin levels were either significantly higher in FTO-4 mice following refeeding or significantly lower before feeding began. Levels of leptin were significantly higher in FTO-4 mice ( $P=0.006$ ) than in wild-types following refeeding suggesting resistance to leptin's anorexigenic effects. Levels of NPY, an orexigenic neuropeptide, were also significantly higher ( $P=0.0003$ ) in FTO-4 mice following refeeding.

This dysregulation may contribute to the hyperphagia seen in the FTO-4 mice. Weekly fasting prevented the increased body weight phenotype, and any early significant differences were reversed. Therefore limiting excess access to food through paired-feeding experiments may prevent obesity in FTO-4 mice. These further experiments should help to establish how the FTO risk allele can affect obesity in humans.

### P334

#### Synergistic post-prandial deregulation of orexigenic and anorexigenic systems in obesity

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#### Introduction

Obese humans have increased plasma levels of endocannabinoids like anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG). However, it is unknown whether these orexigenic signals have a role in human eating behavior.

#### Design

We measured plasma AEA and 2-AG levels in 12 normal weight (10 F, age:  $39.1 \pm 3.7$  years, body mass index (BMI):  $21.0 \pm 0.6$  kg/m<sup>2</sup>, waist circumference (WC):  $73.8 \pm 2.9$  cm) and 12 insulin-resistant obese subjects (8 F, age:  $37.4 \pm 3.4$  years, BMI:  $42.0 \pm 1.4$  kg/m<sup>2</sup>, WC:  $125.0 \pm 4.5$  cm) 1 h before, immediately before and 1 h after the consumption of a balanced meal. The anorexigenic peptide YY (PYY) was measured at the same time points.

#### Results

Basal plasma AEA and 2-AG levels were increased in obese and positively correlated with BMI, WC and insulin for AEA. Before the meal, AEA similarly and significantly increased in both groups. After the meal, AEA decreased significantly in normal weight but not in obese subjects. 2-AG levels did not change over the meal. Post-prandial AEA change negatively correlated with WC and explained 20.7% of the variance observed for waist ( $r=0.455$ ,  $r^2=0.207$ ,  $P=0.04$ ). Likewise, post-prandial PYY change inversely correlated with WC and explained 21.3% of waist variance ( $r=0.462$ ,  $r^2=0.213$ ,  $P=0.04$ ). Post-prandial AEA and PYY changes were not correlated ( $r=0.242$ ,  $P=0.30$ ). A multiple regression analysis showed that post-prandial AEA and PYY changes explained 34% of the waist variance ( $r=0.582$ ,  $r^2=0.34$ ,  $P<0.03$ ), with only 8.2% of the variance commonly explained.

#### Conclusion

The pre-prandial peak of AEA suggests a role for this endocannabinoid in the initiation of the meal. The post-prandial changes of both the orexigenic AEA and the anorexigenic PYY are blunted in obese subjects and inversely and independently correlate with WC.

### P335

#### Long-term effects of normalization of testosterone on variables of the metabolic syndrome in hypogonadal men: the moscow study

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#### Introduction

Men with the metabolic syndrome have low plasma testosterone (T) levels. We aimed to study whether normalization of plasma T in such men improved features of the metabolic syndrome over the longer term.

#### Design/methods

145 men, 35 to 70 years, with the metabolic syndrome and hypogonadism (baseline total testosterone level  $<12.0$  nmol/l or calculated free T level  $<225$  pmol/l). They had received treatment for 30 weeks with either parenteral testosterone undecanoate ( $n=88$ ); TU; 1000 mg i.m.) or placebo ( $n=57$ ) with measurements at baseline, and after 6 and 18 weeks. After 30 weeks all men received TU for an additional 33 weeks.

#### Main Outcome Measures

Body mass index (BMI), waist circumference (WC), hip circumference (HC), insulin, glucose, cholesterol, triglycerides, high (HDL) and low density lipoproteins cholesterol (LDL) and triglycerides (TG).

#### Results

In the men who had received TU for 66 weeks there was a progressive improvement of BMI, WC, HC, insulin, and glucose, with no progressive improvement of cholesterol, LDL, HDL and TG after 30 weeks (Table). In the men who had received placebo for 30 weeks and then shifted to TU for 33 weeks all study variables improved and caught up with the other group over these 33 weeks.

#### Conclusions

The beneficial effects of normalization of T in hypogonadal men with the metabolic syndrome on BMI, WC, HC, insulin, and glucose are progressive over at least 66 weeks, while maximal effects on cholesterol, LDL, HDL and TG have been reached after 30 weeks

**P336****Therapy with exenatide in obese and morbidly obese type 2 diabetic patients: comparative analysis**

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**Aim**

To compare the effectiveness and tolerability of Exenatide twice daily treatment of obese and morbidly obese type 2 diabetic (T2D) patients.

**Material and methods**

To analyze data of obese and severe obese T2D patients initiated on Exenatide in our Endocrine clinics who complete one year of treatment. We separate patients in two groups: group A (BMI < 40 kg/m<sup>2</sup>) and group B (BMI ≥ 40 kg/m<sup>2</sup>). We did not include any modifications in previous lifestyle plans.

**Results**

We included 42 patients, 18 in group A (10 women and 8 men, mean ± s.d. age 57 ± 10 years, 9 ± 4 years of diabetes evolution) and 24 in group B (20 women and 4 men, mean ± s.d. age 61 ± 9 years, 5 ± 4 years of diabetes evolution).

At baseline, HbA1c was 7.2 ± 1.3 vs 7.8 ± 1.6 and weight 92.8 ± 12.8 kg (BMI 36.5 ± 2 kg/m<sup>2</sup>) vs 111 ± 14 kg (BMI 44.4 ± 3 kg/m<sup>2</sup>) in group A and B respectively.

After 12 months of treatment, mean HbA1c reduction was 0.9 ± 2% in group A and 0.6 ± 1.4% in group B, mean weight loss 5.9 ± 6 kg (BMI reduction 2.3 ± 2.8 kg/m<sup>2</sup>) in group A and 10 ± 7.8 (BMI 3.6 ± 3 kg/m<sup>2</sup>) in group B, being all of them statistical significant (*P* < 0.01 Wilcoxon test). Comparing HbA1c, weight and BMI mean reductions of two groups we have not assessed significant differences (*P* > 0.05 Mann-Whitney *U* test).

Adverse events were registered in 9 patients (50%) in group A and in 4 patients (16.7%) in group B (*P* < 0.01  $\chi^2$  test), with similar withdrawal rates (22.2 vs 21.7%).

**Conclusions**

Exenatide treatment significantly reduces HbA1c and weight in our population of obese T2D patients. We have not observed any significant difference in Exenatide efficacy between obese and severe obese T2D patients. Morbidly obese patients showed better Exenatide tolerance profile.

**P338****Glimepiride improves insulin sensitivity in oxidative skeletal muscle of obese rats: possible association between GLUT4 and SUR2A expressions**

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Studies have demonstrated that ATP-sensitive potassium channel (KATP) subunits SUR2A (sulfonylurea receptor) and KIR6.2 are involved in glucose uptake into skeletal muscle. It is known that the glucose transporter 4 (GLUT4) expression is highest in oxidative type I fibers, pointing out the important role of oxidative muscles as glucose uptakes. Considering these aspects the aim of this study was to investigate whether KATP channel can influence glucose uptake in oxidative muscle soleus by regulating GLUT4 expression. Insulin resistance was induced in male Wistar rats by inducing obesity with monosodium glutamate (MSG) (4 mg/g per day). At the age 3 months, half control and MSG-treated animals started receiving 0.1 mg/kg per day of glimepiride in the drinking water, and distributed in 4 groups: control rats (C), control rats treated with glimepiride (OG), 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 SUR2A, KIR6.2 and GLUT4 mRNA expression and GLUT4 protein. In ITT, O rats showed 33% decrease in glucose decay constant (kITT) in response to insulin (*P* < 0.05 versus C and OG) and the glimepiride treatment made the kITT value return to the control level. OG rats presented a ~48% increase in SUR2A mRNA content in comparison to O and C rats (*P* < 0.01). O rats showed 16% increase in GLUT4 mRNA content (*P* < 0.01 versus C), without any increase in GLUT4 protein. After glimepiride treatment, OG rats increased GLUT4 mRNA (19%, *P* < 0.01 versus C) and protein (159%, *P* < 0.01 versus C; and 78%, *P* < 0.05 versus O). Obesity or glimepiride treatment did not alter KIR6.2 mRNA content. We conclude that the glimepiride increased the GLUT4 expression in soleus of the insulin resistant animals which could be associated to SUR2A genes expression, playing an important role in the maintenance of the glycemic homeostasis.

**P339****The relationship between adiposity and carbohydrate metabolism, by age and gender in Vlorë, a city in Albania**

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Objective study was to determine the prevalence of overweight, obesity, type 2 diabetes.

**Methods**

The study in hospital of Vlorë was carried out between January 2008 and December of 2009.

The total study involved 284 patients, 167 women and 137 men, mean age 40–70 years whose height and body weight were evaluated.

They were diagnosed with type 2 diabetes and were tested in addition to anthropometric evaluations.

Each survey comprised a general practitioner, a nurse and a medical secretary.

Body weight and height were measured.

Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters).

All statistical analyses were performed for women and men separately.

**Results**

The prevalence of diabetes showed a steady increase up to the age group 60–69 years, after which the prevalence fell. Overall, the overweight rate was 34.2, 33.5% in women and 36.3% in men; obesity rate was 23.7, 32.4% in women and 14.1% in men. The prevalence of obesity showed steady increase up to the age group 60–69 years in men and 50–59 years in women.

**Conclusion**

We have reported for the first time the prevalence of overweight, obesity, and type 2 diabetes and their association with each other and with age and gender in a hospital of Vlorë, a city in Albania.

**P337****Vitamin D and obesity**

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Low vitamin D levels have been found in patients with diabetes mellitus type 2 and have been correlated with poor glycemic control. There are observations suggesting that vitamin D levels may be low in obesity.

The aim was to study vitamin D levels in obese individuals.

The levels of 25(OH)D<sub>3</sub> were measured in 30 obese individuals aged 42.67 years (mean), range 27–64, BMI 35.49 (mean), range 30–46 and 37 control subjects, aged 58.3 years (mean), range 33–83, BMI 24.9, range 21–29. The levels of 25(OH)D<sub>3</sub> were measured by RIA.

Vitamin D levels were lower in the obese patients than the control subjects, levels being 21.87 ± 12.36 ng/ml (mean ± s.d.) in the obese patients as compared to 38.42 ± 15.31 ng/ml in the control group, *P* < 0.05 (Student's *t* test).

It appears that vitamin D levels may be low in obesity. The aetiology of low vitamin D levels in obesity is still obscure. Vitamin D may be stored in the fat and thus its real levels within the body may be normal, low levels being detected only in the blood. Alternatively, vitamin D may be destroyed in obesity, as a result of increased oxidative reactions occurring in the context of obesity. Thus, low vitamin D levels may be another pathophysiological mechanism resulting in increased morbidity in the obese.

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**Long term low intensity recreational physical exercise attenuates colonic inflammation in rats: the role of heme oxygenase system**

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**Introduction**

Obesity caused health problems are increasing. Metabolic syndrome including obesity with insulin resistance, hypertension, and high blood lipid levels increases the risks of cardiovascular and inflammatory diseases. Many studies have reported that heme oxygenase (HO) inducers reduce insulin resistance and severity of inflammatory bowel disease. We investigate the anti-inflammatory effect, one among many positive effects, of regular recreational physical activity in rat colitis model provoked by trinitrobenzene sulphonic acid (TNBS).

**Methods**

After 6 weeks self-administered physical activity (running wheel) male Wistar rats were treated with TNBS (10 mg). 72 h after TNBS challenge we measured colonic inflammatory parameters and HO activity.

**Results**

The colonic inflammatory damage were significantly decreased by physical activity (extent of lesion: from  $49.7 \pm 0.9$  to  $38.4 \pm 4.4\%$ ; severity of mucosal damage: from  $7.3 \pm 0.1$  to  $6.1 \pm 0.4$ ;  $n=10-12$ ,  $P<0.05$ ). The regular physical activity enhanced HO activity in the running control group compared to the sedentary control group (from  $1.06 \pm 0.16$  to  $5.48 \pm 0.29$  nmol bilirubin/h per mg protein;  $n=10-12$ ,  $P<0.001$ ). The TNBS treatment increased the HO activity both in the non-running sedentary control and running groups.

**Conclusion**

Long lasting recreational physical activity improves body's defense mechanisms. Physical activity-induced increasing activation of HO system may play role of these mechanisms including colonic inflammation.

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**P341**

**Effects of Orexin-A on AMPK activity**

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**Introduction**

The hypothalamic hormone orexin-A (OXA) increases food and water intake, regulates the sleep-wake cycle, muscle tone and energy balance. AMP-activated protein kinase (AMPK) is an important metabolic regulator with direct hypothalamic orexigenic and diverse peripheral effects including fat tissue and muscle.

**Aims**

We aimed to study the influence of OXA on the activity of AMPK in the hypothalamus, subcutaneous and visceral adipose tissues, and skeletal muscle.

**Methods**

Thirty minutes after intraperitoneal (i.p.) or intracerebroventricular (i.c.v.) administration of OXA to male Wistar rats, tissues were rapidly dissected and protein extracts were immunoprecipitated and the AMPK activity was determined by an in vitro kinase assay.

**Results**

AMPK activity significantly increased in the hypothalamus the after i.c.v. OXA administration ( $172 \pm 15.8$  pmol ATP/min per mg protein (control, mean  $\pm$  s.e.m.),  $236 \pm 11.5$  (OXA)  $P=0.0022$ ,  $n=6-8$ ). The AMPK activity decreased in white adipose tissues after i.p. OXA administration (subcutaneous fat:  $52 \pm 14.3$  (control),  $14 \pm 2.9$  (OXA),  $P=0.0121$ ; visceral fat:  $76 \pm 6.8$  (control),  $41 \pm 6.3$  (OXA),  $P=0.0173$ ). In the skeletal muscle neither types of OXA administration had effect on AMPK activity.

**Conclusion**

Central administration of OXA increases appetite by the elevation of the AMPK activity in the hypothalamus. Peripheral administration of OXA reduces the AMPK activity in both subcutaneous and visceral white adipose tissues, which could lead to increased fat stores.

**P342**

**Monitoring the efficacy of long term low intensity physical exercise programmes by the laboratory and hormone profile of obesity**

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**Introduction**

In the human, obesity is one of the leading health problems as declared by the World Health Organization. Obesity is frequently associated with diabetes, hypertension and hyperlipaemia, these acts together as metabolic syndrome (MS). The risk of cardiovascular diseases is 5 to 10 times higher in MS. Lack of daily exercise is a good reason to evolve MS. Several hormones take part in the development of MS. Leptin and insulin, which are produced by the adipose tissue and the pancreas, and in addition the developed resistance to them play key role in the development of MS. We investigated the actions of recreational type of physical exercise on the levels of insulin and leptin in association with glucose- and lipid-metabolism parameters of MS.

**Design**

One hundred and eighty-five volunteers participated in a 5-month-long, low intensity, recreational type of aerobic physical activity program. We measured the levels of serum insulin, leptin, cholesterol, triglyceride, glucose and haemoglobin A1c (HBA1c) in 4 BMI categories (20–25, 25–30, 30–35, 35 <) at the beginning (day 0) in the middle (day 50) and at the end (day 150) of the program.

**Results**

We found decrease of body weight ( $88.6 \text{ kg} + 2.2$  to  $81.0 + 2.0 \text{ kg}$ ) in the high BMI groups ( $30 <$ ), where body fat content (from  $42.7 + 1.0$  to  $38.5 + 1.0\%$ ), cholesterol (from  $6.2 + 0.4$  to  $4.6 + 0.4 \text{ mmol/l}$ ), triglyceride (from  $1.5 + 0.1$  to  $1.0 + 0.1 \text{ mmol/l}$ ), HBA1c (from  $6.4 + 0.1$  to  $5.5 + 0.05\%$ ), leptin (from  $35 \text{ 595} + 4400$  to  $17 \text{ 319} + 1816 \text{ pg/ml}$ ) and insulin (from  $7.6 + 1.6$  to  $3.0 + 0.4 \text{ } \mu\text{IU/ml}$ ) levels were all decreased (at a minimum of  $P < 0.05$  level), while the decrease in glucose level was more moderate (from  $5.7 + 0.4$  to  $4.9 + 0.1 \text{ mmol/l}$ ).

**Conclusions**

Long term recreational physical activity program decreases insulin and leptin resistance. Monitoring of these and other obesity associated hormones seems to be useful to follow up the efficacy of physical exercise programmes.

**P343**

**3-iodothyronamine metabolism and metabolic effects in rat white adipocytes**

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**Introduction**

3-Iodothyronamine (TIAM) is a novel thyroid hormone derivative, which has been detected in rat blood and tissues. *In vivo*, TIAM has been reported to produce several functional effects, including the switch of fuel utilization from carbohydrates to lipids. In the present work we used rat white adipocytes to investigate TIAM uptake, metabolism, and metabolic effects.

**Methods**

Rat white adipocytes were incubated with exogenous TIAM. The concentrations of TIAM, thyronamine, 3-iodothyroacetic acid and thyroacetic acid were assayed by HPLC coupled to tandem mass spectrometry. Palmitate uptake was measured radiochemically using [<sup>3</sup>H]-2-D-dideoxyglucose (1 microM; 1 mCi/ml) and [<sup>14</sup>C]-palmitic acid (100 microM; mCi/ml) respectively. Type A and type B

monoamine oxidase activities (MAO-A and MAO-B) and semicarbazide-sensitive amine oxidase activity (SSAO) were studied radiochemically, using [<sup>14</sup>C]-serotonin or [<sup>14</sup>C]-benzylamine as substrates, in the absence and in the presence of TIAM.

#### Results

TIAM was significantly taken up by rat white adipocytes. Intracellular catabolism included oxidation to 3-iodothyroacetic acid and deiodination to thyronamine. TIAM (from 50 nM to 10 microM) did not affect baseline glucose or palmitate uptake. However, TIAM inhibited insulin-stimulated glucose and palmitate uptake with IC50's of 1.4 microM and 18 nM, respectively. In addition, TIAM inhibited benzylamine oxidation by MAO-B and SSAO with IC50's of 6.5 and 3.3 nM respectively, whereas no inhibition was observed with regard to the oxidation of serotonin, a MAO-A substrate.

#### Conclusions

TIAM antagonizes the effects of insulin on glucose and palmitate uptake by rat white adipocytes. TIAM is taken up by adipocytes, where it is a high affinity target of plasma membrane SSAO and mitochondrial MAO-B.

## P344

### The modulation of brain derived neurotrophic factor (bdnf) during exercise in obesity

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#### Objectives

Brain Derived Neurotrophic Factor (BDNF) plays a fundamental role in regulation of metabolism and energy homeostasis. Low BDNF levels have been identified as possible biological causes for development of obesity and BDNF administration has been shown to induce weight loss and to improve insulin sensitivity in diabetic animal models. Acute exercise is known to induce a transient increase of peripheral BDNF but no information is yet available regarding the exercise-induced modulation of BDNF in obesity.

#### Methods

We studied the BDNF responses to 30 minutes exercise on cyclo-ergometer at a self-selected intensity in 12 obese healthy subjects (OS) (6males; BMI 33.9 ± 1.6 kg/m<sup>2</sup>) and 13 lean controls (LC) (6males; 21.6 ± 0.5 kg/m<sup>2</sup>).

Blood samples were collected every 15 min: before, during exercise and for 90 minutes thereafter. Heart rate (HR) was monitored and serum BDNF concentration was measured with an ELISA.

#### Results

Age, gender, fasting glucose and BDNF levels were comparable between groups. All the subjects concluded the 30 min of exercise at an average peak intensity of 138.4 ± 42.6 W (85.2 ± 10.8% of subjects' HRmax.) that were comparable between groups (*P* > 0.05).

Exercise increased BDNF significantly in both OS (22.8 ± 5.2 ng/ml (baseline) vs 28.7 ± 6.3 (30 min) and 21.4 ± 6.2 (120 min); (*P* < 0.01)) and LC (20.5 ± 7.3 ng/ml (baseline) vs 27.8 ± 9.9 (30 min) and 19.8 ± 6.8 (120 min); (*P* < 0.01)). The BDNF response was comparable between both groups (*P* > 0.05).

#### Conclusions

We show that exercise increases in BDNF levels in lean subjects and that this effect was preserved in obesity.

## P345

### The modulation of atrial natriuretic peptide levels during exercise in obesity

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#### Objectives

Growing interest has been focused on the role of atrial natriuretic peptide (ANP) in the protection of cardiac and vascular integrity. Moreover, ANP enhances lipolysis and fat-oxidation. However, the impact of exercise on ANP in obesity is not yet investigated. The aim of this study was to evaluate the modulation of exercise-induced changes in ANP in obesity.

#### Methods

We studied the hormonal and metabolic responses to 30 min exercise on cyclo-ergometer at a self-selected intensity in 12 obese healthy subjects (OS) (6males; BMI 33.9 ± 1.6 kg/m<sup>2</sup>) and 13 lean controls (LC) (6males; 21.6 ± 0.5 kg/m<sup>2</sup>). Blood samples were collected every 15 min: before, during exercise and for 90 minutes thereafter. Heart rate (HR) was monitored and plasma MR-proANP<sub>53-90</sub> was measured using a chemiluminescence assay.

#### Results

All the subjects concluded the 30 minutes of exercise at an average peak intensity of 138.4 ± 42.6 W (85.2 ± 10.8% of subjects' HRmax.) that were comparable between groups (*P* > 0.05).

Exercise significantly increased ANP levels in both study groups (*P* < 0.01). However, the ANP response was more pronounced in LC as compared to OS (proANP-AUC120: 131.8 ± 5.3 (OS) vs 151.8 ± 5.9 (LC); *P* < 0.01). Similarly, the exercise-induced stimulation of lipolysis was diminished in obese subjects (*P* < 0.01).

#### Conclusions

This study demonstrates that the ANP responsiveness to exercise is reduced in obesity. The mechanisms underlying differential regulation of ANP in obesity and the role of ANP in mediating the exercise-induced lipolysis needs further investigations.

## P346

### AM251, a CB1 receptor antagonist, increases GLUT4 expression in adipocytes

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Evidences have suggested that the endocannabinoid system is overactive in obesity, resulting in enhanced endocannabinoid levels in both circulation and visceral adipose tissue. The cannabinoid CB1 receptor is expressed in the adipose tissue besides the brain. Few studies in vitro suggest that CB1 activation increases glucose uptake in adipocytes. The objective of the present study was to investigate the CB1 receptor modulation on glucose transporter GLUT4 expression, which is encoded by the SLC2A4 gene, and the related mechanisms. For this, 3T3-L1 adipocytes were incubated in the presence of a selective antagonist of CB1 receptor, AM251 (1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide). After 2, 4 or 24 h, cells were harvest to evaluate GLUT4 mRNA (real time PCR) and protein (western blotting), and NF-kappaB activation specifically on the promoter of SLC2A4 gene (EMSA). Acute and chronic incubation for 4 or 24 h with AM 251 expressively increased GLUT4 protein content (*P* < 0.001) but did not altered GLUT4 mRNA expression. Increased GLUT4 mRNA expression was only observed after 2 h of AM 251 treatment (*P* < 0.001). Considering the participation of NF-kappaB in the SLC2A4 gene expression, AM251 was able to increase NF-kappaB activation in adipocytes only after 24 h incubation. In conclusion, the present data shows that the CB1 receptor inhibition markedly increases GLUT4 expression in adipocytes. Evidences point out to an important participation of the inflammatory transcriptional factor NF-kappaB on the modulation of the SLC2A4 gene expression by CB1 receptor. FAPESP (08/09194-4 and 07/50554-1).

## P347

### Serum homocysteine levels in patients with nonalcoholic fatty liver disease

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#### Introduction

High serum homocysteine (Hcy) has been associated with insulin resistance (IR) syndrome and cardiovascular risk, although controversy exists. Nonalcoholic fatty liver disease (NAFLD) is regarded as the hepatic component of IR syndrome, but data on Hcy are limited. The aim of this cross-sectional study was to evaluate serum Hcy levels in patients with NAFLD.



**Methods**

Thirty-one consecutive patients (54 ± 11 years, 8 males) with biopsy-proven NAFLD, 15 of whom with simple nonalcoholic fatty liver (NAFL) and 16 with nonalcoholic steatohepatitis (NASH) according to NAFLD activity score (NAS) histologic system, were recruited. Twenty-two healthy controls (52 ± 9 years, 5 males) matched for gender, age and body mass index (BMI) served as controls. Blood samples for Hcy, folate, vitamin B12, insulin and standard biochemical tests were obtained after overnight fasting. Homeostatic model of assessment-insulin resistance (HOMA-IR) was calculated.

**Results**

The BMI of NAFLD patients was 33.1 ± 5.3 kg/m<sup>2</sup>. There was no difference in mean serum Hcy levels between controls and NAFLD patients (12.6 ± 4.6 vs 13.5 ± 2.6 mmol/l, respectively; *P* = 0.432). Serum folate and vitamin B12 were also similar between the study groups. As expected, NAFLD patients had higher ALT (*P* < 0.001), γ-gt (*P* = 0.001), glucose (*P* = 0.001), insulin (*P* = 0.002) and HOMA-IR (*P* = 0.001). However, NASH patients had lower mean serum HCY levels compared with NAFL patients (14.7 ± 2.1 vs 12.3 ± 2.5 mmol/l; *P* = 0.006). Mean age, BMI, serum folate, vitamin B12, ALT, γ-gt, glucose, insulin and HOMA-IR did not differ between NAFL and NASH patients. In multiple logistic regression analysis, HCY independently predicted NASH (*P* = 0.049) after adjustment for age, BMI and HOMA-IR.

**Conclusions**

Our data suggest that serum Hcy levels are similar between NAFLD patients and controls, but decreased in NASH compared with NAFL patients and can independently predict NASH. Serum Hcy could probably represent another non-invasive marker for the assessment of NAFLD.

**P348****Body mass index, obesity and hyperuricemia in postmenopausal women**

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**Aim of research**

To determine uric acid level in blood serum and incidents of hyperuricemia in postmenopausal women and their relation with body mass index (BMI).

**Object of research**

Patients of clinic by the physiology and pathology of bone and joint disease department and Ukrainian medical-scientific center of osteoporosis: postmenopausal women (*n* = 134). They were divided in the following groups depending on BMI: I group (BMI = 20.0–24.9) – normal weight, II group (BMI = 25.0–29.9) – overweight, III group (BMI = 30.0–34.9) – class I obesity, IV group – class II obesity (BMI = 35.0–39.9). Average age of examined patients was 64.3 ± 0.8 years.

**Methods of research**

Retrospective analysis of patients' case histories. Patients were hospitalized to the clinic of physiology and pathology of bone and joint diseases, Institute of Gerontology AMS of Ukraine. Uric acid level in blood plasma was determined by uricase-peroxidase method, statistic analysis – by Statistica 6.0.

**Results**

Uric acid level among women during postmenopausal period depends on body mass index. The higher level of uric acid were found in patients with obesity of II class. In the I group it was 285.0 ± 13.4; in II group – 285.2 ± 13.5; in III group – 281.7 ± 8.3, in IV group – 329.7 ± 17.7. Incidence of hyperuricemia among women in the I group was 12.9%, in II group – 23.5%; in III group – 18.6%, in IV group – 39.0% (differences between I and IV groups was significantly (*P* < 0.05)).

**Conclusions**

It was determined that uric acid level among women during postmenopausal period depending on body mass index. The higher level of uric acid and incidence of hyperuricemia were found in women with obesity of II class in comparison with women who have normal body mass index.

**Steroid metabolism****P349****Beneficial effects of testosterone supplementation on prostatitis-like alterations in an animal model of metabolic syndrome**A Morelli, S Filippi, L Vignozzi, P Comeglio, E Sarchielli, E Maneschi, I Cellai, G B Vannelli & M Maggi  
University of Florence, Florence, Italy.**Introduction**

Several studies suggested a direct relationship between metabolic syndrome (MetS) and increased risk of benign prostatic hyperplasia (BPH). An

inflammatory component, mainly mediated by IL-8, has been proposed as the primary link between MetS and BPH pathogenesis. MetS, is often associated with testosterone (T) deficiency and an imbalance between the androgen and estrogen ratio characterizes BPH patients.

**Methods**

We investigated the effects of *in vivo* T supplementation on MetS-related prostate alterations, using a previously characterized high fat diet (HFD)-induced rabbit model of MetS. Untreated HFD rabbits and rabbits fed a standard diet (control) were used for comparison. The effect of the not-aromatizable T metabolite, dihydrotestosterone (DHT), was studied in human prostatic stromal cells (hBPH).

**Results**

HFD rabbits developed all features of MetS, including dyslipidemia, hyperglycemia, glucose intolerance, hypertension, visceral obesity and hypogonadism. In addition, HFD rabbits showed prostatitis-like symptoms, such as prostate fibrosis, hypoxia, lymphocyte infiltration and formation of corpora amylacea, a typical sign of inflamed prostate. Accordingly, mRNA expression of several inflammatory (TNFα, IL8, IL6, IL1β, TLR2, TLR4), T-lymphocyte (CD4, CD8), and fibrosis/myofibroblast activation (TGFB, MMP9, RhoA, ROCK1) markers was significantly increased in prostate samples from HFD-rabbits, when compared to control rabbits. T supplementation prevented not only some MetS features (glucose intolerance and visceral fat accumulation) but also all HFD-induced prostate alterations. Moreover, features of liver steatosis and inflammation, observed in HFD rabbits, were not affected by T treatment, indicating a tissue-specific effect of T on prostate. hBPH cells exposed to TNFα stimulus showed a significantly increased secretion of IL8 and other cytokines/chemokines (IL6, MCP1, eotaxin, IP10). Preincubating cells with 30 nM DHT prevented TNFα-induced cytokine secretion.

**Conclusion**

Overall, our results suggest that anti-inflammatory properties of androgens could counteract the development of MetS-related prostatitis-like alterations.

**P350****Testosterone supplementation improves adipose tissue function in an animal model of metabolic syndrome**A Morelli<sup>1</sup>, E Maneschi<sup>1</sup>, L Vignozzi<sup>1</sup>, S Filippi<sup>1</sup>, M Marchetta<sup>1</sup>, B Mazzanti<sup>1</sup>, A Calcagno<sup>2</sup>, P Comeglio<sup>1</sup>, I Cellai<sup>1</sup>, G B Vannelli<sup>1</sup> & M Maggi<sup>1</sup><sup>1</sup>University of Florence, Florence, Italy; <sup>2</sup>University of Padoa, Padoa, Italy.**Introduction**

Adipose tissue dysfunction is associated to metabolic syndrome (MetS), a clustering of cardio-metabolic risk factors, including hypogonadism. We recently demonstrated that T supplementation was able to ameliorate the metabolic profile and reduce visceral fat accumulation in high-fat diet (HFD)-induced rabbit model of MetS.

**Methods**

We evaluated the differentiation capacity of preadipocytes (rPAD) obtained from visceral fat of the following rabbit groups: HFD (rPAD-HFD), T-supplemented HFD-rabbits (rPAD-T), standard diet (rPADcontrol) and HFD treated with INT-747 (rPAD-I), an agonist of the nuclear receptor FXR which previously showed the ability to reduce visceral fat accumulation in HFD-rabbits. rPAD were exposed to differentiating mixture (DIM) for 10 days and adipogenic potential was evaluated by quantitative analysis of triglyceride content and expression of adipocyte-specific genes.

**Results**

Histomorphometric analysis of visceral fat sections from all groups evidenced that adipocyte size was significantly increased in HFD-rabbit compared to control, indicating adipocyte dysfunction. Accordingly, rPAD-HFD showed a reduced capacity to accumulate triglyceride when exposed to DIM (84% over untreated cells) in comparison with rPADcontrol (318%). Moreover, glucose uptake in response to insulin was also reduced in rPAD-HFD. Interestingly, visceral adipocyte size was normalized in both T- and INT747-treated HFD rabbits. Functionally, rPAD-T and rPAD-I showed a triglyceride accumulation capacity after DIM (247 and 129%, respectively) and a glucose uptake ability comparable to that of rPADcontrol.

These findings were confirmed in terms of mRNA expression of adipocyte-specific genes (DKK1, FABP4, adiponectin and leptin), which were significantly induced in rPADcontrol, rPAD-T and rPAD-I, while remained unchanged in rPAD-HFD after exposure to DIM.

**Conclusion**

Overall, our results indicate that T-supplementation in the animal model of MetS may positively affect adipose tissue functions through the restoration of adipocyte commitment. This could reflect the ability of T in counteracting metabolic alterations most likely restoring adiponectin and insulin sensitivity in experimental MetS.

**P351****Evaluation of a method to measure long term cortisol levels in health and disease**

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**Introduction**

Elevated levels of cortisol are known to induce a wide range of pathology, e.g. abdominal obesity, type 2 diabetes and cardiovascular disease. Measuring serum and saliva cortisol is limited to one time point. Measurement of cortisol in scalp hair is a recently developed method to measure long term cortisol levels and might reflect cortisol exposure in patients with hyper- or hypo-cortisolism and in healthy persons. Our aims were to explore this measurement of cortisol by determining factors influencing hair cortisol levels in healthy controls. We also studied whether hair cortisol levels correspond to features of cortisol exposure in healthy persons and clinical course in patients with hyper- or hypo-cortisolism.

**Methods**

Hair samples were collected from 192 healthy individuals, 10 patients with Cushing's syndrome (CS) and two patients with Addison's disease (AD). Cortisol was extracted using methanol and cortisol levels were measured using a salivary ELISA kit. Measurements of waist and hip circumference were performed in 46 healthy subjects. A questionnaire was used to collect data concerning hair features.

**Results**

Cortisol levels were slightly influenced by hair treatment, but not by other factors such as gender, hair color, frequency of hair wash or hair products. In long hair, cortisol levels showed variation over time, without an overall washout effect. In patients with AD and CS, cortisol levels in hair corresponded with clinical course. In healthy individuals hair cortisol correlated positively with WHR ( $r=0.506$ ,  $P<0.001$ ) and waist circumference ( $r=0.360$ ,  $P=0.01$ ).

**Conclusions**

Hair cortisol concentrations seem to be slightly influenced by hair treatment. No washout effect of cortisol in distal hair segments was observed and hair cortisol levels correspond with clinical course in patients with AD and CS. This suggests that cortisol in scalp hair reflects cortisol exposure, which is supported by the positive correlation between hair cortisol and abdominal fat.

**P352****Effect of smoking cessation on steroid balance in postmenopausal women**

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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 function in women, induce changes in female hormonal balance and likely contributes to reported associations of smoking with adverse reproductive outcomes, including menstrual dysfunction, infertility, and earlier menopause. Studies on the effects of smoking and smoking cessation on female sex hormones have concentrated mainly on premenopausal women. In our study, we monitor changes of hormonal levels in postmenopausal women during smoking cessation.

**Methods**

We examined 60 postmenopausal women (average age 57.7 years, average BMI 26), before initiating of smoking cessation and after 6, 12, 24 and 48 weeks of abstinence. In the data analysis, we included seven women (average age 63.3 years), who got through. A broad spectrum of steroid metabolites, LH, FSH and SHBG, also basic anthropometric data were measured using GC-MS or immunoanalysis. Repeated measures ANOVA model was used for evaluation of the data. The local Ethics Committee approved the study, and all patients signed an informed consent form before taking part in the study.

**Results**

We found increasing levels of androgens (testosterone, androsterone) during smoking cessation, increasing of DHEA was insignificant. Conjugated to nonconjugated 20alpha pregnanolon ratio was significantly decreasing. Changes in the levels of other C21 steroids were not significant.

**Conclusion**

Smoking causes higher androgen levels in women, our results indicate that smoking discontinuation leads to their further increase. The study was supported by Grant No. NS 10125-3 of the IGA MZCR and GAUK.

**P353****Activin A regulates local testosterone production and growth in prostate cancer through 17β-hydroxysteroid dehydrogenase**

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**Introduction**

Local androgen synthesis in prostate cancer (PC) could contribute to the development of castration-resistant PC (CRPC). Pathways controlling intratumoral steroidogenic enzyme expression in PC are unknown. Since activin has been implicated in regulation of PC growth and steroidogenic enzyme expression in other steroidogenic tissues, we investigated the effect of activin on intratumoral steroidogenesis in PC.

**Methods**

Activin A effects and regulation of the activin-signaling pathway molecules were investigated in the PC cell lines LNCaP, VCaP and PC3 and in 13 PC xenograft models. Expression levels of activin subunits (INHBA and INHBB) and of the activin antagonist follistatin (FST) were studied in patient PC samples.

**Results**

Activin A induced the expression of AKR1C3 and HSD17B3, both 17β-hydroxysteroid dehydrogenase enzymes, in LNCaP and VCaP. Activin A also increased the enzyme activity as measured by androstenedione to testosterone conversion. Inhibition of endogenous activin A action in PC3 decreased AKR1C3 and HSD17B3 levels and testosterone synthesis. Androgens suppressed activin A expression in both VCaP cells and xenografts. Anti-proliferative effects of activin A were opposed in the presence of androstenedione. INHBA expression correlated with AKR1C3 levels in patient PC samples. It was increased in CRPC samples and high levels of a ratio the INHBA plus INHBB to FST were associated with a worse metastasis-free survival.

**Conclusions**

Activin in the prostate is controlled by androgens and regulates local androgen production. Activin antagonism could form a novel therapeutical target in CRPC since activin is a possible intermediate between castration and residual intratumoral androgen levels.

**P354****Standardized high throughput LC-MS/MS steroid-quantification for clinical metabolomics**

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Mass spectrometry based clinical metabolomics needs standardization of analytical assays to improve future inter-laboratory comparability and to become a successful new and established technology in clinical routine laboratories such as mass spectrometry based therapeutic drug monitoring (TDM) over the past 10 years. Steroid hormones are one of the most interesting endogenous metabolite class in clinical metabolomics today. Standardized determination of concentrations of steroid hormones in serum may aid in improvements in the clinical environment, e.g. IVF, diagnosis and treatment of steroid-related diseases in children and adults. The application of this assay will be focused on analysis of premenopausal women, i.e. the quantitation range of steroids is optimised for this clientele.

We will present a newly developed high throughput and standardized steroid LC-MS/MS based assay intending the quantitative determination of 16 steroids in human serum samples for clinical application. The steroid metabolites are testosterone, progesterone, cortisol, estradiol (E<sub>2</sub>), DHEAS androstenedione, 17-OH progesterone, corticosterone, 11-deoxycortisol, estrone (E1), DHEA, 11-deoxycorticosterone, aldosterone, cortisone, etiocholanolone, androsterone. The assay includes standardized sample preparation and LC-MS/MS analysis in 96-well plate format. The sample preparation, needed to clean up and pre-concentrate the sample, is performed by a solid phase extraction (SPE) procedure

in 96-well plate format. Serum (500 µl) sample volume is needed. Two different elution steps are necessary (first fraction: all steroids except DHEAS, second fraction: DHEAS) for highly pure extraction fractions resulting in minimal matrix effects and improved accuracy. As the result there are two subsequent LC-MS/MS runs with a total run time of 22 min.

Validation data of the assay for human serum will be presented including inter-laboratory comparisons demonstrating the precision, accuracy, stability, and reproducibility of the newly developed standardized assay.

### P355

#### Salivary cortisol and testosterone: a comparison of salivary sample collection methods in healthy controls

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#### Context

In population and psychosocial studies, saliva becomes increasingly popular, mainly due to its non-invasive collection methods and availability of free hormone fractions. However, few studies have evaluated the impact of collection devices on steroid hormone analysis; and none with the currently used methods.

#### Objective

The aim of this pilot study was to minimize pre-analytical errors based on sample collection methods.

#### Methods

We tested two saliva collection methods: chewing on a cotton swab (Salivette, Sarstedt) and passive drooling. Thirty healthy males with a median age of 27 years (range 19–65 years) participated. Morning saliva and serum samples were simultaneously collected after overnight fasting. Serum and salivary cortisol, serum total testosterone and SHBG were analyzed by electrochemiluminescent immunoassay (ECLIA, Roche Modular) and transcortin by a RIA. Serum free cortisol and testosterone were calculated. Salivary testosterone was determined using liquid chromatography–mass spectrometry.

#### Results

Strong positive correlations between the cotton-based and the passive drooling collection method were observed for cortisol as well as testosterone (respectively  $r=0.70$  and  $r=0.88$ ). For cortisol, we observed a mean positive bias of 65% for passive drooling versus Salivette. In addition, cortisol determined on passive drooling samples correlated much worse with calculated free cortisol than cortisol analyzed on the cotton samples (respectively  $r=0.34$  versus  $r=0.70$ ). However, for testosterone passive drooling correlated well with the calculated free fraction ( $r=0.66$ ). A positive bias of 21% for testosterone collected by passive drooling versus Salivette was observed. In contrast to earlier reports, we found no artefactually high testosterone results with the cotton-based collection method.

#### Conclusion

Our findings have important implications for the use and potential misuse of saliva collecting methods. Cotton swabs and passive drooling both seem adequate saliva collection methods for the analysis of testosterone, but passive drooling does not appear suitable for cortisol analysis.

### P356

#### Hormonal parameters and sex hormone receptor gene polymorphisms in males with autoimmune diseases

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#### Introduction

Autoimmune disease (AD) is more common in women than in men. Sex hormones may play a role. Sex hormone receptors (SHR) are expressed in cells of the autoimmune system. We investigated the possible role of hormonal

parameters and of common gene polymorphisms of the estrogen receptor alpha (*ESR1*), beta (*ESR2*) and androgen receptor (*AR*) in the presence of AD in men. Methods

In total 227 men were studied; 125 had at least one AD: Hashimoto's ( $n=65$ ), Graves' ( $n=12$ ), SLE ( $n=10$ ), RA ( $n=38$ ). 152 were controls. Estradiol, testosterone, SHBG, insulin, glucose and lipid levels were measured after discontinuation for  $\geq 1$  month of any corticosteroid therapy. Molecular analysis of the PvuII *ESR1*, AluI *ESR2* and the number of the AR CAG repeats ((CAG) $n$ ) was performed.

#### Results

The frequency of *ESR1 PvuII* and *ESR2 AluI* did not differ between patients with AD and controls (allele frequency 52.2%, 40.2% in AD; 51.0% and 46.4% in control). The distribution of (CAG) $n$  did not differ between the two groups: (AD: (CAG) $n$  16–30, mean 21.38, control: 9–30, mean 20.7). Men with AD had higher estradiol levels (AD  $31.32 \pm 12.1$ , control  $20.37 \pm 7.9$ ,  $P < 0.001$ ). No other significant differences in hormone, biochemical and clinical parameters were found. In AD group the (CAG) $n$  was positively associated with estrogen ( $r=0.39$ ,  $P=0.002$ ); *AluI* carriers had lower SHBG levels ( $27.20$  vs  $39.49$ ,  $P=0.001$ ), higher waist perimeter, BMI and weight compared to non carriers ( $99.09$  vs  $91.58$  cm,  $P=0.025$ ,  $27.36$  vs  $24.55$ ,  $P=0.008$  and  $83.86$  vs  $75.21$  kg,  $P=0.006$  respectively).

#### Conclusions

Higher estradiol levels may play a role in the appearance of AD in men. Distribution of common polymorphisms of SHR genes does not differ between patients and controls. We confirmed the positive association of estrogen levels with the CAG repeat length. The *ESR2 AluI* may adversely affect adiposity parameters in men with AD.

### P357

#### Inhibitory effect of glucocorticoids on GLUT4 expression in visceral adipose tissue may be determined by increased 11β hydroxysteroid dehydrogenase 1 (11β-HSD1) expression

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Local glucocorticoids action depends on 11β hydroxysteroid dehydrogenase (11β-HSD) enzymes. Adipose tissues mainly express the 11β-HSD1, which activates inactive cortisone. Altered 11β-HSD1 and GLUT4 expressions have been reported in obesity. GLUT4 participates in the genesis and maintenance of insulin resistance/obesity, and could also be involved in the insulin resistant state observed in hypercortisolism. The aim of this study was to investigate the 11β-HSD1 and GLUT4 expressions in visceral (VAT) and subcutaneous (SAT) adipose tissues of insulin resistant, hypercorticotestosterone MSG-obese rats, considering the tissue-specific action of glucocorticoids.

#### Methods

Male Wistar rats (C) were rendered obese (O), by neonatal treatment with monosodium glutamate (4 mg/g per day). Insulin tolerance tests (ITT), plasma insulin and corticosterone confirmed the obesity features. GLUT4 and 11β-HSD1 mRNAs were analyzed in VAT and SAT, by northern blotting and RT-PCR respectively. Sterol regulatory element binding protein-1c (SREBP-1c) expression and binding activity were also investigated.

#### Results

Obese rats were hyperinsulinemic ( $O=61.4 \pm 5.0$  µU/ml,  $P < 0.05$  vs  $C=40.1 \pm 5.5$  µU/ml), hypercorticotestosterone ( $O=168.3 \pm 9.5$  ng/ml  $P < 0.001$  vs  $C=80.3 \pm 9.8$  ng/ml) and insulin resistant ( $kITT=3.42\%/min$ ,  $P < 0.01$  vs  $C: 5.06\%/min$ ). GLUT4 mRNA was decreased in obese VAT ( $O=80.9 \pm 3.8$  UA,  $P < 0.01$  vs  $C=100.2 \pm 4.2$  UA), where 11β-HSD1 mRNA was increased ( $O=142.5 \pm 6.9$  UA,  $P < 0.01$  vs  $C=100.0 \pm 5.8$  UA). On the contrary, in obese SAT GLUT4 mRNA was increased ( $O=122.1 \pm 9.4$  UA,  $P < 0.05$  vs  $C=100.0 \pm 5.3$  UA) while 11β-HSD1 was decreased ( $O=78.0 \pm 7.5$  UA,  $P < 0.05$  vs  $C=100.0 \pm 5.2$  UA). SREBP1-c binding activity to GLUT4 promoter sequence was unaltered. However, SREBP-1c expression was significantly lower in obese VAT ( $O=82.0 \pm 4.0$  UA,  $P < 0.05$  vs  $C=100.0 \pm 4.4$ ), remaining unchanged in obese SAT ( $O=94.8 \pm 3.2$  UA,  $P < 0.05$  vs  $C=100.0 \pm 6.3$  UA).

#### Conclusion

Data suggest an inhibitory effect of glucocorticoids on GLUT4 gene expression in VAT, determined by a higher expression of the 11β-HSD1 enzyme. This effect may involve mechanisms at transcriptional level, since the expression of SREBP1-c, a transcriptional factor known to increase GLUT4 gene transcription, was shown to be decreased in VAT. Financial support: 07/59722-4.

**P358****Chronic effect of 17 $\beta$  estradiol on GLUT4 expression in 3T3-L1 adipocytes**

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**Introduction**

Insulin resistance results from a combination of genetic and environmental factors and it may be considered the primary cause of the development of metabolic disorders, such as type 2 diabetes mellitus. It is characterized by a reduced ability of insulin sensitivity tissues to respond to normal levels of the hormone. In these tissues, glucose transport stimulated by insulin occurs through the glucose transporter 4 and alterations in its expression are related to changes in insulin sensitivity. It is known estradiol (E<sub>2</sub>) modulates insulin sensitivity and previous studies in skeletal muscle revealed it also participates in the GLUT4 regulation. Thus, the aim of this study was to investigate the effect of 17 $\beta$  Estradiol on GLUT4 expression in 3T3-L1 adipocytes.

**Methods**

3T3-L1 cells were treated with increasing doses of 17 $\beta$  Estradiol (0, 0.1, 1, 10 and 100 nM), for different periods of time (12, 24 and 48 h). After treatment, GLUT4 protein content was quantified by western blotting and mRNA expression by real time-PCR.

**Results**

GLUT4 protein content increased significantly (19–56%) after 24 h of E<sub>2</sub> treatment, in all of the concentrations analyzed, as compared to control (0 nM). Furthermore, after 48 h of treatment, cells exposed to E<sub>2</sub> (0.1 nM) also presented a significantly increase (16%) in GLUT4 protein content, comparing to the other doses. GLUT4 mRNA content increased significantly (20–30%) after 12 h of treatment in intermediate doses (1 and 10 nM), as compared to control (0 nM). However, after 24 or 48 h, no significant difference was observed in GLUT4 mRNA content in any of the doses evaluated.

**Conclusion**

Results suggested a time and dose dependent E<sub>2</sub> effect on GLUT4 expression in 3T3-L1 adipocytes, in addition to a possible post-transcriptional modulation in the presence of the hormone.

**Funding**

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**Nuclear receptors****P359****Oleic and linoleic fatty acids reduce the SLC2A4 gene expression: the involvement of the NF $\kappa$ B and HIF-1 $\alpha$  transcriptional factors**

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**Introduction**

High circulating levels of some but not all unsaturated fatty acids (UFAs) can be related with reduced insulin sensitivity and glucose uptake in skeletal muscle. The real mechanisms by which UFAs impair the insulin sensitivity need to be clarified; nevertheless the UFA's effects in the regulation of activity and/or expression of some transcriptional factors such as, nuclear factor  $\kappa$ B (NF $\kappa$ B), hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), peroxisome proliferator-activated factor  $\gamma$  (PPAR $\gamma$ ), liver-X-receptor alpha (LXR alpha) and sterol regulatory element binding protein 1c (SREBP-1c) which are related with SLC2A4 gene expression have been proposed. Therefore the aim of this study is to investigate the modulation SLC2A4 gene expression by oleic (OFA) (C18:1) and linoleic (LFA) (C18:2) unsaturated fatty acids. At the present moment the involvement of NF $\kappa$ B and HIF-1 $\alpha$  in this mechanism has been analyzed.

**Methods**

L6 muscle cells were maintained in medium DMEM with BSA 1% and exposed to OFA (12.5–400  $\mu$ M) and LFA (50–400  $\mu$ M) for 16 h. GLUT4 protein content was analyzed by western blotting; mRNA expression by real time-PCR; NF $\kappa$ B and HIF-1 $\alpha$  activation by gel shift.

**Results**

The GLUT4 protein and mRNA expression significantly decreased in the presence of OFA and LFA in a dose-dependent way. A significative enhancement of the NF $\kappa$ B mRNA expression and activity was verified at the presence of both fatty acids. However only an increase of the HIF-1 $\alpha$  mRNA expression was observed by OFA and LFA.

**Conclusion**

The treatment with OFA and LFA reduce the SLC2A4 gene expression in L6 muscle cells. The present findings suggest that NF $\kappa$ B, but not HIF-1 $\alpha$ , may be involved in this modulation.

**Funding**

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**Adrenal medulla****P360****Pheochromocytoma: a complex case**

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**Introduction**

Pheochromocytoma is an uncommon tumor. Non-classical forms can make diagnosis difficult and delay correct management strategies.

**Case report**

A 20-years old caucasian male presented to the emergency room with unproductive cough, fever and tiredness for 7 days. He was eupneic, afebrile, BP 176/115 mmHg, 104 bpm with inspiratory crepitations in the left lung base. Leucocyte–14.1  $\times 10^9/l$  (N 70%); platelets – 659  $\times 10^9/l$ ; CRP – 6.9 mg/dl. Normal renal and hepatic function tests and no biochemical markers of myocardial lesion. X-ray: cardiomegaly, opacities in both lungs. Echocardiogram: dilated ventricles, LVH and LVEF 37%. Diagnosed as bilateral pneumonia, dilated cardiomyopathy and hypertension. On day 4, acute right limb ischemia, hemodynamic instability, high fever and rapid deterioration of renal function. CT scan: 7 cm left adrenal tumor, aortic enlarged lymph nodes, right kidney hyperdensity suggesting embolism. Pheochromocytoma was confirmed by urinary epinephrine 202  $\mu$ g/day (<27) norepinephrine 311 (<97), and 131MIBG hyperfixation. After phenoxibenzamine he underwent left adrenalectomy and loco-regional lymphadenectomy. Three days later a left nephrectomy was necessary due to irreversible ischemia. Abdominal hematoma and accentuation of thrombocytosis supervene. Hystology: pheochromocytoma ('unknown behaviour'). One month later, he developed hypercalcemia 11.9 mg/dl, PTH <3 pg/ml, with normal PTHrp and chromogranin A. Thrombocytosis and hypercalcemia both subsided in the next 3 months. No gene mutations were found. After 30 months the patient is asymptomatic.

**Conclusions**

Dilated cardiomyopathy with heart failure in a young hypertensive patient should lead to consider pheochromocytoma. Occlusive arterial phenomena and hypercalcemia made this case even more complex. Tumor size and histology justifies lifelong control.

**P361****Head and neck paragangliomas: genetic spectrum and clinical variability in 101 consecutive patients**

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**Purpose**

To genotype patients with head-neck paragangliomas (HNPG) and evaluate the percentage and types of germ-line mutations in patients classified according to family history (FH) and clinical presentation.

**Design**

In total, 101 consecutive patients with HNPGL were examined for mutations in *SDHB*, *SDHC*, *SDHD*, *SDHAF2* and *VHL* genes by PCR/sequencing. According to a careful FH, clinical, laboratory (including metanephrines) and instrumental examinations patients were divided in three groups: a) with a positive FH for HNPGL; b) with a negative FH and multiple/recurrent HNPGL; c) with negative FH and single HNPGL.

**Results**

Group a) included 33 patients (8 index cases, 25 affected relatives) resulted *SDHD* mutation carriers; seven presented a single HNPGL, 26 presented HNPGL which were multiple (18 patients) or associated with a secreting paraganglioma (sPGL) (eight patients).

All the 16 patients (100%) of group b) resulted *SDHD* mutation carriers. Four patients presented also a sPGL. Among the 52 patients of group c) 9 (17.3%) presented germ-line mutations (two *SDHB*, three *SDHD*, two *VHL*, two *SDHAF2*). Of the two *SDHAF2* mutations one is missense and the same described in one Dutch and Spanish family and one is a novel frame-shift mutation.

A sPGL was found at diagnosis or during the follow up in 12 patients (11.9%); all were *SDHD* mutation carriers and none presented a missense mutation. No *SDHC* mutations were found.

**Conclusions**

A positive FH or the presence of multiple/recurrent HNPGL are strong predictors for germ-line mutations, which are also present in 17.3% of patients clinically classified as sporadic.

By far, the most frequently mutated gene is *SDHD* but other, including *SDHB*, *SDHAF2* and *VHL*, may be affected

In *SDHD* mutation carriers the risk of an associated sPGL is significantly higher for nonsense, frame-shift or insertion/deletion than for missense mutations.

**P362****Succinate dehydrogenase (SDH) mutations: biochemical and functional effects in pheochromocytoma/paraganglioma tissues**

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Pheochromocytoma (PHEO)/paraganglioma (PGL) are neural crest-derived tumors, and about 25% of the apparently sporadic cases are due to a germ line mutation in one of the susceptibility genes. The group of these genes includes the genes encoding three of the four subunits of the SDH, and the recently discovered gene, *SDHAF2/SDH5*, responsible for the flavination of the SDHA subunit.

Although several hypotheses have been proposed, the molecular events by which an impaired SDH function causes the onset of these tumors are still unknown. In the present study, 43 samples of PHEOs and 15 samples of HN-PGLs were evaluated for SDH, citrate synthase (CS) activity, and mitochondrial content.

Our results demonstrate that in tumors of SDH-mutated patients SDH activity is always decreased and that a decreased SDH activity is present also in some wt patients tumors. In any event, the decrement of complex II activity always correlates with a decrease in SDH content. The DNA of all the wt patients with a decreased SDH activity were screened for large deletions and *SDHAF2/SDH5* mutations, but resulted wt once again. Interestingly, CS activity was comparable among all the tissues, thus suggesting that mitochondrial function was not specifically impaired. The expression of COX, which is another enzyme of the respiratory chain, has been found extremely variable in SDH mutated as well as in SDH-wt and SDH-polymorphic tumoral tissues, while VDAC content, that is a channel present on the outer mitochondrial membrane, was comparable among all the samples.

In conclusion, impairment of SDH activity in SDH-wt tissues suggests that these tumors might be mutated for other still unknown susceptibility genes affecting SDH function.

Moreover, the extreme variability in COX expression levels strongly suggests to evaluate functions and expression of other mitochondrial complexes and enzymes to better understand mitochondrial role in the pathogenesis of these tumors.

**P363****Expression of IGF/mTOR pathway components in human pheochromocytomas and *in vitro* inhibition of PC12 rat pheochromocytoma cell growth by mTOR inhibitors**

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The pathogenesis of pheochromocytomas (pheo) is poorly understood and malignant pheo need new treatment options. mTOR inhibitors, as sirolimus (S) and everolimus (E), are new promising antineoplastic drugs.

**Aim**

To evaluate whether the IGF/mTOR pathways have a role in the pathogenesis and whether S and E may have antiproliferative effects in pheo.

In 24 human pheo and two normal adrenal medulla (NM), we evaluated the mRNA expression of: IGFI, IGFI, IGFI-receptor (IGF-R), insulin-receptor (IR) A, IRB, IGFIIR, IGF-binding-proteins (BP) 1, 2, 3 and 6, mTOR, 4EBP1 and p70S6K (qPCR). We tested the dose- and time-dependent effects of S and E on cell growth in the PC12 rat pheo cell line.

In pheo, we observed a high expression of IGFI mRNA and an increased IRA/IRB ratio. The IGFI-R mRNA levels and the IRA/IRB ratio were higher in pheo than in NM. The mRNA levels of mTOR, IGFIIR, BP1 and 6 were lower in pheo than in NM. S and E were able to suppress PC12 growth in a dose and time-dependent manner, but S was more potent than E.

The results of the current study suggest a role of the IGF system in the pathogenesis of pheo. mTOR inhibitors can inhibit the *in vitro* cell proliferation of rat pheo cells, suggesting that mTOR inhibitors and/or other compounds targeting the IGF system may have a role in the treatment of malignant pheo.

**P364****Unusual mutation spectrum in Hungarian patients with apparently sporadic pheochromocytomas**

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**Background**

Pheochromocytomas (Pheo) and paragangliomas (PGL) are rare, mostly benign tumors. Most of these tumors are sporadic but a significant percentage can be found as components of hereditary tumor syndromes caused by germline mutations of the *VHL*, *RET*, *NFI*, *SDHD*, *SDHC*, *SDHB* and the recently identified *SDHAF2* genes. Germline mutations of these genes can be also found in several cases with apparently sporadic Pheo/PGL.

**Aim**

The aim of our study was to analyze the prevalence of germline mutations in patients with apparently sporadic Pheo/PGL.

**Materials and methods**

Mutations analysis of the *RET*, *VHL*, *SDHC*, *SDHB*, *SDHD* and *SDHAF2* genes were performed in 69 patients (27/42 male/female, age: 38.2 (14–72) mean range, years) with apparently sporadic Pheo/PGL consecutively admitted for evaluation. Patients were clinically evaluated and genetic analysis was performed using PCR, direct DNA sequencing and multiplex ligation-dependent probe amplification (MLPA) of the *VHL* gene.

**Results**

Of the 69 patients 14 patients (20.2%) had pathogenic germline mutation. Six patients had mutation of the *RET* protooncogen (Cys611Tyr, Cys634Tyr, Cys634Trp, Cys634Arg and in two unrelated cases Ser649Leu), five patients showed *VHL* gene (Pro63Leu, Arg79Gly, Tyr156Cys and in two unrelated patients Arg167Gln), one patient *SDHD* gene (c147–148 insA) and two patients *SDHB* gene mutations (Cys243Tyr and Cys253Tyr). Of the 14 mutation carriers four patients (one with *VHL* and three with *RET* mutation) were older than 40 years at the time of diagnosis of Pheo/PGL.

**Conclusion**

The prevalence of disease-causing mutations among Hungarian patients with apparently sporadic Pheo/PGL is similar to those reported in the literature. However, Cys649Leu mutation of the *RET* gene classified as a mutation causing mild phenotype has not been previously associated with Pheo. Both Arg79Gly of the *VHL* gene and Cys243Tyr of the *SDHB* gene are novel disease-causing mutations. In addition, our results suggest that complex genetic testing is useful not only in younger but also in older cases.

**P365****Pheochromocytoma occurs in Saudi patients with MEN 2A**

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**Objective**

To evaluate pheochromocytoma that occurs in Saudi patients with MEN 2A  
**Patients and methods**

Of 44 patients belonging to 5 MEN 2A families, 14 (31.8%) presented with pheochromocytoma. The following variables have been studied: clinical and diagnostic data (age, mutation, clinical features, and laboratory studies included measurements of 24-h urinary catecholamines, epinephrine, metanephrine, norepinephrine, dopamine, vanillylmandelic acid, and plasma metanephrine, norepinephrine, and epinephrine. Laboratory studies were considered abnormal in cases where these values were elevated. Tumor location and dimensions were obtained from computerized tomography (CT) scan and iodine-131 meta-iodobenzylguanidine (MIBG) scintigraphy results. The means of diagnostic, clinical, or genetic screening were also studied as well as surgical treatment and follow-up and recurrence. The mean follow-up time was 7 years (range: 12–108 months).

**Results**

The mean age of the 14 patients was 33.5 years (range: 12–52 years); 10 were women. Most (71.4%) mutations were found in exon 11. The most frequent mutations were Cys634Tyr (in eight cases (57.1%)) and Cys634Arg (in three (21.4%)). The diagnosis of pheochromocytoma was made after the diagnosis of MTC in seven cases (50%), simultaneously in five (35.7%), and prior in the two remaining cases (14.3%). At the time of diagnosis eight patients (57.1%) were asymptomatic and six (42.9%) had clinical features related to pheochromocytoma. The most useful isolated laboratory studies were measurements of plasma metanephrine followed by 24-h of urinary metanephrines (82%). The CT scan did not provide a correct diagnosis in one patient with bilateral lesions, and one patient with a unilateral tumor was not diagnosed by MIBG. The combination of CT scan and MIBG diagnosed 100% of cases. The pheochromocytoma was bilateral in two cases. The most commonly used medication for preoperative treatment was phenoxybenzamine, which was utilized in 11 patients (78.6%) and prazosin was used in three patients (21.4%). In two patients (14.2%), beta-blockers were administered in addition to an alpha-blocker. The length of preoperative management was variable with a range of 2–8 weeks.

A laparoscopic approach was used in nine cases and a laparotomy in five. The mean tumor size was 3.5 cm (range: 1.5–10 cm). There was a greater frequency of pheochromocytoma in those subjects who had the Cys634Arg mutation ( $P < 0.03$ ). In addition, the Cys634Arg mutation is more frequent in bilateral cases. There are no prognostic factors for recurrence.

**Conclusions**

Pheochromocytoma in MEN 2A is related to the type of mutation, which can be early onset and is frequently asymptomatic. Its diagnosis requires catecholamines determinations as well as a CT scan. Laparoscopic adrenalectomy is the treatment of choice.

**P366****Impact of surgery upon lipid profile in a case series of pheochromocytoma**

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In pheochromocytoma, intense lyplotic activity may lead to altered lipid profile, which can reverse after tumour removal.

**Aim**

In this study, we evaluated before and at 3 months after surgery for a catecholamine secreting tumour, a group of 19 patients diagnosed with pheochromocytoma (17) or paraganglioma (2).

**Patients and methods**

13 women and 6 men, aged  $53.68 \pm 12.75$  years (36–75), presented with a clinical picture of paroxysmic high blood pressure and decrease of body weight, associated with other signs suggesting excess of catecholamines in 16/19, three cases being oligosymptomatic. The initial evaluation confirmed excess of plasma metanephrines ( $736.11 \pm 886.51$  pg/ml), normetanephrines ( $2423.26 \pm 1907.6$  pg/ml) and chromogranin A ( $790.44 \pm 697.85$  ng/ml).

**Results**

Lipid profile was evaluated by total plasma cholesterol ( $220.27 \pm 50.39$ ) and triglycerides ( $135.11 \pm 74.84$  mg/dl), being abnormal for total cholesterol in 11/19 cases. A CT scan detailed the tumour, with tumour diameters of  $68 \pm 20$  mm (110–40 mm), the local invasion and allowed surgical approach. The patients were prepared for surgery using alpha (phenoxybenzamine) and alpha-beta (carvedilol) blockade for 2–3 weeks. Surgery was uneventful in all cases. After surgery, all cases showed a normalisation of catecholamine levels, for plasma metanephrines ( $21.32 \pm 18.41$  pg/ml),  $P = 0.001$ , normetanephrines ( $67.79 \pm 48.82$  pg/ml)  $P < 0.001$  and chromogranin A ( $95.83 \pm 81.28$  ng/ml),  $P < 0.001$ . The lipid profile showed an improvement in cholesterol levels after surgery ( $187.82 \pm 44.14$ ),  $P = 0.01$ , in nine cases being improved but in two worsened. In addition, three of the cases with normal preoperative values presented an increased cholesterol post surgery. Triglyceride levels decreased but did not reach statistical significance ( $109.5 \pm 41.02$   $P = NS$ ). Tumour diameter was strongly correlated with metanephrine and normetanephrine ( $P < 0.001$ ) values.

**Conclusion**

In pheochromocytoma, tumour removal improves also the hypercholesterolemia. Definitive metabolic treatment should be decided at 3 months after surgery.

**Signal transduction****P367****Transmembrane potential ( $\Delta\psi_p$ ) slight changes in non-excitabile individual cells at short-term hormone action**

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Early events in activated by adrenaline ( $10^{-6}$  M) and phenylephrine ( $10^{-5}$  M) single hepatocytes of rat have been investigated by quantitative image microfluorometry and microspectrofluorometry. Membrane potential-sensitive cationic DiOC<sub>2</sub>(3) and anionic SqSC4 probes have been used for image analysis and transmembrane potential ( $\Delta\psi_p$ ) estimation in real-time studies. Fluorescence spectra resulting from the accumulation of dyes in single cells were recorded. A special data acquisition program allowed measuring fluorescent cell parameters. Based on the mean fluorescence intensity the magnitude of  $\Delta\psi_p$  was calculated by Nernst equation adapted for lipophilic cationic probes.

DiOC<sub>2</sub>(3) has revealed that both hormones induced biphasic hyperpolarization of hepatocytes membrane with alpha-agonist phenylephrine causing a higher amplitude of  $\Delta\psi_p$  changes.

The first increase of  $\Delta\psi_p$  between 2 and 5 min ( $\Delta\Delta\psi_p = -8.6 \pm 4.2$  mV) after the Ca<sup>2+</sup>-mobilizing hormone addition evidently depends on Na<sup>+</sup>/K<sup>+</sup>-ATPase activation. The second peak of hyperpolarization ( $\Delta\Delta\psi_p = -13.2 \pm 3.2$  mV) between 25 and 30 min, after a transient decrease of  $\Delta\psi_p$  ( $\Delta\Delta\psi_p = 10.9 \pm 4.3$  mV) at 15 min of experiment, probably is mediated by phenylephrine stimulating action on K<sup>+</sup>-channels.

Ca<sup>2+</sup> chelating with EDTA didn't influence the curve character; however it slightly decreased the hormonal effect on the second peak amplitude.

Modulation of PLD-dependent signal transduction pathway by 0.4% butanol had a weak influence on the first increase of  $\Delta\psi_p$  but it abolished the second phase of hyperpolarization. It points to PLD involvement in the mediated by K<sup>+</sup>-channels  $\Delta\psi_p$  fluctuations in response to phenylephrine.

Based on SqSC4 fluorescent parameters estimation of  $\Delta\psi_p$  relative changes showed the same character of time dependence curve with two phase of hyperpolarization. Synchronic fluctuation of this parameter determined by oppositely charged probes demonstrates that quantitative microfluorimetry allows estimation of slight  $\Delta\psi_p$  changes in non-excitabile individual cells at short-term hormone action.

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**Research resource for analysing functional data of glycoprotein hormone receptors: novel tools to gain deeper insights into molecular mechanisms**

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The sequence–structure–function-analysis of glycoprotein hormone receptors (SSFA-GPHR) database provides a comprehensive set of mutation data for the glycoprotein hormone receptors (covering the lutropin – (LHCGR), the follitropin (FSHR) and the thyrotropin receptor (TSHR)). Numerous databases present functional information along with pooled data from various public resources, but tools to analyse the molecular effects of genetic variations are as yet poorly provided. Besides extending the dataset (> 1100 mutations), the database has been completely redesigned and several novel features and analysis tools have been added. These tools allow the focused extraction of semi-quantitative mutant data from the GPHR subtypes and different experimental approaches. Functional data of the GPHRs are now linked interactively at the web interface with protein structure data and novel tools for data search and visualization are provided. A user defined 2D-search option via snake plots highlights the requested mutated residue(s) in a two dimensional representation of the sequence of interest and hyperlinks them to the mutation information. A 3D-search serves as interactive picking option of any residue position of interest on a 3D structure and provides information about functional effects of available genetic variations that can be mapped onto the structure. The new receptor morphing simulation tool permits tracing of potential structural changes from the basal to an activated receptor conformation for any individual wild type residue and provides clues to the local structural environment, including potentially relocated spatial counterpart residues. Furthermore, double and triple mutations are newly included to allow the analysis of their functional effects related to their spatial interrelationship in structures or homology models. These new tools can be used to identify interacting residues as counterparts and to evaluate potential mechanisms of molecular malfunction caused by specific mutations and give thereby deeper insights in the mechanisms of hormone binding, signal transduction and signaling regulation of GPHRs.

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**Different and common transmembrane activation mechanisms between glycoprotein hormone receptors and other G-protein coupled receptors**

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The aim of our study is to identify signalling sensitive residue positions of the glycoprotein hormone receptors (GPHR), especially the thyrotropin receptor (TSHR) on the transmembrane helices (TMH) 5 and 6, knowing to be involved in GPCR activation. We performed modelling driven site-directed mutagenesis to pinpoint residues that are responsible for stabilization of active and inactive conformations of the TSHR.

We highlight two amino acids that are highly conserved in other family A GPCRs but are different in the GPHRs. Proline at position 5.50 causes a conformational-twist in TMH5 at all available GPCR X-ray structures. This proline is replaced in GPHRs by an alanine. However, mutation of alanine to proline at position 5.50 (TSHR-A593P) results in a 20-fold decreased cell surface expression and a folding defect of TSHR. This implies structural consequences because of different interactions between TMH5 and neighbouring helices 3, 4, and 6 in the TSHR compared to other GPCRs. Our results favour for TSHR and homologous FSHR and LHCGR rather a regular alpha-helix conformation than a proline-twisted conformation of TMH5.

Position 6.48 that is highly conserved in other family A GPCRs by a tryptophan known as a 'toggle switch' is replaced in GPHR by a methionine. Substitution of this methionine by tryptophan (TSHR-M637W) results in constitutive activation and indicates an alternative micro-switch at TSHR position 6.48.

Our functional data highlight the importance of hydrophobic amino acids at the interface between TMH5 and 6. We confirmed this hypothesis by a statistical analysis of residue properties between the TMHs of a vast number of family A GPCR sequences. We revealed that interacting polar residues between TMH2, 3, 6 and 7 likely constrain the inactive conformation, whereas their release plus the formation of hydrophobic interactions between TMH5 and 6 may constrain the active state conformation of GPCRs and also of GPHRs.

P370

**Distribution of 3-iodothyronamine and trace amine-associated receptors in mouse**

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**Introduction**

3-Iodothyronamine (TIAM) is a novel endogenous relative of thyroid hormone able to interact with specific G protein-coupled receptors, known as trace amine-associated receptors (TAAR). Metabolic, endocrine and cardiac effects of exogenous TIAM have been reported.

**Methods**

We synthesized radiolabelled TIAM, [<sup>125</sup>I]-TIAM, and explored its distribution in different mouse organs after injection in the tail vein. The specificity of [<sup>125</sup>I]-TIAM was checked in parallel experiments, in which a 100-fold excess of unlabeled TIAM was injected 5 min before the radioligand. We also performed an *in vivo* evaluation of TAAR expression by quantitative real-time PCR, using specific primers for the nine known TAAR subtypes.

**Results**

[<sup>125</sup>I]-TIAM preferentially distributed to the gastrointestinal tract, liver and kidney: in particular at 30 min the highest levels of radioactivity (expressed as percentage of injected dose per g wet weight) were detected in gallbladder, stomach, intestine, liver and kidney (23.3, 8.6, 7.4, 6.1 and 7.2 respectively) and at 60 min top values were still present in gallbladder, liver and kidney (14.7, 6.1 and 6.5 respectively). At 24 h however the highest residual concentration was detected in liver and adipose tissue (1.2 and 0.5 respectively). TAAR expression investigations showed that in most tissues TAARs were expressed only at trace amounts (< 10 copies per µg mRNA). Higher expression was detected in stomach and testis for TAAR1 (83 and 55 copies per µg mRNA) and in small bowel, spleen and testis for TAAR8 (16, 19 and 12 copies per microg mRNA). Notably, TAAR expression was negligible in liver and kidney.

**Conclusions**

Exogenous [<sup>125</sup>I]-TIAM is preferentially taken up by the liver, kidney and gastrointestinal tract. TAAR binding seems unable to account for TIAM distribution suggesting that TIAM binds to different targets, particularly in liver and kidney.

**Thyroid (non cancer)**

P371

**Minimally invasive video-assisted thyroidectomy versus conventional thyroidectomy: a single-blinded, randomized controlled clinical trial**

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**Aim**

We aimed to test the hypothesis that the minimally invasive video-assisted thyroidectomy (MIVAT) affords comparable safety and efficacy as to the open conventional surgery in patients with unilateral thyroid nodules or follicular lesions in terms of cosmetic results, intraoperative and postoperative complications, postoperative pain, and hospital stay.

**Methods**

This was a single-blinded randomized controlled trial comparing the MIVAT with conventional thyroidectomy. The primary endpoints of the study were measurement of postoperative pain after 24 and 48 h from operation and cosmetic outcome 3 months postoperatively. The secondary outcome measures were operative time, incidence of recurrent laryngeal nerve injury, length of incision, and hospital stay.

**Results**

Operative time was less with open thyroidectomy than with MIVAT, while MIVAT was associated with less pain 24 h postoperatively. Pain score depicted statistically significant differences in favor of the MIVAT after 24 h. MIVAT was associated with less scarring and more satisfaction with cosmetic results. There was no difference between both procedures for presence of transient recurrent laryngeal nerve palsy and hypoparathyroidism.

**Conclusion**

MIVAT is a safe procedure that produces outcomes similar to those of open thyroidectomy, and is superior in terms of immediate postoperative pain and cosmetic results.

**P372****Complications after thyroidectomy: a 10 years experience in a District General Hospital**

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**Background**

Patients undergoing thyroid surgery are consented for a 1% risk of permanent hypocalcaemia and voice changes. These complications are considered to be permanent if they persist for more than 6 months. The aim of this study is to determine the incidence of complications in patients undergoing thyroid surgery in our hospital.

**Methods**

From January 1999 to March 2009, all patients who underwent thyroid surgery under the care of one surgeon were identified from the database. Data on the surgical procedure, indication, final histology and follow-up were recorded from case notes.

**Results**

Data were recorded on 559 patients who underwent surgery during this period, 498 for benign thyroid disease and 61 for malignancy. Thirty-five patients (6.3%) with benign pathology and 20 (32.8%) with thyroid malignancy had postoperative complications ( $P=0.0001$ ). Two patients (0.4%) in benign group and one patient (1.64%) with malignancy had permanent hoarseness. Permanent hypocalcaemia was noted in five patients (1.0%) in benign group and one patient (1.64%) in malignant group. The overall incidence of permanent hoarseness and hypocalcaemia was 0.54 and 1.07% respectively.

The incidence was higher in patients over the age of 60 years (17/284 versus 38/275,  $P=0.002$ ) and in those having completion thyroidectomy (13/50 versus 42/509,  $P=0.004$ ). Multivariate logistic regression revealed only thyroid malignancy to be significantly associated with postoperative complications ( $P<0.0001$ ).

**Conclusion**

The incidence of permanent complications in thyroid surgery is low, patients with thyroid malignancy being at a higher risk. Thyroid surgery appears to be safe in the hands of a general surgeon with a subspecialist interest.

**P373****Risk factors for morbidity after thyroid surgery: analysis of 559 cases**

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**Background**

Surgery remains the mainstay of treatment for both benign and malignant thyroid disease. Permanent hypoparathyroidism and recurrent laryngeal nerve injury are the two most commonly occurring complications post thyroid surgery. The aim of this study is to determine the risk factors for developing post-operative complications.

**Methods**

From January 1999 to March 2009, all patients who underwent thyroid surgery under the care of one surgeon were identified from the database. Data on the surgical procedure, indication, final histology and follow-up was recorded from medical notes.

**Results**

The study included 559 patients out of which 391 patients underwent total thyroidectomy, 118 had hemithyroidectomy and 50 had completion total thyroidectomy. Fifty-five patients had postoperative complications, 17 of which were <60 years and 38 were more than 60 years old ( $P=0.002$ ). 35/498 patients with benign disease and 20/61 with thyroid malignancy had postoperative complications ( $P=0.0001$ ). Two patients in benign group and one patient in malignant group had permanent hoarseness ( $P=0.29$ ), whereas five patients in benign group and one patient with malignancy had permanent hypocalcaemia ( $P=0.50$ ). 22/119 patients with compressive symptoms and 33/440 with non-compressive symptoms had postoperative symptoms ( $P=0.564$ ). The incidence was significantly higher in completion thyroidectomy as compared to total thyroidectomy and thyroid lobectomy ( $P=0.0004$ ). Multivariable logistic regression identified only thyroid malignancy to be significantly associated with postoperative complications ( $P<0.0001$ ).

**Conclusion**

The risk of postoperative complication is significantly higher in malignant cases. On multivariate analysis, age, symptoms and the type of procedure did not have statistically significant association with postoperative complications.

**P374****Acoustic radiation force impulse imaging of thyroid gland**

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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 the feasibility of ARFI-measurements in the thyroid tissue.

**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  $\geq 1$  cm, non-functioning or hypo-functioning on radionuclide scanning, and cytological/histological assessment. All patients received conventional ultrasound, real-time elastography (RTE) and ARFI-imaging.

**Results**

Sixty nodules in 55 patients were available for analysis. Fifty-seven nodules were benign on cytology/histology, and three nodules were papillary carcinoma. The median velocity of ARFI-imaging in the healthy nodule-free thyroid gland, as well as in benign and malignant thyroid nodules was 1.98 m/s (range: 1.20–3.63 m/s), 2.02 m/s (range: 0.92–3.97 m/s), and 4.30 m/s (range: 2.40–4.50 m/s) respectively. While no significant difference in median velocity was found between healthy thyroid tissue and benign thyroid nodules, a significant difference was found between malignant thyroid nodules on the one hand and healthy thyroid tissue ( $P=0.018$ ) or benign thyroid nodules ( $P=0.014$ ) on the other hand. A specificity of ARFI-imaging of 91% could be achieved using a cut-off of 3.1 m/s. This was comparable to the specificity of RTE with 91% in the present study.

**Conclusions**

ARFI can be performed in the thyroid tissue with reliable results. This novel quantitative elastography-method can be performed with high specificity in the diagnostic work-up of thyroid nodules. Larger studies are awaited.

**P375****The influence of cure of subclinical hyperthyroidism on left ventricular walls thickness and mass index**

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**Introduction**

Subclinical hyperthyroidism (SH) affects about 1% of world human population at least. The diagnosis of this disease leans on the laboratory criteria only: decreased of TSH and normal FT<sub>3</sub> and FT<sub>4</sub> concentrations. SH increases mortality mostly due to cardiovascular diseases including overload of the heart and ventricular arrhythmia. These symptoms are anticipated by increasing of left ventricular walls thickness and its mass index.

**Aim**

To estimate an influence of cure of SH on left ventricular walls thickness: interventricular septum diameter (IVSD), posterior wall diameter (PWD) and left ventricular mass index (LVMI) measured by echocardiography. And also to find the correlations between these parameters and TSH, FT<sub>3</sub> and FT<sub>4</sub> concentrations.

**Method**

Forty-four patients (37 women, 7 men) aged  $45.9 \pm 11$ , with  $12.8 \pm 9.8$  month history of only autonomous endogenous SH (TSH =  $0.16 \pm 0.1$  mIU/l), were examined with echo twice: before and  $5.7 \pm 4.2$  months after TSH normalization (TSH =  $1.32 \pm 0.1$  mIU/l) with radioiodine treatment (dose  $12.1 \pm 5.7$  mCi). The average time between examinations was  $12.5 \pm 6$  months. The Local Ethical Committee approval has been obtained.

**Results**

The cure of SH caused decrease of IVSD ( $P<0.00001$ ), PWD ( $P<0.00001$ ), LVMI ( $P<0.0001$ ). During SH the level of FT<sub>3</sub> was positively correlated with IVSD ( $P=0.045$ ) and PWD ( $P=0.038$ ).

**Conclusions**

i) Cure of autonomous subclinical hyperthyroidism with radioiodine decreases left ventricular walls thickness and its mass index. ii) These results can indicate



decrease of heart after load and the risk of ventricular arrhythmia. iii) In autonomous subclinical hyperthyroidism, the level of FT<sub>3</sub> positively correlates with diameter of interventricular septum and left ventricular posterior wall. iv) Above findings support the decision to treat endogenous subclinical hyperthyroidism especially in patients with cardiovascular risk.

### P376

#### The use of lithium and perchlorate therapy in special cases of hyperthyroidism

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#### Objective

To study the efficacy of lithium and perchlorate in special cases of hyperthyroidism: thioamide-resistant or iodine-induced thyrotoxicosis/thyrotoxic pre-crisis, hematological adverse effects of methimazole, leukopenia of other causes.

#### Material and methods

Twenty-seven hyperthyroid patients received lithium carbonate and/or potassium perchlorate, majority after methimazole-therapy (associated with folic acid in leukopenia). We followed the clinical course, TSH, FT<sub>4</sub>, TT<sub>3</sub> and hemogram during therapy.

#### Results

Thyrotoxic pre-crisis appeared in 14.8% and moderate overt hyperthyroidism in 85.2% of patients (majority due to autoimmune thyroid diseases). Methimazole induced leukopenia in 18 cases: in 14 the initial high doses (30–60 mg/day), in 4 the chronic treatment. In four subjects interferon provoked leukopenia. Methimazole-therapy was insufficient in 3 and caused allergy in 2 cases. We introduced lithium carbonate in 250–1000 mg daily doses, and the leukocyte count begun to rise on the second day, with normalization on fifth day. Lithium-therapy (perchlorate in 4 cases) normalized thyroid function on the 3–4th week in 8 cases, and reduced the plasma levels of thyroid hormones with about 38.8% in 19 patients. Four subjects underwent thyroidectomy after euthyroid status was reached, 6 continued lithium-therapy with lower doses, and 9 patients received gradually reduced doses of lithium and methimazole (5–15 mg/day) necessary to keep thyroid function and bloodcount normal. Lithium was ceased due to adverse effects in 6 cases (3 gastrointestinal, 3 psychical); we lost the contact with 2 patients. Mean duration of treatment was 4.4 months for lithium and 1.1 month for perchlorate.

#### Conclusion

Lithium/perchlorate with or without small doses of thioamide may be a therapeutic option when thioamides alone are insufficient or not tolerated; they improve both the hyperthyroid state and the hematological disorders. Euthyroid status was reached, thus thyroidectomy could be performed if was necessary. Lithium provoked gastrointestinal and psychic complications, while the short-term perchlorate therapy did not cause any side effects.

### P377

#### Comprehensive symptom profile in patients with nontoxic goiters: practicability and sensitivity of the new symptom assessment tool CSP-ThSF22

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The value of patient-reported outcomes in treatment efficacy assessment of thyroid disorders is of great importance. Recently, a new tool, Comprehensive Symptom Profile (CSP-ThSF22), has been developed to assess symptoms specific for patients with nontoxic goiters. We aimed to test practicability and sensitivity of CSP-ThSF22 in patients with euthyroid goiter undergoing thyroidectomy. A total of 78 patients with uninodular/multinodular euthyroid goiter were enrolled in this study (mean age 48.0, male/female – 14/64). All the patients underwent thyroidectomy. Patients filled out CSP-ThSF22 before surgery and at different time-points thereafter. CSP-ThSF22 is developed to assess the severity of 22 symptoms specific for patients with euthyroid or hypothyroid goiter. Wilcoxon's matched pairs test was used to compare symptom severity at different time-points. The prevalence of moderate-to-severe symptoms was studied using  $\chi^2$  test or Fisher's exact test. Practicability of CSP-ThSF22 was shown: patients needed 5–7 min to answer it; the proportion of missing values

was <1.5% for all questions; the questionnaire found high acceptance reflected by no refusals. Usefulness of CSP-ThSF22 to distinguish patients in terms of severity and number of symptoms experienced was demonstrated. The information obtained was used by the surgeons at follow-up. After thyroidectomy, reduction in the vast majority of symptoms was observed. At 3 months after surgery, the severity of 11 out of 22 symptoms decreased significantly ( $P < 0.05$ ). Remarkable decrease in the prevalence of moderate-to-severe symptoms after surgery was shown ( $P < 0.05$ ). CSP-ThSF22 is a sensitive and practical tool to assess symptom profile and severity in patients with nontoxic goiters. Comprehensive symptom monitoring is strongly recommended to clearly determine treatment outcomes in patients with euthyroid goiter undergoing thyroidectomy.

### P378

#### Selenium treatment in chronic autoimmune thyroid diseases

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#### Objective

To evaluate the effect of selenium on autoimmune thyroid diseases through ATPO-titers.

#### Material and methods

Selenium (0.1–0.2 mg/day) was associated to basic treatment of thyroid dysfunctions in 21 female patients with high ATPO-levels (normal <34 U/ml). We followed the thyroid function and ATPO-values usually after 2–6 months of selenium administration.

#### Results

Ten hypo-thyroid patients had Hashimoto's thyroiditis, 6 were diagnosed with Hashitoxicosis and 3 with active Graves' disease, while two female patients were euthyroid without any treatment. Initial ATPO-levels were between 185 and 2090 U/ml with mean value of 802 U/ml, in the majority (15 cases) being 10-times above the upper normal limit. Eleven patients received firstly L-T<sub>4</sub> or antithyroid therapy and selenium was associated later (first group), in other ten cases basic thyroid treatment and selenium were combined from the beginning (second group). In the first group initial mean ATPO-value was 942 U/ml, during efficient basic therapy decreased to 808 U/ml, but individual ATPO-levels decreased only in five cases (from mean of 1166 to 634 U/ml), in the other six patients increased considerably (from mean of 756 to 1060 U/ml); differently, selenium treatment during 2–6 months reduced mean ATPO-value to 237 U/ml, without increase in any patient. In the second group mean ATPO was 578 U/ml before treatment, and the combined therapy applied for relatively short period (1–4 months) reduced it gradually to 296 U/ml. We observed considerable individual differences in the response to selenium treatment. It seems that the effect of selenium was stronger in patients having very high initial ATPO-levels compared to those with moderately increased titers. The response was also influenced by the duration of selenium administration.

#### Conclusion

In autoimmune thyroid diseases with or without hypo-/hyperthyroidism, selenium in about 0.1 mg/day dose reduces substantially the high ATPO-levels and may slow the evolution of autoimmune thyroid processes.

### P379

#### Comprehensive symptom profile in patients with toxic goiters: practicability and sensitivity of the new symptom assessment tool CSP-ThHyperSF27

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The value of patient-reported outcomes in treatment efficacy assessment of thyroid disorders is of great importance. Recently, a new tool, Comprehensive Symptom Profile (CSP-ThHyperSF27), has been developed to assess symptoms specific for patients with toxic goiters. We aimed to test practicability and sensitivity of CSP-ThHyperSF27 in patients with diffuse/ multinodular toxic goiter undergoing thyroidectomy. A total of 42 patients with diffuse/multinodular toxic goiter were enrolled in this study (mean age 42.8, male/female – 7/35). All the patients underwent thyroidectomy. Patients filled out the CSP-ThHyperSF27 before surgery and at different time-points thereafter. The CSP-ThHyperSF27 is developed to assess the severity of 27 symptoms specific for patients with toxic

goiter. Wilcoxon's matched pairs test was used to compare symptom severity at different time-points. The prevalence of moderate-to-severe symptoms was studied using  $\chi^2$  test or Fisher's exact test. Practicability of the CSP-ThHyperSF27 was shown: patients needed 5–7 min to answer it; the proportion of missing values was <2% for all questions; the questionnaire found high acceptance reflected by no refusals. Usefulness of the CSP-ThHyperSF27 to distinguish patients in terms of severity and number of symptoms experienced was demonstrated. The information obtained was used by the surgeons at follow-up. After thyroidectomy, reduction in the vast majority of symptoms was observed. At 3 months after surgery, the severity of 16 out of 27 symptoms decreased significantly ( $P < 0.05$ ). Remarkable decrease in the prevalence of moderate-to-severe symptoms after surgery was shown ( $P < 0.05$ ). The CSP-ThHyperSF27 is a sensitive and practical tool to assess symptom profile and severity in patients with toxic goiters. Comprehensive symptom monitoring is recommended to clearly determine treatment outcomes in patients with toxic goiters undergoing thyroidectomy.

### P380

#### A case of Riedel's thyroiditis

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#### Introduction

Riedel's thyroiditis (RT) is a rare and chronic fibro-inflammatory disease firstly described by Riedel in 1896. It is characterized by a dense fibrosis that replaces normal thyroid parenchyma. The fibrotic process invades adjacent structures of the neck and extends beyond the thyroid capsule, which differentiates RT from other inflammatory or fibrotic disorders of the thyroid. The overall incidence in outpatients is 1.06 per 100 000. We presented here a case of Riedel's thyroiditis, which is operated for a suspicion of thyroid malignancy.

#### Case report

A 48-year-old woman was admitted to the hospital with shortness of breath and swelling in the neck. Her thyroid was firm and multiple nodules were palpated. She was euthyroid. Anti-TPO and anti-Tg antibody levels were in normal ranges. In her thyroid ultrasonography, right lobe's dimensions were increased. There were hypoechoic areas with irregular margins with dimensions of 9 mm at left upper pole, 23×15 mm at right middle pole and 9×9 mm at left lower pole, which were totally interpreted as thyroiditis. Multinodular hyperplasia with hypoactivity in the nodules of right lobe was obtained in the thyroid scintigraphy. In the fine-needle aspiration biopsy, there were no atypical cells. Surgery was performed to exclude malignancy and to relieve the patient's complaints. On pathological examination, Riedel's thyroiditis was diagnosed.

#### Conclusion

The main diagnostic modality in Riedel' thyroiditis is pathological examination. The criteriae most commonly used to diagnose Riedel's thyroiditis are gross description of visible fibroinflammatory process involving all or a portion of the thyroid gland, gross or histological evidence of extension into adjacent structures, absence of granulomatous reaction and neoplasm. It may occur in a multinodular goiter, mimicking thyroid cancer. So such a rare disorder may also be encountered in the investigation of thyroid malignancy.

### P381 – ESE Young Investigator Award

#### Molecular analysis and functional significance of TSHR gene mutations in pediatric patients

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TSH receptor (TSHR) is a G-protein-coupled seven-transmembrane domain receptor located in the basolateral membrane of thyroid follicular cells. The activated receptor couples to two major signal transductions pathways: the Gs/adenylate cyclase and the Gq/11/phospholipase C signalling. Many loss of function mutations have been identified in this gene leading to a wide spectrum of thyroid abnormalities, ranging from hyperthyrotropinemia and TSH resistance to severe hypothyroidism. We identified in one child a non-sense mutations in exon 10 for the substitution of tryptophan at position 520 with a stop codon (W520X).

Because the mutated receptor loses its C-terminal portion necessary for the cellular signalling, the functional significance of this variation was assessed *in vitro*.

#### Methods

Molecular analysis of TSHR gene was performed in 112 pediatric patients (M/F: 56/56; age range: 1–18 years) showing elevated serum TSH levels and normal thyroid hormones and in 112 healthy subjects. W520X mutation was introduced into the pSVL vector by mutagenesis. Wild-type and mutated vectors were expressed in CHO cells and cAMP assay, inositol phosphate (PI) assay and immunofluorescence analysis were performed.

#### Results

We identified in exon 9 the new synonymous variation D232D and the intronic substitution A/G at position IVS9+3 bp. Both were found in heterozygous state and were not present in our group of controls. We observed that CHO cells expressing the W520X mutated receptor showed a lower cAMP and PI production with respect to wild-type receptor. Moreover, a reduced expression of mutated receptor on the cell surface was observed.

#### Conclusion

Our data demonstrate that the premature stop codon introduced by the W520X mutation causes a reduction of TSHR expression at the cell surface. This reduces TSHR signalling pathway, thus being responsible of the hormonal pattern of subclinical hypothyroidism observed in our child.

### P382

#### Evolution of thyroid function and antibodies in Hashimoto thyroiditis after 10 years observation. Study on 1000 patients

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#### Study

Retrospective, cohort.

#### Material and methods

A. Hashimoto thyroiditis (HT) diagnosis: i) antithyroperoxidase antibodies (ATPO) criteria; cut-off > 34 IU/ml. ii) If ATPO=normal (N), HT was considered only antithyroglobuline antibodies (ATG) were > 50 IU/ml (†) and ultrasound was pattern 1 (pseudonodular, intense hypoechogenic, inhomogeneous – Peretianu, Endocrine Abstracts, 2007, 14, P340; 2008, 16, P772; 2009, 20, P79).

B. Patients: 1000 patients. HT: women: 950, men 5%.

#### Results

A. At the diagnostic moment (DiMo). i) Function: euthyroid (EUT): 460 (46%), hypothyroid (HOT): 397 (39%), hyperthyroid: 143 (15%) – from these: 95 (66%) associated certainly (TRAB<sup>+</sup>) Graves-Basedow disease (GBD). ii) ATPO: average: 620, s.d.: 1060. min: 1, max: 13 000. In 7 (0.7%), ATPO high, even no thyroid was detected. 28 with ATPO=N. iii) 26 (33%) HT with TRAB<sup>+</sup> were EUT/HOT. iv) Amiodarone association: 14 (1.4%): 6 EUT, 4 HOT, 2 HIT.

B. Follow-up. i) Function: a. 15 (3.25%) with EUT became HOT after 0.2(1)-5 years (average=2.16, s.d.=1.72). b. 100% HOT remained HOT. c. HIT without treatment at 3 years become EUT in only 3, HOT in only 1. ii) ATPO: a. Evolution type: undulatory: 118 (45%), decreasing: 34 (34%), increasing: 23 (56%). b. Linear correlation with thyroid hormone/TSH: none;  $r=0.5/0.17$ ,  $P=0.9$  (NS).

#### Discussion

i) Ultrasound thyroid patterns. ii) ATPO histogramme by intervals. iii) Most common association in HT was GBD (see also Poiană, Endocrine Abstracts, 2008, 16, P7822).

#### Conclusions

i) HT presented more as EUT than HOT. ii) Only 2.5% EUT-HT become HOT, during first 5 years. iii) For searching new HOT, TSH at 3 months. iv) ATPO is not correlated to thyroid (function and morphology). v) No EUT-HT after 5 years modified function. vi) Amiodarone did not affect thyroid function. vii) HT-GBD is more difficult to control nonradically than GBD-no HT; if medical treatment, > 3 years.

### P383

#### Hashimoto's thyroiditis is not associated with breast cancer more than other thyroid condition or more than general population

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#### Premises

Numerous recent medical journals and communications insist on the high prevalence of Hashimoto's thyroiditis (HT) associated with breast cancer in women (see [www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/) key words: breast cancer, thyroiditis, #91).

**Objective**

We proposed to present data from our casuistry related to HT (over 2000 patients and control, including men), observed during last 10 years.

**Materials and methods**

A. Patients: 950 consecutive women with HT, compared with 860 women with other thyroid diseases or normal thyroid, as controls, were investigated for breast cancer. B. All the patients had: i) ATPO analysis, for inclusion or exclusion HT diagnostic; ii) thyroid ultrasound; iii) FT<sub>4</sub> and TSH; iv) breast specific investigations (ultrasound and/or mammography). C. Statistical analysis was done by  $\chi^2$ -test.

**Results**

Breast cancer was registered in only 19 women (prevalence 1.05%): 11 with HT (prevalence 1.16%), 8 with other thyroid diagnostics (prevalence 0.93%).

	Breast cancer	No breast cancer	Total
HT	11	939	950
Non HT	8	852	860
	19	1210	1225

Statistical analysis:  $\chi^2=0.55$ ,  $P=0.36$  (nonsignificant).

**Discussion**

Breast cancer prevalence in general population is around 0.85% (see, for USA, [seer.cancer.gov/statfacts/html/breast](http://seer.cancer.gov/statfacts/html/breast)). Breast cancer in HT seems to be more than in general population: 1.05 vs 0.85%. But, introducing the data in a  $\chi^2$  test, the result will be:  $X^2=0.36$ ,  $P=0.83$  (nonsignificant). Considering all our patients (TH and control) versus general population,  $\chi^2$  test will be:  $X^2=0.51$ ,  $P=0.42$  (nonsignificant).

**Conclusion**

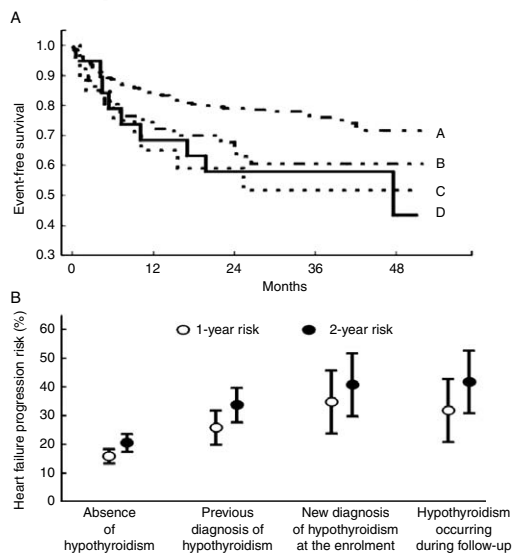
i) Women with Hashimoto's thyroiditis did not associated more breast cancer than other women with other thyroid clinical conditions. ii) Breast cancer in women with Hashimoto thyroiditis is no more prevalent than breast cancer in general population. iii) Breast cancer in women with Hashimoto thyroiditis and other thyroid clinical condition is no more prevalent than breast cancer in general population.

**P384****Occurrence and prognostic impact of hypothyroidism in chronic heart failure**

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The aim of this study was to define the occurrence of hypothyroidism and its impact on CHF progression. Four hundred and twenty-two consecutive



**Figure 1** (A) Heart failure progression in subgroups with euthyroid status at the enrolment and during follow-up (Group A), previous diagnosis of hypothyroidism (Group B), new diagnosis of hypothyroidism at the enrolment (Group C) and occurrence of hypothyroidism during follow-up (Group D). (B) Probability of 1- and 2-year occurrence of heart failure progression in different subgroups.

outpatients (326 males, aged  $65 \pm 12$  years) with CHF in stable condition and conventional therapy were evaluated. Thyroid status was checked at least every 4 months. The progression of heart failure was defined as death, urgent heart transplantation, or hospitalisation due to worsening HF.

**Results**

A total of 51 patients (12%) had a previous diagnosis of hypothyroidism while 21 (5%) were newly diagnosed at the enrolment (prevalence of hypothyroidism at the first evaluation: 17%; 33% in females vs 13% in males;  $P < 0.001$ ). During follow-up (median 28 months) hypothyroidism occurred in further 19 patients (incidence rate: 26/1000/year) and it was mainly attributable to amiodarone therapy. In patients affected by hypothyroidism a significantly greater occurrence of heart failure progression was observed (Fig. 1A/B).

**P385****Serum lipid oxidation and paraoxonase enzyme activity in subclinical thyrotoxicosis**

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Changes in serum lipid and lipoprotein concentrations occur frequently in disorders of thyroid function. LDL-C oxidation susceptibility is higher in these patients than in normal population. This study aims at assessing lipids, lipoproteins, lipid oxidations and serum paraoxonase activity in patients with subclinical hyperthyroidism. Forty patients were compared with an age and sex matched control group. A fasting blood sample was obtained and serum total cholesterol, triglycerides, lipid oxidation parameters, and paraoxonase activity were measured. In patients with subclinical hyperthyroidism, significantly lower serum paraoxonase activity ( $52 \pm 26$  vs  $76 \pm 35$  IU/ml,  $P < 0.001$ ), triglycerides, LDL, Total cholesterol, Total antioxidant capacity, B-carotene, Vitamin A and E were found. The results show significant changes of lipid levels in subclinical thyrotoxicosis. In addition, significant reduction in serum paraoxonase activity was observed in these patients, this can be due to the hypermetabolic state in the patients with subclinical thyrotoxicosis.

**P386****Undiagnosed subclinical hypothyroidism, health indicators and cardiovascular risk factors in a Mediterranean population**

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**Introduction**

The clinical significance of subclinical thyroid diseases is debated.

**Design**

To assess the relationship between undiagnosed subclinical hypothyroidism (USH), health indicators (HI) and cardiovascular risk factors (CVRF), this cross-sectional study was conducted in 1100 adults representatives of non-institutionalized Catalan population (44.5% men; mean age:  $44.8 \pm 15.2$  years) in Catalonia (Spain); 40 USH (18 men) and 1060 euthyroid (474 men). Patients were given a 165 items health questionnaire (one specifically asking about depression – D or anxiety – A), collected the anthropometric parameters, TA, and FC and glucose, total cholesterol and fractions, triglycerides, urate, ferritin, sideremia, transferrin, adiponectin, insulin, thyrotropin (TSH), free thyroxine (FT<sub>4</sub>), thyroperoxidase (TPOAb) and thyroglobulin (TgAb) antibodies were measured. Obesity was diagnosed if BMI  $> 30$  kg/m<sup>2</sup> and diabetes mellitus (DM), high blood pressure (HTA) and HC if there saw knowledge of them and/or patients took specific drugs and/or basal glucose  $> 126$  mg/dl, TA  $> 135/85$  mmHg, or cholesterol  $> 200$  mg/dl, respectively. Student *t*-test, ANOVA,  $\chi^2$  and a logistic regression model with USH as the dependent variable and the rest as independent were used for the statistical analysis. A *P* value  $< 0.05$  was considered statistically significant.

**Results**

D or A, obesity, DM and HC were more frequent in the USH group ( $P=0.01$ ; 0.01; 0.00; 0.01), while smoking was less frequent ( $P=0.02$ ). In this group, age and LDL-C were higher ( $P=0.02$ ; 0.05). USH was only associated with depression or anxiety (OR 2.57; 95% CI 1.16–5.37), with the fact of not smoking (OR 3.14; 95% CI 1.25–9.6) and HC (OR 2.16; 95% CI 1.1–4.4).

## Conclusions

Depression or anxiety, not being a smoker and hypercholesterolemia are the variables independently associated with undiagnosed subclinical hypothyroidism.

**P387****Current results on urinary iodide excretion in pregnant women on the day of childbirth in Germany**

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## Objectives

Recent Germany-wide studies have shown that urinary iodide excretion in schoolchildren as well as in adults meet WHO targets. No current results for pregnant women in Germany are available.

## Methods

We investigated urinary iodide excretion in 1003 pregnant women on the day of childbirth in the greater Rostock area. Iodide was measured by cerarsenite method modified by Lorenz. Domestic use of iodized table salt and intake of iodine supplement tables was tracked using a questionnaire. We also determined urinary nitrate excretion (u.v. detection at 245 nm following ultrafiltration of the urine and isocratic separation by ion chromatography) and the serum thiocyanate concentration (pyridine/barbituric acid method).

## Results

Iodine contamination occurred in 168 women after caesarean section due to the use of the skin disinfectant povidone-iodine (Braunol). These women were excluded from analysis. In the remaining sample population of 714 women that had a spontaneous vaginal delivery, the median urinary iodide excretion was 103.3 µg I<sup>-1</sup>/l. Relative iodine excretion distribution: 46% >100 µg I<sup>-1</sup>/l, 32% 50–99 µg I<sup>-1</sup>/l, 12% 20–49 µg I<sup>-1</sup>/l, 10% >20 µg I<sup>-1</sup>/l.

Median urinary iodide excretion in women using iodized table salt (91%) was 104 µg I<sup>-1</sup>/l and 102.8 µg I<sup>-1</sup>/l in women that did not use iodized table salt. Iodide excretion relative to iodine supplement intake: no intake – 92.3 µg I<sup>-1</sup>/l; 100 µg per day – 95.8 µg I<sup>-1</sup>/l; 200 µg per day – 112.9 µg I<sup>-1</sup>/l. The values for thiocyanate (5.08 mg SCN<sup>-</sup>/l) and nitrate (44.22 mg NO<sub>3</sub><sup>-</sup>/ml) were below the respective goitrogenic threshold and correlated with neither urinary iodide excretion nor thyroid volume obtained by sonography.

## Conclusions

In Germany, women during pregnancy and in early puerperium remain significantly undersupplied with iodine (recommended daily amount 230–260 µg). These women require intake of iodine supplements particularly if their diet is low on iodine. The relevant goitrogens SCN<sup>-</sup> and NO<sub>3</sub><sup>-</sup> do not interfere.

**P388****Tear cytokines in endocrine ophthalmopathy (EOP)**

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## Introduction

Human tear is rich in cytokines, i.e. in healthy individuals it exhibits a higher ratio of cytokines to total proteins than that in the serum of the same individual. EOP related to Graves-Basedow disease is thought to develop due to elevation of serum cytokine levels and to an altered cytokine response of the intra- and periorbital tissues. In our present study we focused on the measurement of IL6, IL8 and TNF-α, the relative amount of a lipocalin-type protein (α-1-acid glycoprotein, α-1AG or orosomucoid) in tear samples of EOP patients.

## Methods

Tear was collected by the Schirmer method from 26 EOP patients and 20 control individuals without autoimmune and thyroid diseases. Eighty percent of them were females. The mean age was 45 (25–71) years. The total tear protein content was measured by the Bradford method followed by SDS-PAGE electrophoresis with silver intensification (1.8 µg/lane). Tear α-1AG was detected by chemiluminescent Western blot technique. Tear cytokine levels were quantified by a chemiluminescent immunoassay method (Immulite, Beckman-Coulter).

## Results

TNF-α and IL6 increased significantly in the tear samples of EOP patients (TNF-α: 513 ± 291 vs 217 ± 124 pg/mg, IL6: 39 ± 31 vs 12 ± 5 pg/mg, EOP patients versus control individuals, respectively). IL8 and α-1AG levels did not differ significantly between the two groups.

## Conclusions

The content of some cytokines in the tear of EOP patients is different from that measured in healthy individuals. Increased tear TNF-α (having a primary role in the regulation of immune cells) and IL6 levels (an *interleukin* that acts as both a pro-inflammatory and anti-inflammatory cytokine secreted by *T cells* and *macrophages*) are considered to be a new finding in EOP patients. Mass spectrometric identification of some tear proteins is in progress to answer the question if tear as a non-invasive sample might be used for monitoring disease activity and/or effectiveness of treatment in EOP.

**P389****The CT60 and Jo31 polymorphisms of CTLA-4 gene are associated with disease progression in Graves' disease**

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## Introduction

Both genetic and environmental factors contribute to clinical manifestation of Graves' disease including the response to medical therapy. *CTLA-4* gene is an important inhibitor of T-cell activation and its polymorphisms may influence the course of the disease.

## Goal

We investigated the association between *CTLA-4* gene polymorphisms: c.49A>G, g.319C>T, g.\*642AT(8\_33), CT60, and Jo31 and the response to pharmacological therapy.

## Materials

One hundred and seventy-two unrelated GD patients were genotyped and categorized into two groups: 96 patients responded to medical treatment and 76 subjects required surgical or I<sup>131</sup> therapy because of a relapse.

## Methods

The polymorphisms g.319C>T (rs5742909) in the promoter region, c.49A>G (rs231775) in exon 1, and CT60 (g.\*6230G>A, rs3087243) in the 3'UTR of the *CTLA-4* gene were examined by PCR-restriction fragment length polymorphism (PCR-RFLP) using *TruI*, *BseXI*, and *TaqII* enzymes. The Jo31 (g.\*10223G>T; rs11571302) polymorphism in the 3'UTR region of the *CTLA-4* gene was genotyped using PCR amplification followed by minisequencing using the commercial kit SNaPShot. The *CTLA-4* 3'UTR containing an (AT)<sub>n</sub> repeat was studied by PCR and fluorescence based technique.

## Results

CT60[G] allele (genotype [GG] or [GT]) carriers were 4.51-fold more prone to disease progression ( $P=0.01$ , 95% CI: 1.25–16.20). CT60[G] allele and [GG] genotype increased risk of disease progression ( $P=0.002$ , OR=2.05, 95% CI: 1.29–3.27 and  $P=0.01$ , OR=2.24, 95% CI: 1.21–4.15, respectively).

Also presence of one or two Jo31[G] allele was associated with relapse ([G] allele:  $P=0.008$ , OR=1.82, 95% CI: 1.17–2.83 and [GG] genotype:  $P=0.02$ , OR=2.07, 95%CI: 1.10–3.89, respectively). The CT60 and Jo31 markers were subjected to multivariate analysis. It appeared that only the CT60 polymorphic marker is an independent risk factor for disease progression ( $P=0.02$ , OR=4.51, 95% CI: 1.24–16.30).

## Conclusion

These data suggest that the variation within *CTLA-4* gene may discriminate Graves' disease patients with different clinical manifestation and help to identify subjects at risk of a relapse.

**P390****Hyperthyroidism in pregnancy: therapeutic decisions**

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## Objectives

Hyperthyroidism in pregnancy presents a challenge for medical doctor, patient and products of conception.

**Aim of the study**

To specify the peculiarities of expression of hyperthyroidism in pregnant women, diagnosis, monitoring, treatment, prognosis of pregnancy and effect on the fetus. Materials and methods

The study included a total of 27 women with hyperthyroidism of which 15 (group A) had thyrotoxicosis before conception and 12 (group B) were diagnosed during pregnancy. All pregnant women with thyrotoxicosis received treatment with propylthiouracil in average dose: 200 mg/day ( $n=5$ , 26.31%), those in maintained remission during pregnancy received 50 mg/day ( $n=14$ , 73.68%).

**Results**  
Dosage anti TSH-receptor antibodies (TRAb) revealed pathological values in a number of 9 patients (60%) including 4 pregnant with ophthalmopathy. Of the 27 pregnancies studied initially remained in development a number of 19 (70.37%), three cases with known hyperthyroidism did not want pregnancy preferred therapeutic abortion, all presenting also ophthalmopathy; three cases had suffered spontaneous abortion in weeks 13, 17 and 19 respectively; for two cases it was decided to practice therapeutic abortion in week 16 and 21 due to heart rhythm abnormalities correlated with persistent thyrotoxicosis with increased necessary for propylthiouracil and beta-blocker. In pregnant women who completed pregnancy were found two birth before term. For patients with ophthalmopathy remaining pregnant ( $n=4$ ) were not found symptoms and signs of progressive disease, correlated with serum levels of thyroid hormones and TRAb in remission. Mentioned one case with esophageal atresia in a female infant whose mother received propylthiouracil monotherapy 200 mg/day initiated within 16 weeks of pregnancy.

**Conclusions**  
Hyperthyroidism in pregnant woman poses problems of diagnosis and monitoring due to maternal-fetal risk. Abortion rate is generally high both in terms of the decision, therapeutic and pathological. After the first trimester there is often an 'improvement' of clinical symptoms and autoimmune process: ophthalmopathy evolution; incidence of pathological TRAb serum levels.

**P391****The association between serum TSH levels and hypertension in 12 353 children and adolescents**

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**Objective**

Although subclinical thyroid dysfunction is associated with cardiac endpoints such as endothelial dysfunction, left ventricular hypertrophy, thickened artery walls or atrial fibrillation, it is currently under debate whether subclinical hyper- or hypothyroidism as well as thyroid function within the reference range are associated with an increased risk of hypertension. So far no study investigated the association between thyroid function and blood pressure in children and adolescents. Thus, we investigated the association between serum TSH levels and hypertension in a large-scale population-based study conducted in children and adolescents aged 3–17 years.

**Material and methods**

Data from 6528 children and 6134 adolescents of the 'Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland' (KiGGS) were analyzed. Two readings of systolic, and diastolic pressure were taken in an upright position after 5 min of rest. Hypertension was defined as a systolic blood pressure  $> 120$  mmHg or a diastolic blood pressure  $> 80$  mmHg. Serum TSH levels were measured with the ECLIA method on an Elecsys E2010. Decreased and increased serum TSH levels were defined according to age-specific reference limits for the assay used. Continuous as well as categorized serum TSH levels were associated with hypertension by multivariable logistic regression.

**Results**

Serum TSH levels were significantly associated with hypertension in adolescents (odds ratio (OR)=1.19;  $P<0.001$ ) and in children (OR=1.12;  $P=0.045$ ). Decreased and increased serum TSH levels were not associated with hypertension in adolescents and children.

**Conclusion**

In conclusion, we demonstrate a weak relationship between serum TSH levels and hypertension in adolescents and children. The conflicting results compared to studies conducted in adults might be related to lower prevalences of thyroid diseases and intake of medication in our population. Furthermore, previous studies might have had not sufficient power to detect a weak association between serum TSH levels and hypertension.

**P392****Increased Th1 response and regulatory CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low/-</sup>FoxP3<sup>+</sup> T cells in patients with chronic hepatitis C virus infection developing interferon-induced thyroiditis**

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**Introduction**

Interferon- $\alpha$  (IFN- $\alpha$ ) exerts different immune effects and is used in current therapy for chronic hepatitis C virus (HCV) infection. One of the side-effects of IFN- $\alpha$  therapy is interferon-induced thyroiditis (IIT). The role of lymphocyte subpopulations in IIT remains to be defined.

**Objective**

This study was conducted to assess different peripheral blood lymphocyte subpopulations, mainly regulatory CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low/-</sup>FoxP3<sup>+</sup> T cells (Tregs), in patients with chronic HCV infection who developed IIT.

**Patients and methods**

From 120 patients with HCV who started antiviral treatment, those who developed IIT (IIT patients) were selected and compared with patients who did not develop IIT (Co-HCV). Peripheral blood mononuclear cells (PBMC) were obtained before treatment (BT), mid-treatment (MT), end of treatment (ET), 24 weeks post-treatment (PT) and at appearance of IIT (TT).

**Results**

Eleven patients developed IIT: 3 Hashimoto's thyroiditis, 1 Graves' disease, 1 positive antithyroidal antibodies, 1 non-autoimmune hypothyroidism and 5 destructive thyroiditis. During combined antiviral treatment, a significant increase in CD8<sup>+</sup> lymphocytes and in Tregs was observed in both groups. A significant decrease in CD3<sup>+</sup>, CD19<sup>+</sup> and NKT lymphocyte subpopulations was also observed (all  $P<0.05$ ). However, no changes were observed in the percentage of CD4<sup>+</sup>, CD3<sup>+</sup> $\gamma\delta$ <sup>+</sup> and iNKT lymphocytes, Th1/Th2 balance and Bcl2 expression on B cells when BT was compared with ET. At the appearance of IIT (TT), IIT patients had a higher TH1 response (CCR5<sup>+</sup>CCR7<sup>-</sup>) ( $P<0.01$ ) and a higher Tregs percentage ( $P<0.05$ ) than Co-HCV.

**Conclusions**

Our results point to the immunomodulatory effects of IFN- $\alpha$  on different lymphocyte subpopulations and a possible role of Th1 response and Tregs in HCV patients who developed IIT.

**P393****Study of the relation between subclinical hypothyroidism and diabetic complications**

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**Introduction**

There were some studies on the relation between subclinical hypothyroidism and arteriosclerosis, but few studies between thyroid function and diabetic microangiopathy. This study was aimed at investigating the influence of subclinical hypothyroidism on diabetic complications in Japanese population.

**Description of methods/design**

We retrospectively surveyed a total of 106 type 2 diabetic patients with regard to thyroid function and microvascular complications. The patients were divided into two groups of subclinical hypothyroidism group (SCH group; TSH  $\geq 3$   $\mu$ U/ml), and euthyroid group (TSH  $< 3$   $\mu$ U/ml). There were 16 patients of SCH group and 90 patients of euthyroid group.

**Results**

In the SCH group and euthyroid group, those with proliferative and proliferative retinopathy were 5 (31.2%) and 10 patients (11.1%), overt nephropathy were 5 (31.2%) and 10 patients (11.1%), respectively. Those had neuropathy were each 14 patients (87.5%) and 46 patients (51.1%), respectively. The trends for the three diabetic complications were all significantly higher in the SCH group than euthyroid group ( $P=0.015$ , 0.015, 0.007). Serum TSH level was associated with greater prevalence of diabetic retinopathy in multiple logistic-regression analysis after an adjustment for age, duration of diabetes, HbA1c (OR 1.54,  $P=0.037$ ).

**Conclusion**

Subclinical hypothyroidism contributes to development of diabetic microangiopathy.

**P394**

**Melatonin drug use in complex treatment of hypothyroidism manifest**  
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**Aim**

The aim of the study was to evaluate the clinical efficacy of melatonin in patients with hypothyroidism.

**Materials and methods**

In study were taken 51 patients from 25 to 63 years to manifest hypothyroidism (on a background of autoimmune thyroiditis), with anxiety-depressive syndrome and sleep disturbance. Thyroid hormones were determined by ELISA (Vector-Best Russia) Anxiety-depressive syndrome in patients with hypothyroidism were analyzed on hospital Anxiety and Depression Scale HADS. Patients were divided into 2 groups. First group with manifest hypothyroidism (25 patients) received standard replacement therapy with L-thyroxine and symptomatic therapy for 1 month. Second group with manifest hypothyroidism (26 patients) along with L-thyroxine and symptomatic therapy additionally received melatonin (15 mg/night) Statistical analysis was done by SPSS, to analyze the reliability of the Student T criteria.

**Results**

Thyroid hormones levels in the blood of patients with hypothyroidism group 1 and 2 before treatment showed no differences. With combination therapy of melatonin in group 2, serum TSH fell from the baseline to  $82.2 \pm 1.5\%$ , the level of free serum  $T_4$  increased from baseline to  $56.8 \pm 2.4\%$ , which differ from that of patients of group 1 after treatment. After the treatment, noted that in both groups there is significant reduction in anxiety,  $P < 0.05$ , especially subclinical anxiety ( $P < 0.05$ ). Found that only after treatment of melatonin in the treatment of hypothyroidism is significantly reduced depressive states with  $10.9 \pm 0.9$  points to  $6.9 \pm 0.5$  points ( $P < 0.05$ ), with significantly reduced subclinical depression.

**Conclusion**

The inclusion of melatonin in the standard therapy for symptomatic hypothyroidism showed that the drug can affect the pituitary-thyroid system, removing anxiety and depression.

**P396**

**Epidemiologic study of iodine deficiency in 6 regions of the Republic of Uzbekistan**

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**Introduction**

Iodine deficiency disorders are considered as the most wide spread non infectious diseases of the humanity. Uzbekistan is in the zone of the severe iodine deficiency (ID). According to epidemiologic studies performed in 1998 endemic goiter (EG) prevalence was 80% whereas urinary iodine was  $< 10 \mu\text{g/l}$ . In May, 2007 Uzbek government legislated the Salt Iodization Law and in 2009 ID evaluation was performed in the Uzbekistan using sentinel approach.

**Materials and methods**

As recommended by World Health Organization we performed epidemiologic studies using sentinel method in six regions of the country, i.e. examination of children from 3 rural public schools and 3 urban public schools (randomized design). We collected 180 urinary samples and 180 salt samples on the level of households' iodized salt consumption (HISC) from schoolchildren aged 6–12 years-old from each region. Both total urinary samples number and salt samples on the level of HISC constituted 6480 samples for each group. EG evaluation was performed among schoolchildren aged 6–15 years-old where total number of children constituted 19 212 children in 6 regions of the country (Namangan, Syrdarya, Djizak, Surkhandarya, Andijan and Republic of Karakalpakstan).

**Results**

Urinary iodine measurements in evaluated regions demonstrated ID in 39.0% of schoolchildren while HISC constituted 61.0%. Of 19 212 children aged 6–15 years-old 9626 (47.63%) had EG. Normal level of HISC registered in 52.0% of schoolchildren whereas 42.0% of children had insufficient salt consumption as 6.0% of evaluated salt was not iodized.

**Conclusion**

According to our results we consider that the iodine deficiency situation remains as severe in the Republic of Uzbekistan, although, in comparison with the 1998 data there is a positive trend as the endemic goiter prevalence.

**P395**

**MPV levels in patients with overt hypothyroidism before and after levothyroxine treatment**

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**Objective**

Platelet activation and aggregation play major role in the pathophysiology of atherothrombosis. Mean platelet volume (MPV), a determinant of platelet function, is a newly emerging risk factor for atherosclerosis. The present study was designed to evaluate levels of MPV before and after the levothyroxine (L-T<sub>4</sub>) treatment in patients with overt hypothyroidism.

**Materials and methods**

The study included 30 Hashimoto's thyroiditis patients with overt hypothyroidism and age-matched 20 healthy control subjects.

**Results**

The mean MPV levels of hypothyroidic patients was higher than control group ( $P < 0.001$ ). A significant decrease in the mean MPV level was detected after the maintenance of euthyroidism with L-T<sub>4</sub> treatment ( $P < 0.05$ ).

**Conclusion**

Our study suggests that subjects with overt hypothyroidism tend to have increased platelet activation. This activation may cause increased risk of atherothrombotic complications that may be reversed by treatment of hypothyroidism.

**P397**

**Thyroid status and health-related quality of life in the LifeLines-cohort**

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**Background**

Thyroid disorders are prevalent in western society, yet many subjects experience limited symptoms at diagnosis, especially in hypothyroidism.

**Design**

In order to compare the health-related quality of life (HR-QOL) of subjects with suppressed TSH-levels (TSH  $< 0.5$  mU/l) or elevated TSH-levels (TSH  $> 10$  mU/l) to subjects with normal TSH-levels (TSH 0.5–4 mU/l), a cross-sectional study was performed within the Dutch adult population who participated in the LifeLines-cohort from December 2009 until August 2010. We measured thyroid hormone status (serum TSH, free T<sub>4</sub> and free T<sub>3</sub>, Roche Modular) and HR-QOL (RAND-36 item Health Survey) in 9491 Caucasian participants, 3993 men and 5498 women (median age 45, range 18–88 years), without current or former use of thyroid medication.

**Results**

Suppressed TSH-levels ( $< 0.5$  mU/l) were found in 114 participants (1.2%), while 70 participants (0.7%) had TSH  $> 10$  mU/l. No relationship between increasing TSH-levels or reduced HR-QOL and ageing could be found. Men had a higher HR-QOL than women ( $P < 0.001$ ) except for the domain 'general health' ( $P = 0.692$ ). None of the domains of the RAND-36 was significantly reduced in men with suppressed or elevated TSH-levels ( $P \gg 0.05$ ) compared to euthyroid men. Only the domains 'physical functioning' and 'general health' were significantly reduced in women with suppressed TSH-levels versus euthyroid women ( $P = 0.013$  and  $P = 0.036$  respectively). Women with TSH  $> 10$  mU/l had similar HR-QOL compared to euthyroid women ( $P \gg 0.05$ ). In both men and women no significant differences could be observed between HR-QOL and subjects with decreased, normal or elevated FT<sub>4</sub>-levels ( $P \gg 0.05$ ).

**Conclusions**

HR-QOL of subjects with suppressed TSH-levels or elevated TSH-levels is not significantly reduced compared to subjects with normal TSH-levels.

**P398****Subclinical hypothyroidism and thyroid autoimmunity: risk factors for coronary heart disease?**Z Milicevic<sup>1</sup> & J Ciric<sup>2</sup><sup>1</sup>Laboratory for Molecular Biology and Endocrinology, Institute for Nuclear Sciences Vinca, Belgrade, Serbia; <sup>2</sup>Clinic of Endocrinology, Diabetes and Metabolic Disease, Clinical Centre, Belgrade, Serbia.

It has been claimed that an increased level of thyroid autoantibodies in serum is associated with a higher risk for coronary heart disease (CHD). We explored whether this risk was mediated by subclinical hypothyroidism and hypercholesterolemia. We evaluated 945 consecutive hospital patients (mean age 59 years) according to strict electrocardiographic and clinical criteria for the presence of CHD. Serum cholesterol, LDL and HDL cholesterol and sensitive TSH were measured in all patients. In 245 patients antithyroglobulin and thyroid peroxidase antibodies were also determined. Patients were divided into group I (normal TSH, i.e. below 4 mU/l), group II (borderline TSH, i.e. 4.0 to 5.9 mU/l) and group III (high TSH, i.e. 6.0 mU/l or more). There were 398, 45 and 22 women and 410, 50 and 20 men in group I, II and III respectively. Patients of group III were randomly age- and sex-matched with patients of group I and II combined as controls. Alternately the groups II and III were randomly age- and sex- matched with group I as controls. Both procedures gave on identical prevalence of CHD in control and index groups in both sexes. Cholesterol was significantly lower in group III compared to matched controls. The prevalence of thyroid antibodies was 8.3, 14.3 and 48% in women and 4.5, 2 and 4.8% in men of group I, II and III respectively. In women with antibodies mean cholesterol was lower than in age-matched controls without antibodies ( $P < 0.05$ ) irrespective of whether they belonged to group I, II or III and the presence of antibodies was not associated with the prevalence of CHD. In men the prevalence of antibodies was too low to permit analysis of their contribution to CHD. We conclude that neither subclinical hypothyroidism nor thyroid antibodies are risk factors for CHD.

**P399****Faster response to cholestyramine added in treatment of patients with hyperthyroidism**

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**Introduction**

Faster decline in serum thyroid hormone levels in hyperthyroidism is seen adding cholestyramine at conventional treatment in some studies (1,2).

**Design/methods**

Retrospective controlled study. Follow-up:  $\pm 3$  weeks.

Patients with hyperthyroidism: 17 treated with antithyroid drugs (mean dose 35 mg/day) combined with cholestyramine (mean dose 12 g/day).

Control group: 23 only treated with antithyroid drugs (mean dose 20 mg/day).

Patients with Quervain disease: 5 treated with cholestyramine (mean dose 12 g/day). Control group: 5 without therapy.

FT<sub>4</sub> and FT<sub>3</sub> values between groups at baseline and after follow-up period were compared. Statistical analysis: Student's *t*-test.

**Results**

Patients with hyperthyroidism: cholestyramine vs control group showed decline: FT<sub>4</sub>: 61 vs 20%,  $P < 0.01$  and FT<sub>3</sub>: 72 vs 48%,  $P < 0.02$ . Patients with severe hyperthyroidism were all treated with cholestyramine and higher doses of antithyroid drugs. Subgroup analysis in mild hyperthyroidism and equal dose of medication (5 versus control 6 patients) showed: FT<sub>4</sub>: 48 vs 19%,  $P < 0.05$  and FT<sub>3</sub>: 57 vs 25%,  $P < 0.2$ . Patients with Quervain disease: cholestyramine versus control group showed: FT<sub>4</sub>: 51 vs 41%,  $P < 0.3$  and FT<sub>3</sub>: 54 vs 11%,  $P < 0.05$ .

**Discussion**

Cholestyramine added in treatment of hyperthyroidism shows faster decline in thyroid hormone levels during the first weeks. In this study, the effect is less in mild hyperthyroidism, probably because of smaller increase in T<sub>3</sub>-concentration in bile and faeces, but seems more pronounced in severe hyperthyroidism.

**Conclusion**

Cholestyramine is an option as adjunctive therapy especially in severe hyperthyroidism, obtaining faster decline in thyroid hormone levels.

**P400****The expression of keratin 34βE12 in ectopic thymus tissue**R Cornea<sup>1</sup>, M Cornianu<sup>1</sup>, E Lazar<sup>1</sup>, A Dema<sup>1</sup>, S Taban<sup>1</sup>, A Muresan<sup>1</sup>, C Lazureanu<sup>1</sup> & I Golu<sup>2</sup><sup>1</sup>Pathology Department, University of Medicine and Pharmacy Victor Babes, Timisoara, Romania; <sup>2</sup>Endocrinology Department, University of Medicine and Pharmacy Victor Babes, Timisoara, Romania.**Introduction**

The ectopic thymus tissue (ETT) represents a migration and descending deficiency of the organ during embryogenesis.

HMWK have a strong positive reaction in normal anatomical localized thymus and thymomas. A similar reaction pattern is seen in thyroid solid cell nests (SCNs) suggesting that these cells share a common origin with thymic tissue. Moreover, can be very helpful in differentiate CASTLE from other thyroid tumors.

Our aim was to investigate the expression of CK34βE12 in the normal ectopic thymic tissue which was not yet described.

**Materials and methods**

We analyzed 5 cases of ETT, from female patients with age between 14 and 49 years, 2 intrathyroidal and 3 adjacent to thyroid and parathyroid. The tissue was incidentally discovered in patients with diverse thyroid and parathyroid pathology.

Slides of paraffin-embedded specimens were HE stained and the immunohistochemical investigation used monoclonal antibodies against HMWK, clone 34βE12 (Dako).

**Results**

The thymic tissue was microscopically identified inside and adjacent to thyroid and parathyroid. In one case, within the thyroid were identified areas of SCNs. Also in one case ETT had the appearance of partial involution. In the intrathyroidal ETT, we observed an intense positive reaction for CK 34βE12 with strong, granular pattern, in the epithelial cells of the subcapsular region, medullar areas and Hassall corpuscles. In the ETT adjacent to thyroid the intensity of reaction was smaller. The lower intensity was seen in the partial involution thymus.

**Conclusions**

Our results suggest that might be difference of CK34βE12 expression in the ectopic cervical thymic tissue according with the location and age.

**P401****Parathyroid cyst, a rare cause of cystic thyroid lesion**

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**Introduction**

Parathyroid cyst is a very rare abnormality, representing 0.6% of all thyroid and parathyroid lesions. The diagnosis can be made on the basis of sonography and analysis of aspirated cystic fluid for parathyroid hormone. Parathyroid cysts are divided in two groups: functional (associated with clinical hyperparathyroidism) and non-functional. In the case of non-functional parathyroid cysts, fine needle aspiration is the elective treatment, but for functional parathyroid cysts, surgery is the recommended approach.

**Case report**

We report the case of a big, non-functional, parathyroid cyst in a 50-year-old female patient, with an anterior neck mass. The patient was complaining of pain in the anterior cervical region, dysphagia, dyspnea and dysphonia accompanying a cervical mass rapidly developed in the left cervical region. Sonography showed an anechoic and a vascular lesion of 20 ml, located in the left lobe of the thyroid. The rapid development of the cyst suggested a blood cyst but the fine needle aspiration (FNA) revealed a clear colourless and watery fluid, suggesting a parathyroid cyst. In fact, the fluid showed a high concentration of parathyroid hormone (PTH) in both the native and the diluted content of the cyst, while serum PTH was normal, indicating a non-functional parathyroid cyst. The patient was cured by percutaneous aspiration and there was no relapse of the parathyroid cyst after 1 year follow-up.

**Conclusion**

The case illustrates a very rare cause of cystic lesion in the thyroid area and its cure by percutaneous aspiration.

**P402****Clinical aspects in Graves' disease associated with infiltrative orbitopathy**M Balas<sup>1</sup>, I Golu<sup>1</sup>, M Vlad<sup>1</sup>, C Gurban<sup>2</sup> & I Zosin<sup>1</sup><sup>1</sup>Department of Endocrinology, University of Medicine and Pharmacy 'Victor Babes', Timisoara, Romania; <sup>2</sup>Department of Biochemistry, University of Medicine and Pharmacy 'Victor Babes', Timisoara, Romania.**Aim**

The aim of the study was to present some epidemiological data about Graves' ophthalmopathy in a tertiary centre.

**Material and method**

The retrospective study included 98 patients with Graves' disease associated with infiltrative ophthalmopathy, evaluated in our Department of Endocrinology in the period 2008–July 2010.

The mean age of the group was  $45.2 \pm 12.4$  years, with a female/male ratio = 84/14.

The diagnosis of thyrotoxicosis was established on clinical criteria, hormonal data (TSH, FT<sub>3</sub> and FT<sub>4</sub>) and immunological data (TSH receptor antibodies).

The evaluation of the ophthalmopathy comprised exophthalmometry, clinical activity score (CAS) and severity criteria.

**Results**

In 72 cases, thyrotoxicosis developed concomitantly with orbitopathy, 25 patients presented first the thyrotoxicosis and in one case the eye disease was diagnosed 2 years before thyrotoxicosis.

At admission, the evaluation of thyroid functionality during ophthalmologic assessment, showed 22 cases with subclinical hyperthyroidism, 15 euthyroid patients, 3 with subclinical hypothyroidism, the remaining presenting overt thyrotoxicosis (59.1%).

CAS values were comparable in euthyroid and dysthyroid groups ( $3.6 \pm 2.0$  vs  $3.2 \pm 1.85$ ,  $P=0.47$ , *t*-test).

According to EUGOGO severity criteria, the ophthalmopathy was classified as follows: 73 mild forms (9 men, 64 women), 20 moderate (4 men, 16 women) and 5 severe forms (1 man, 4 women).

Based on clinical activity score, the patients were divided into active ophthalmopathy group (31 cases) and inactive eye disease group (67 cases).

There were no statistically significant differences regarding age ( $P=0.08$ , *t*-test), smoking ( $P=0.07$ , Fisher's exact test) or thyrotoxicosis severity ( $P=0.40$ , Mann-Whitney test) in mild ophthalmopathy group, as compared to moderate-severe group.

**Conclusions**

The studied Graves' cases were dominated by mild ophthalmopathy forms. Thyroid ophthalmopathy (CAS, severity) does not correlate with thyrotoxicosis onset.

**P403****Thyroid nodules in children: review of 67 patients**J Mesquita, S Paco Lima, M J Matos, F Neiva, S Paupério, S Martins, C Costa, C Castro Correia, Davide Carvalho & Manuel Fontoura  
São João Hospital, Porto, Portugal.**Introduction**

Thyroid nodules in children and adolescents are less prevalent, but entail greater risk of malignancy than in adults. Most are asymptomatic because they are associated with normal thyroid function, but their prevalence is increasing due to the incidental diagnosis by neck ultrasound.

**Aims**

To evaluate the characteristics of a pediatric population with thyroid nodules submitted to fine-needle aspiration (FNA) biopsy.

**Methods**

We evaluated 67 children and adolescents followed in the Pediatric Endocrinology consultation of Hospital de São João-EPE because of thyroid nodules. We determined the demographic parameters of patients, thyroid function, nodules' size by neck ultrasound, the result of FNA and histology (in patients who underwent surgery).

**Results**

Fifty-six patients were females (83.6%). Overall they had mean age of  $14.6 \pm 3.7$  years (4–18 years) and thyroid nodules mean size was  $11.2 \pm 5.2$  mm (9–21 mm). Thyroid function was normal in 60 patients (89.55%), with the remaining having hypothyroidism. Mean TSH was  $2.3 \pm 1.1$   $\mu$ U/ml ( $N$ : 0.35–4.94) and free T<sub>4</sub> was  $1.1 \pm 0.16$  ng/dl (0.7–1.48), after exclusion of patients with hypothyroidism. FNA revealed lymphocytic thyroiditis in 14 patients (20.9%), papillary carcinoma in 5 patients (7.46%) and follicular tumor in 8 patients (11.94%). In the latter case,

histology showed that 4 were follicular adenomas (50.0%), 3 were follicular variant of papillary carcinomas (37.5%) and 1 was follicular carcinoma (12.5%). Two of the patients whose FNA showed benign lesions underwent thyroidectomy for multinodular goiter and its histology showed that they were adenomatous goiters. Malignancy was confirmed in a total of 9 patients (13.4%).

**Conclusions**

The prevalence of malignancy in our sample was high, which is consistent with previous studies.

**P404****The etiologic profile of thyromegaly in children in the south west of Romania between 2005 and 2010**Marginean Otilia<sup>1</sup>, Varcus Flore<sup>2</sup>, Simedrea Ioan<sup>1</sup>, Dora Andor<sup>3</sup>, Tamara Marcovici<sup>1</sup> & Rodica Ilie<sup>1</sup><sup>1</sup>1st Pediatric Clinic, 'Louis Turcanu' Children Hospital, Timisoara, Romania; <sup>2</sup>Chirurgery Department, Country Hospital, Timisoara, Romania; <sup>3</sup>Cardiology Department, University of Medicine and Pharmacy 'Victor Babes', Timisoara, Romania.**Aim**

To show the etiology of thyromegaly in our region to assess the rationale treatment and follow up.

**Material and methods**

Between 2005 and 2010, we have studied the prevalence and etiologic profile of the children with thyromegaly admitted in the Endocrinology Department of our hospital. The working protocol included: weight and length birth, gestational age, auxological parameters and pubertal stages after Tanner's criteria, morphogram, laboratory studies (usual BCC, serum cholesterol, triglycerides, urinary iodine, the thyroid function tests (TSH, free T<sub>4</sub>, free T<sub>3</sub>), immunologic parameters (antiTPO and antithyroglobulin antibodies)); thyroid ultrasound (thyroid volume and aspect). The bone age, thyroid MRI and iodine uptake were performed in selected cases. All the cases were followed at last 3 years under treatment.

**Results**

The study a group consisted of 72 children (F/M: 51/21; age: 2–17 years). According to the clinical exam, thyroid tests, imagistic exams the patients were divided in four groups. Group A: 36 patients euthyroid diffuse thyromegaly, treated with iodine and after 1 year the goiter disappeared in 27 patients (75%). Group B: 27 hypothyroid patients, (25 with chronic autoimmune thyroiditis and 2 cases of dishormonogenesis). All the cases were treated with L-thyroxin. The goiter decreased after 1 year, especially in the CAT cases. Group C: 4 cases with autoimmune hyperthyroidism treated, initially, with thiamazol. Group D: 5 patients with thyroidian nodule/chist who follow surgical intervention, 3 of them had subclinical hypothyroidism after intervention; in this group only the children with high levels of the serum cholesterol were treated with L-thyroxin; 2 cases no needed substitute therapy after 2 years of follow up.

**Conclusions**

1. In our region the etiologic profile is quite various. 2. The treatment depends on the etiology and thyroid function. 3. The response of the treatment in childhood is satisfied.

**P405****Awareness of secondary hypothyroidism in GH deficient hypopituitary adults has improved their lipid status**M-L Hartoft-Nielsen<sup>1</sup>, M Klose<sup>1</sup>, O Klefter<sup>1</sup>, V Gavan<sup>2</sup>, Å K Rasmussen<sup>1</sup> & U Feldt-Rasmussen<sup>1</sup><sup>1</sup>Department of Endocrinology, Rigshospitalet, University Hospital of Copenhagen, PE-2131 Copenhagen, Denmark; <sup>2</sup>Department of Thyroid Related Disorders, National Institute of Endocrinology 'C.I. Parhon', Bucharest, Romania.**Introduction**

To evaluate if improved thyroid status in patients with hypopituitarism would improve cardiovascular risk markers.

**Patients and methods**

A total of 85 hypopituitary patients (45 women) with GH deficiency; 7 had isolated GH deficiency, 25, 16 and 22 patients had 1, 2 and 3 additional deficits respectively. Fifteen patients had panhypopituitarism. Biochemical and body composition assessment was performed at baseline (when patients were GH



naïve), and at follow-up (median 4.7 years (3.0–5.5) after initiation of GH replacement). Patients were divided into TSH sufficient (TSHsuff) (not on levothyroxine and free  $T_4 > 12$  ( $n=23$ )), and TSH deficient (TSHdef), that were further divided into tertiles according to baseline free  $T_4$ .

#### Results

Baseline free  $T_4$  was negatively associated to BMI ( $P=0.003$ ) and total fat mass ( $P=0.01$ ). TSHdef patients with lowest tertile free  $T_4$  had higher total ( $P=0.01$ ) and LDL cholesterol ( $P=0.05$ ) and triglycerides ( $P<0.01$ ) compared to TSHsuff patients, also after adjustment for gender, age, BMI and IGF1. At follow-up 9 patients initially defined as TSHsuff had initiated levothyroxine treatment. 53 and 28% of patients with free  $T_4$  from the low and median tertiles respectively, received an increased levothyroxine dose compared to baseline, whereas 40% of patients with free  $T_4$  from the median/high tertile groups had had a dose reduction. None recovered from TSHdef. Free  $T_4$  at follow-up was not significantly correlated to body-composition or cholesterol variables, nor were there any group differences comparing patients in the four groups according to TSH and free  $T_4$ . Delta free  $T_4$  (follow-up – baseline) was negatively correlated to delta total cholesterol ( $r = -0.23$ ;  $P=0.06$ ) and LDL cholesterol ( $r = -0.30$ ;  $P=0.01$ ) and remained so after adjustment for the change in IGF1 ( $P=0.02$ ).

#### Conclusion

After ~5 years of GH replacement also the secondary hypothyroidism was better adjusted with an improvement of the lipids of a higher magnitude than seen after GH replacement alone.

## P406

### Cytological evaluation of pyramidal lobe and thyroglossal duct cysts

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#### Aim

Thyroid pyramidal lobe and thyroglossal duct cysts were reported to be associated with increased risk of thyroid cancer. In this study, we aimed to evaluate ultrasonography (US) guided fine needle aspiration biopsy (FNAB) cytology results of pyramidal lobe and thyroglossal duct cysts.

#### Material and methods

Patients with pyramidal lobe and thyroglossal duct cysts detected in US were included. Thyroid functions, thyroid autoantibodies, Tc 99m thyroid scintigraphy and US guided FNAB cytology results were evaluated.

#### Results

There were 79 (97.5%) female and 2 (2.5%) male patients with a mean age of  $52.0 \pm 12.3$  (20–82). Thyroglossal duct remnant was observed in 26 (32.1%) and pyramidal lobe in 55 (67.9%) patients. There were 74 (91.4%) patients with a previous history of thyroidectomy and all were histopathologically benign. In 7 patients, there was not a history of thyroid operation. FNAB results of all thyroglossal duct and pyramidal lobe were benign.

#### Conclusion

Pyramidal lobe is an embryological remnant of thyroglossal duct and its incidence varies between 15 and 75% in surgical and autopsy series. It was reported that this tissue becomes hypertrophic under the influence of increased thyrotrophin in thyroidectomized patients. Thyroglossal duct cysts develop in front of the neck. Increased risk of occult malignancy risk was reported in solid lesions, particularly when they include ectopic thyroid tissue. In our series, we did not observe any malignant cytology result.

## P407

### The outcome of radioiodine therapy after 10 years in patient with toxic nodular goiter

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We treated 4150 patients, aged 30–70 years; 1950 patient with toxic multinodular goitre (TMNG), and 2200 patient with autonomous toxic nodule (ATN).

Qualification of these patients was based on high levels of serum  $fT_3$  and  $fT_4$ , low levels of serum TSH. Malignant changes were excluded in all nodules by fine needle aspiration biopsy. All the patients had serum TSH levels  $\leq 0.1$  mU/l and the effective T-half was  $\geq 3$  days at the time of treatment. The activity dose was calculated by the use of Marinelli's formula and ranged between 200 and 800 MBq. The absorbed dose (Gy) for TMNG ranged between 150 and 260, and for ATN 200–300. Follow up control was done every 6 weeks in the first year, then every 6 months for 9 years.

#### Results

In general the success of treatment after 1 year was: 97% of patient with ATN and 91% of patient with TMNG achieved euthyroidism. 2% of patient with ATN and 8% of patient with MNG develop hypothyroidism. 1% of the patients had persistent or relapse of hyperthyroidism and received second dose of radioiodine therapy. After 3 years of radioiodine therapy 4% of patient with ATN and 10% of patient with TMNG develop hypothyroidism. After 5 years of radioiodine therapy 6% of patient with ATN and 11% of patient with TMNG develop hypothyroidism. After 8 years of radioiodine therapy 8% of patients with ATN and 11% of patients with MNG develop hypothyroidism. After 10 years of radioiodine therapy 9% of patients with ATN and 12% of patients with TMNG develop hypothyroidism. The volume of thyroid glands decreased about 55% average.

#### Conclusions

The achievement of euthyroidism and the remission of the symptoms and signs of hyperthyroidism, were due to good diagnosis, well preparation of the patients; accurate measurement of administered activity, effective half-life, and well-organised follow up.

## P408

### The effect of radioiodine therapy in patients with non-toxic goiter with hot nodules on thyroid scan

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Radioiodine therapy is non-invasive, safe and cost effective method of therapy for reduction of goitre. We aimed to determine whether radioiodine therapy has benefit effect on the symptoms mimicking hyperthyroidism in nontoxic goitre patients.

#### Material and methods

We studied 20 patients with non toxic nodular goitre, aged 42–79 years, 12 females and 8 males. All the patients complained of some symptoms of hyperthyroidism (palpitation, hot intolerance, sweating, tiredness and tachycardia). In all the patients serum TSH was in the lower normal range, serum levels of  $fT_3$ , and  $fT_4$  were in the normal range, initial RAIU after 24 h was ranged between 23 and 47%, and thyroid volume ranged between 40 and 80 ml. The serum  $fT_4$ ,  $fT_3$ , and TSH were evaluated before and monthly up to 12 months after RIT. Thyroid ultrasound, and thyroid scan with thyroid RAIU were done before and after 12 months of radioiodine therapy. The activity dose was calculated by Marinelli's formula and was ranged between 280 and 800 MBq. The absorbed dose ranged between 180 and 260 Gy and was proportional to thyroid volume.

#### Results

After 12 months of radioiodine therapy a mean thyroid volume reduction of 46% was achieved in all the patients, euthyroidism persist in 95% of patients, and hypothyroidism develop in one patient (5%). All patients were highly satisfied; all the symptoms relieved and exercise tolerance improved.

#### Conclusions

The hot nodules may contribute to the symptoms complained by the patients, and this was confirmed when all the symptoms relieved after radioiodine therapy. Radioiodine is non-invasive, safe and cost effective method of therapy for reduction of goitre and should be used as first choice in every patient with non-toxic nodular goitre (>40 ml) with and without symptoms. The reduction of thyroid volume with low percent of hypothyroidism were due to accurate measurement of administered activity, and relatively high effective half-life.

**P409****The induction of hyperthyroidism in patient with non-toxic goiter after radioiodine therapy: a case report**

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A 44-year-old female, with non-toxic goiter was referred to our Department for thyroid volume reduction. Serum levels of FT<sub>4</sub>, FT<sub>3</sub> and TSH were within normal range, high resolution ultrasonography show enlarged thyroid glands (50 ml), with 2 nodules one in the left and one in the right lobe. Malignancy was ruled out by ultrasound-guided fine-needle aspiration biopsy. Thyroid radioiodine scintigraphy showed homogenous and diffuse uptake in the right lobe, with very low uptake in the left lobe, radioiodine uptake (RAIU) after 24 and 48 h was 32.3 and 33%. The effective half-life measured by the use of RAIU was about 7 days. The activity dose was calculated by Marinelli's formula and the patient received 280 MBq of I-131. The absorbed dose was about 200 Gy. Follow up control was done every 4 weeks. After 3 months of radioiodine therapy, the patient developed hyperthyroidism TSH serum levels decreased and serum FT<sub>4</sub> and FT<sub>3</sub> increased, the TSH receptor antibodies increased, anti thyroglobulin antibodies and anti peroxidase antibodies were within normal range. The patient received antithyroid drugs to control the hyperthyroidism, after 6 months of radioiodine therapy the patient was in subclinical hyperthyroid state, thyroid scintigraphy showed homogenous and diffuse uptake in both lobes with small reduction in the thyroid volume. RAIU after 24 and 48 h was 53 and 48% respectively. The patient received more doses of antithyroid drugs to achieve euthyroidism before the second dose of radioiodine therapy. Radioiodine therapy is non-invasive, safe and cost effective method of therapy for reduction of goiter even in patient with low radioiodine uptake and should not be restricted to elderly, or to patients with high operative risk. In this case radioiodine therapy induce hyperthyroidism, maybe due to the activation of Graves' disease.

**P410****Lithium in the acute preoperative preparation of thyrotoxicosis: a case series**

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**Introduction**

Thionamides are first line treatment in the management of thyrotoxicosis; nevertheless several weeks of treatment is needed before euthyroidism is restored. This delayed and gradual action makes thionamides insufficient as sole treatment when acute control of the hyperthyroid status is mandatory, hence the need for potent yet rapid acting adjuvant antithyroid agent. Lithium is a monovalent cation that inhibits thyroid iodine uptake and hormone release with maximum effect in 3–5 days making it a suitable adjuvant agent to conventional preoperative antithyroid treatment.

We here report a series of three cases where lithium was used successfully as adjuvant treatment in the acute preoperative control of hyperthyroidism prior to emergency thyroid surgery.

Case 1: 28 years female with young family and severe symptomatic Graves hyperthyroidism resistant to high doses of carbimazole was admitted for primary thyroid surgery. She was treated with Lithium 1200 mg dose in addition to her current carbimazole treatment, KI and steroids. T<sub>3</sub> levels were down by 78% on day 8 and she underwent uneventful total thyroidectomy with good recovery.

Case 2: 32 years old female admitted acutely with large toxic retrosternal goitre, stridor and near complete dysphagia. She was counselled for urgent total thyroidectomy. Lithium tablets were crushed along with regular pre operative medication. On day eight T<sub>3</sub> and T<sub>4</sub> were down by 70 and 51%. She underwent an uncomplicated total thyroidectomy.

Case 3: 51 years female admitted with Grave's thyrotoxicosis and progressive ophthalmopathy resistant to high dose carbimazole and oral steroids. Lithium was added at a dose of 800 mg od, by day six T<sub>3</sub> was down by 70.9% and subsequently underwent a successful total thyroidectomy.

**Conclusion**

Our series demonstrates that euthyroidism can be restored quickly, effectively, and safely with short-term co-administration of lithium (800–1200 mg/day) to conventional antithyroid agents in the acute preoperative preparation of thyrotoxic patients.

**P411****Thyroid peroxidase antibodies in euthyroid children: is long term follow up required?**K Tharian<sup>1</sup>, C Evans<sup>2</sup> & J T Warner<sup>1</sup><sup>1</sup>Children's Hospital for Wales, Cardiff, UK; <sup>2</sup>University Hospital of Wales, Cardiff, UK.**Introduction**

The presence of thyroid peroxidase (TPO) antibodies in euthyroid children poses a potential risk for the development of autoimmune hypothyroidism. Little is known about the ontogeny of this process. This retrospective study aims to estimate the risk of developing hypothyroidism in euthyroid children with raised TPO antibodies and provide a guideline for follow up.

**Methods**

Children 0–16 years with raised TPO antibodies (1996–2005) were identified from the biochemistry database of a University Hospital. In those that were euthyroid on initial screen, follow up clinical details and thyroid function tests (TFT) were obtained from case records.

**Results**

Two hundred and eight children were identified with raised TPO antibodies, 164 had concurrent TFT results. 104 were excluded as they either had frank hypothyroidism, compensated hypothyroidism or thyrotoxicosis, leaving 60 euthyroid children (19 males, 41 females). 9/60 had no further follow up results. Within 2 years 2/51 (4%) had developed hypothyroidism requiring treatment. By 5 years a further 2 children had developed hypothyroidism (one had type 1 diabetes (T1DM) and the other Down syndrome). At 10 years another two children were being treated, one with T1DM and one with both T1DM and Down syndrome.

**Conclusion**

Risk of hypothyroidism in healthy euthyroid children with raised TPO antibodies is minimal after the first 2 years. Therefore a suggested policy of follow up with TFT at 3–6 months and then annually up to 2 years before discharging back to primary care would seem appropriate. However, children with chronic conditions like Down's syndrome and autoimmune illnesses like T1DM need periodic monitoring.

**P412****Increasing saturation with thyroid hormones is associated with haemostatic system activation**J Horacek<sup>1</sup>, J Maly<sup>1</sup>, I Svilias<sup>1</sup>, M Zidkova<sup>1</sup>, Z Pavlikova<sup>1</sup>, M Pecka<sup>1</sup>, E Malirova<sup>2</sup> & J Vizda<sup>2</sup><sup>1</sup>Department of Internal Medicine, Faculty of Medicine and University Hospital Hradec Kralove, Charles University Prague, Hradec Kralove, Czech Republic; <sup>2</sup>Department of Nuclear Medicine, Faculty of Medicine and University Hospital Hradec Kralove, Charles University Prague, Hradec Kralove, Czech Republic.

While increasing levels of thyroxine may be a risk factor for venous thromboembolism (van Zaane *et al.* *Blood* 2010 **115**(22) 4344–4349), there is a relative lack of detailed data on the effect of thyroid hormones on haemostatic system. We analyzed multiple markers of haemostasis in a cohort of patients shifting from severe hypothyroidism to mild hyperthyroidism during their differentiated thyroid cancer treatment.

In 94 patients following total thyroidectomy for cancer, selected tests were performed on two occasions: i) before the radioiodine remnant ablation, in hypothyroidism (TSH, median 92.2 mIU/l; interquartile range 72.2–135.9), and ii) 6–8 weeks later on levothyroxine treatment, with low-normal to suppressed TSH (0.29 mIU/l; 0.11–0.82).

While the values for D-dimer (medians, 0.34 and 0.32 mg/l, respectively), antithrombin (100 and 109.5%), and tissue plasminogen activator (2.9 and 2.8 µg/l) were not changed during levothyroxine treatment, other markers, i.e. fibrinogen (3.5 and 3.9 g/l; Wilcoxon  $P < 0.001$ ), plasminogen activator inhibitor 1 (6.8 and 13.2 µg/l;  $P < 0.001$ ), von Willebrand factor (95.141%;  $P < 0.001$ ) and factor VIII (105.184%;  $P < 0.001$ ) were increased significantly, suggesting a shift towards pro-coagulation activities in the haemostatic balance. Also, the activation times of primary haemostasis (i.e. platelet adhesion and aggregation), evaluated by the PFA-100 System after stimulation with collagen and epinephrine (136 and 116 s;  $P = 0.002$ ) or with collagen and ADP (89 and 82 s;  $P = 0.017$ ), were significantly shortened.

Increasing saturation with thyroid hormones is thus associated with multiple changes in haemostatic balance, suggestive of haemostatic system activation.

#### P413

##### **Sclerosing Hashimoto's thyroiditis: a case presentation**

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##### **Introduction**

Autoimmune thyroiditis is rarely painful. A tender goitre usually indicates viral thyroiditis. Here, we present a case of a persistent painful goitre due to sclerosing-type Hashimoto's thyroiditis.

##### **Case presentation**

Our patient is a 27-year-old mother, with no significant past medical history. She was diagnosed with autoimmune hypothyroidism in October 2005 when 4 months *post-partum*. At diagnosis her anti-TPO titre was 2041 U/ml, and she was asymptomatic. She was managed in the community until January 2009 when she presented with exquisite thyroid tenderness and dysphagia. A diagnosis of viral thyroiditis was made and managed with analgesia and steroids. Her anti-TPO titre was 3754 U/ml, and inflammatory markers raised (CRP 125). An ultrasound revealed a diffusely enlarged gland, with no evidence of abscess, nodules or lymphadenopathy. The nuclear medicine scan showed diffusely low uptake at 0.4%, consistent with thyroiditis.

Six months later despite a prolonged course of steroids the pain persisted and her anti-TPO titre was >6500 U/ml. In view of ongoing pain she was referred to the surgeons for biopsy, and consideration of a total thyroidectomy. The biopsy demonstrated small islands of follicles separated by dense fibrous stroma, with an intense chronic lymphocytic infiltrate (polyclonal B and T cells) and an intact basement membrane. The staining demonstrated that 57% of the IgG positive cells were positive for IgG4. This is in keeping with fibrosing-type Hashimoto's thyroiditis, and she underwent a total thyroidectomy for symptom relief.

##### **Conclusion**

We present a case of a rare sclerosing-type of Hashimoto's thyroiditis. Thyroid pain is rarely associated with autoimmune hypothyroidism. We conclude that ongoing pain is an indication for consideration of thyroid biopsy. Sclerosing-type Hashimotos may require thyroidectomy for pain relief.

#### P414

##### **Does levothyroxine plus thyrostatic drug titrated individually offer better control of hyperthyroidism than thyrostatic alone?**

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Clinical Hospital 'Merkur', Zagreb, Croatia.

In order to compare effects of treatment in hyperthyroid patients treated with thyrostatic drug alone and those treated with thyrostatic plus levothyroxine we reviewed data from 60 consecutive patients attending our outpatient clinic. 27 patients, 25 ♀ and 2 ♂, aged 27–68 years were treated with thyrostatic drug alone, while 33 patients, 31 ♀ and 2 ♂, aged 19–88 years received thyrostatic plus levothyroxine. Number of remissions was greater in thyrostatic plus levothyroxine group (16 vs 8) but these patients were treated for longer period than patients in thyrostatic only group: 8.2 years (1–21) vs 4.5 years (1–14). Number of euthyroid findings on every single visit in thyrostatic plus levothyroxine group – 68.34% – was greater than in thyrostatic alone group – 51.28%, but this did not reach statistical significance ( $P=0.28$ ). Numbers of both hypothyroid and hyperthyroid findings were nonsignificantly greater in thyrostatic alone group – 29.09 vs 17.84% ( $P=0.47$ ) and 24.17 vs 18.18% ( $P=0.80$ ) respectively. Although number of hormone findings outside normal range may seem great it has no clinical impact on patients because over 90% findings do not deviate from euthyroidism more than 25% what is generally symptom free. There were no serious side effects of drugs in both groups.

Authors conclude that addition of levothyroxine is inevitable in cases of discordant levels of thyroxine and triiodothyronine, when decreased thyroxine is accompanied by normal or even slightly elevated triiodothyronine irrespective of TSH values.

#### P415

##### **Free T<sub>4</sub> results in neonates: large differences between Immulite/VISTA and Vitros ECI**

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##### **Introduction**

Thyroxine is essential for growth and brain development in neonates. Early detection of hypothyroidism by measurement of free T<sub>4</sub> (fT<sub>4</sub>) is therefore paramount. Moreover, the initial monitoring of Thyrox supplementation requires accurate fT<sub>4</sub> measurements.

##### **Methods**

In the Maasstad Hospital fT<sub>4</sub> has been measured with the Immulite 2500 and from 2010 onward with the Dimension VISTA. Both instruments show good agreement when tested with adult samples. Samples of neonates are measured in our hospital and in addition are sent to the university hospital of Rotterdam for measurement with the Vitros ECI. We compared the Vitros fT<sub>4</sub> results with the measurements in our hospital.

##### **Results**

In all patients, fT<sub>4</sub> results were lower than measured by the Vitros. We found an age dependent, very variable difference (range 0.4–28.5 pmol/l) between methods. In neonates 2–5 days of age the difference was larger than in cord blood and decreased with age. The differences between patient results obtained with VISTA/Immulite and Vitros could not be explained by the results from external quality control surveys. Neither could they be explained by variations in TBG concentration: TBG is not increased in young neonates. Illustrative is a difference of 14 pmol/l in fT<sub>4</sub> results, found in an 8 days old, TBG deficient neonate.

##### **Conclusion**

Although an explanation of our observation is still missing, it is important for pediatricians to be aware of the fact that neonatal fT<sub>4</sub> results can be very method dependent. In our hospital, this could potentially lead to overtreatment with T<sub>4</sub>. The phenomenon is largely unknown in our country.

#### P416

##### **N-terminal pro B type natriuretic peptide levels in thyroid dysfunctions**

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##### **Introduction**

Aminoterminal pro B type natriuretic peptide (NT-pro BNP) is the inactive degradation product of pro BNP. Thyroid diseases are known to have effect on NT-pro BNP levels. In this study, we aimed to determine possible changes in NT-pro BNP levels in patients with thyroid dysfunctions.

##### **Material and method**

Fifteen patients with hyperthyroidism, 8 with subclinical hyperthyroidism, 12 with hypothyroidism, 15 with subclinical hypothyroidism and 35 healthy controls were included in the study. The patients were evaluated for cardiac disease by physical examination, electrocardiography, and echocardiography. Those with systemic diseases were excluded from the study. Serum samples were taken for free triiodothyronine (fT<sub>3</sub>), free thyroxine (fT<sub>4</sub>), TSH, NT-pro BNP before and after achievement of euthyroidism in patients. Control subjects were tested for once.

##### **Results**

Basal mean NT-pro BNP level of hyperthyroid group was significantly higher than the control group (101.8±63.0 vs 41.40±23.66 pg/ml,  $P<0.001$ ). In hypothyroid, subclinical hypothyroid, subclinical hyperthyroid and control groups mean NT-pro BNP levels were similar. Mean NT-pro BNP significantly decreased after achievement of euthyroidism in hyperthyroid group (49.0±20.0 pg/ml,  $P=0.002$ ). In hypothyroid group, NT-pro BNP levels significantly increased after achievement of euthyroidism (52.75±37.99 vs 79.00±57.96,  $P=0.015$ ). There was no significant change in NT-pro BNP levels after treatment in subclinical hypothyroidism and hyperthyroidism. Basal NT-pro BNP level was positively correlated with heart rate in hyperthyroid group ( $r: 0.569$ ,  $P=0.027$ ) and it was negatively correlated with heart rate in hypothyroid group ( $r: -0.616$ ,  $P=0.033$ ).

##### **Conclusion**

Studies to date suggest that thyroid hormone is one of the important factors that regulate serum NT-pro BNP level. In hyperthyroidism, NT-pro BNP levels reach the levels observed in severe heart failure. Thus, in patients without severe cardiac dysfunction, elevated NT-pro BNP level may be suggestive for hyperthyroidism.

**P417****Thyroid hormone levels are associated with body composition in healthy men**

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**Introduction**

Thyroid disorders affect body composition; however, few studies have addressed the effect of variation of thyroid hormone status within the euthyroid range on body composition. Therefore, we investigated the relationship between indices of thyroid status and body composition in a population of euthyroid men.

**Methods**

Healthy male siblings ( $n=677$ , 25–45 years, mean BMI 25.2) were recruited in a cross-sectional, population-based study; a history or treatment of thyroid disease and positive thyroid auto-immunity were exclusions. Body composition was determined using DXA, total ( $TT_3$ ,  $TT_4$ ) and free thyroid hormones ( $FT_3$ ,  $FT_4$ ), TSH, TBG and  $rT_3$  using immunoassays.

**Results**

Both  $FT_3$  and  $TT_3$  were positively associated with weight, BMI and fat mass (all  $P$  values between 0.0008 and 0.02), whereas  $FT_4$  and  $TT_4$  were positively associated with fat mass only ( $P=0.02$ ). Inverse associations of lean mass with  $TT_3$  ( $P=0.01$ ) and  $FT_4$  were observed ( $P=0.04$ ). We observed no association of TSH with body composition. Concordant positive associations were observed for  $TT_3$ ,  $rT_3$  ( $P=0.006$ ) and TBG ( $P<0.001$ ) with weight and fat mass, and inverse associations with lean mass (both  $P<0.0001$ ).

**Conclusion**

In our study, both free and total thyroid hormone concentrations and TBG were positively related to body weight and BMI (for  $T_3$ ) and to fat mass (for  $T_3$ ,  $T_4$  and  $rT_3$ ), and negatively to lean mass ( $TT_3$ ,  $FT_4$ ,  $rT_3$  and TBG). The cross-sectional design does not allow establishing the direction of these associations. In contrast with prior reports, TSH was not associated with body composition in this population of non-obese young men with well characterized euthyroidism.

**P418****The efficacy of real-time ultrasound elastography in evaluation of thyroid nodules**

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**Introduction**

Real-time elastography (RTE) is a noninvasive ultrasound method of estimation of tissue stiffness by measuring the degree of local tissue displacements after a small compression. Recent data has shown indicate on its ability to differentiate benign and malignant tumors. The aim of the study was to evaluate the diagnostic accuracy of RTE in the diagnosis of malignant and benign thyroid nodules.

**Material and methods**

A total of 75 thyroid nodules in 54 patients: 44 females and 10 males aged 28–77 years were examined using conventional ultrasonography (US), fine-flow CD imaging and RTE. Thyroid US and RTE were performed by 3 examiners using a real-time Hitachi Hivision Preirus machine with linear transducer of 5–12 MHz. All nodules underwent fine-needle aspiration biopsy (FNAB) and patients with malignant and suspicious cytological results were referred to surgery. The final diagnosis was based on FNAB results in patients with benign cytology and on the histopathology reading in those who underwent surgery. The elasticity score (ES) from 1 to 5 was determined for each nodule according to Ueno classification.

**Results**

Elasticity score (ES) 4 or 5 was found in 19/22 (86.5%) thyroid cancers and in only 1/34 (3%) benign nodule. This was strongly indicative for malignancy ( $P<0.0001$ ) with sensitivity 86%, specificity 97%, positive predictive value (PPV) 95% and negative predictive value (NPV) 91%. Among 9 suspicious nodules 3 appeared to be malignant (1 oxyphilic cell type carcinoma, 2 papillary carcinomas) and 6 were benign. All malignant nodules had ES 4 and benign ones had ES 2 or 3.

**Conclusions**

RTE is a highly sensitive and specific method of diagnosing thyroid nodules. This technique can be employed in selecting thyroid nodules for the fine-needle aspiration biopsy.

**P419****The incidence of thyroid nodules in autoimmune thyroiditis in one endemic area**

G E Krassas<sup>1</sup>, N Pontikides<sup>1</sup>, E Memi<sup>1</sup>, M Bougoulia<sup>1</sup>, K Tziomalos<sup>2</sup> & K K Bororidis<sup>2</sup>

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**Introduction**

The coexistence of thyroid nodules and autoimmune thyroid disease (ATD) has been widely reported. The aim of our study was to retrospectively evaluate the incidence of thyroid nodules associated with autoimmune thyroiditis. Sibiu county is a well known mild iodine deficiency area from Romania.

**Material and methods**

We retrospectively analysed data from 600 patients (25–79 years) with autoimmune thyroiditis in University County Hospital in last 2 years (2008–2010). We recorded clinical, ultrasonographic, histopathological and laboratory features of these patients.

**Results**

The frequency of thyroid nodules was statistically significantly higher at women (95%) than in men (5%), with a maximum for the group with age between 40 and 60 years. The incidence of thyroid nodules was higher at patients from urban residence (61.9%). Our study show a incidence of nodules about 39.47% from subjects with autoimmune thyroid disease. 16.66% from this patient had a level of TPO between 101 and 200 IU/ml and 13.33% between 1000 and 2000 IU/ml. The TSH was high in 33.33%, low in 19.60% and normal in 47.05% from our patients. The ultrasonographic thyroid volume was higher than normal value in 55% patient. FNAB was performed in 34% of the patient. The results of the cytological examination were: non-diagnostic cytology, benign, malignant and indeterminate in 19.5, 73.8, 2.2 and 4.5% of the nodules, respectively.

**Conclusions**

The incidence of thyroid nodules in patients with ATD was high. So, in Sibiu county is important to evaluate completely the nodular goite, based on the fact this area is known as one with mild iodine deficiency.

**P420****Analysis of T regulatory cells in young patients with dynamic of Graves' disease and Hashimoto's thyroiditis**

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<sup>1</sup>Medical University in Bialystok, Bialystok, Poland; <sup>2</sup>Department of Allergology and Internal Medicine, Bialystok, Poland; <sup>3</sup>Department of Hematology Diagnostic, Bialystok, Poland; <sup>4</sup>Department of Pediatric Ophthalmology, Bialystok, Poland; <sup>5</sup>Division of Cardiology, Internal Affairs and Administration Ministry Hospital, Bialystok, Poland.

Autoimmune thyroid disease (AITD) is the most common organ-specific autoimmune disorder. Genetic background, environmental and endogenous factors are play important roles in determining the activation of immune cells or the efficacy of the immunoregulatory pathways. In recent years underlined the immunosuppressive role of T lymphocytes with expression of CD4+CD25+ (Tregs) in regulation mechanisms of peripheral immune tolerance.

The aim of the study was to estimate the expression of CD4+CD25<sup>high</sup>, CD4+CD25+CD127<sup>low</sup> and CD4+FoxP3 T cells in patients with Graves' disease (GD) ( $n=20$ , mean age 16.3 years old), in patients with Hashimoto's thyroiditis (HT) ( $n=20$ , mean age 15.8 years old) in comparison with sex- and age-matched healthy control subjects ( $n=20$ , mean age 15.9 years old). The expression of the immune cells populations were analyzed by the four-color flow cytometry using a FACSCalibur (BD Biosciences) cytometer.

In untreated patients with Graves' disease and HT we observed a significant decrease of CD4+FoxP3 ( $P<0.001$ ,  $P<0.01$ ) and CD4+CD25<sup>high</sup> ( $P<0.01$ ,  $P<0.048$ ) T lymphocytes in comparison to the healthy control subjects. The analysis of CD4+CD25+CD127<sup>low</sup> T cells in the peripheral blood revealed comparable percentages of these cells in patients with thyroid autoimmune diseases to the controls. In untreated Graves' patients negative correlation between percentage of CD4+CD25<sup>high</sup> T cells and serum level of anti-TSH-R ( $P<0.017$ ) antibodies was found, while no such correlation were detected in relation to CD4+CD25+CD127<sup>low</sup> or CD4+FoxP3 T cells.

We conclude that the defective function and the reduction number of Treg cells with expression of CD4+CD25<sup>high</sup> and CD4+FoxP3 could be responsible for loses immune tolerance and development of autoimmune process in thyroid disorders.

**P421****Effects of thyroxin on pituitary and plasma GH levels in thyroidectomized: diabetic rats**Z Milicevic<sup>1,2</sup> & J Ciric<sup>1,2</sup><sup>1</sup>Laboratory for Molecular Biology and Endocrinology, Institute for Nuclear Sciences Vinca, Belgrade, Serbia; <sup>2</sup>Clinic of Endocrinology, Diabetes and Metabolic Disease, Clinical Center, Belgrade, Serbia.

The effects of thyroid hormone deprivation and restitution on growth rate and pituitary and plasma GH levels have been studied in control and streptozotocin (STZ) – diabetic rats. GH was measured by a specific RIA using the NIAMOD rat-GH kit. Male Wistar rats which had been surgically thyroidectomized for 30 days and which had been stopped growing were injected i.p. with either saline or STZ (4 mg/100 g b.wt). Eight days after saline or STZ, groups of thyroidectomized control (Thx-C) and thyroidectomized diabetic (Thx-D) rats were injected twice daily at 9 a.m. and 5 p.m. for 7 days with either saline or L-thyroxin (T<sub>4</sub>) 0.25, 0.5, or 1.0 µg/100 g b.wt/inj.

**Table 1**

Data: mean ± s.d.	Δb.wt g/7 days	Pituitary GH (µg/mg)	Plasma GH (ng/ml)
Thx-D+saline	-2.0±0.9	1.8±0.8	5.3±2.3
Thx-D+0.5 µg T <sub>4</sub>	1.1±0.8	2±0.9	9.3±4.5
Thx-D+1 µg T <sub>4</sub>	2.2±1.3	9.4±4.5	12.9±7.5
Thx-D+2 µg T <sub>4</sub>	6.4±2.5	23.4±10.2	20.8±13.9
Thx-C+saline	1.3±1.1	1.6±1.3	7±2.9
Thx-C+0.5 µg T <sub>4</sub>	11.6±3.2	32.8±9.3	38.4±10.5
Thx-C+1 µg T <sub>4</sub>	17.9±4.3	50.8±19.2	45.8±17.5
Thx-C+2 µg T <sub>4</sub>	21.3±5.6	69.4±14.9	41.4±14.1

Present data show that when thyroid hormones are replaced in intensely hypothyroid control and diabetic rats both the pituitary GH concentration and the circulating GH levels increase. However, our data show that for comparable doses, T<sub>4</sub> is less efficient on restituting the pituitary and plasma GH levels in the Thx-D than in Thx-C. Thus, it appears that normal insulin levels are required for biological action of thyroid hormones, at least on pituitary GH synthesis.

**P422****The incidence of thyroid nodules in autoimmune thyroiditis in one endemic area**Mihaela Stanciu, Florina Popa, Alina Pascu & Ioan Gheorghe Totoian  
Clinical County Hospital, University Lucian Blaga, Sibiu, Romania.**Introduction**

The coexistence of thyroid nodules and autoimmune thyroid disease (ATD) has been widely reported. The aim of our study was to retrospectively evaluate the incidence of thyroid nodules associated with autoimmune thyroiditis. Sibiu county is a well known mild iodine deficiency area from Romania.

**Material and methods**

We retrospectively analysed data from 600 patients (25–79 years) with autoimmune thyroiditis in University County Hospital in last 2 years (2008–2010). We recorded clinical, ultrasonographic, histopathological and laboratory features of these patients.

**Results**

The frequency of thyroid nodules was statistically significantly higher at women (95%) than in men (5%), with a maximum for the group with age between 40 and 60 years. The incidence of thyroid nodules was higher at patients from urban residence (61.9%). Our study show a incidence of nodules about 39.47% from subjects with autoimmune thyroid disease. 16.66% from this patient had a level of TPO between 101 and 200 IU/ml and 13.33% between 1000 and 2000 IU/ml. The TSH was high in 33.33%, low in 19.60% and normal in 47.05% from our patients. The ultrasonographic thyroid volume was higher than normal value in 55% patient. FNAB was performed in 34% of the patient. The results of the cytological examination were: non-diagnostic cytology, benign, malignant and indeterminate in 19.5, 73.8, 2.2, and 4.5% of the nodules respectively.

**Conclusions**

The incidence of thyroid nodules in patients with ATD was high. So, in Sibiu county is important to evaluate completely the nodular goitre, based on the fact this area is known as one with mild iodine deficiency.

**P423****The regulatory role of neuroprotective nerve growth factor in Graves' ophthalmopathy**Ildiko Molnar<sup>1</sup> & Agota Bokk<sup>2</sup><sup>1</sup>EndoMed, Debrecen, Hungary; <sup>2</sup>Genzyme Europe BV, Budapest, Hungary.

Nerve growth factor (NGF) belongs to the neurotrophic family and maintains the normal function of peripheral sympathetic and sensory neurons. NGF can synthesize locally in the neuronal and nonneuronal tissues. The main cell sources of NGF restrict to bone-marrow derived cells (lymphocytes, mast cells, eosinophils, monocytes, macrophages) as well as to structural cells (fibroblasts, epithelial cells, smooth muscle cells, adipocytes, keratinocytes). The sympathoadrenal activity initiated NGF-liberation is linked to the hormone-releases from the anterior pituitary. NGF plays a potent role in the highly sympathetic innervated organs such as in glands, adipose tissues and orbits, where it participates in the development of retina, corneal and conjunctival epithelium, lens, iris, ciliary bodies and optic nerves. NGF can induce a local inflammation with the liberation of cytokines, chemokines, active peptides and influence the cell survival and apoptosis processes via its two classes of receptors, tyrosine kinase A and p75. Maintaining the integrity of the sympathetic innervation, NGF contributes to tissue reparative responses.

Graves' ophthalmopathy represents a complexity of endocrine, autoimmune and inflammatory events with an increased sympathoadrenal activity. We investigated the serum NGF levels in the patients with Graves' disease ( $n=95$ ), Hashimoto's thyroiditis ( $n=19$ ), toxic nodular goitre ( $n=17$ ) and controls ( $n=20$ ). Our findings demonstrated elevated NGF levels in Graves' hyperthyroidism in comparison with Graves' euthyroidism ( $1831.29 \pm 39.32$  vs  $1649.33 \pm 75.63$  pg/ml,  $P < 0.023$ ). The presence of TSH receptor autoantibodies ( $P < 0.009$ ) and the levels of FT<sub>3</sub> ( $P < 0.01$ ) gave a strict association with the elevation of NGF. Surprisingly, hyperthyroid Graves' patients with ophthalmopathy showed lower NGF levels than those who had no eye symptoms ( $1910.47 \pm 55.62$  vs  $1746.65 \pm 51.98$  pg/ml,  $P < 0.036$ ).

The results support, that the lower serum NGF due to a failed neuroprotectivity may contribute to the onset of ophthalmopathy.

**P424****Biochemical abnormalities in MCT8 patients are due to abnormal thyroid hormone release from the thyroid and altered D1 activity**W E Visser, P Vrijmoeth, H van Toor & T J Visser  
Erasmus MC, Rotterdam, The Netherlands.

The importance of thyroid hormone (TH) transporters for brain development has been unequivocally demonstrated by the identification of mutations in the transporter MCT8. Patients have mental retardation, hypotonia and low body weight (BW). Their biochemical phenotype typically shows high serum T<sub>3</sub>, low serum T<sub>4</sub> and normal TSH levels. Recently, it has been shown that MCT8 is prerequisite for normal TH release from the thyroid. To date, no curative therapy is available, although block-and-replace therapy was reported to normalize thyroid parameters in a boy with an MCT8 mutation.

We identified a loss-of-function MCT8 mutation (p.F501del) in a 40-year-old male with MR. Initial serum levels were: FT<sub>4</sub> 8.9 pmol/l ( $N$ : 11–25), T<sub>3</sub> 2.84 nmol/l ( $N$ : 1.4–2.5) and TSH 1.78 mU/l ( $N$ : 0.4–4.3). BW had decreased towards 50 kg. Furthermore, he showed progressive behavioural changes. Therefore, we initiated block-and-replace therapy. Thyroid parameters did not change after 2 months treatment with methimazole (MMI) 30 mg/day. During initial treatment weight loss continued, requiring additional feeding via a gastric catheter. Switching to propylthiouracil (PTU) 200 mg/day resulted in a decrease in T<sub>3</sub> and T<sub>4</sub> levels and elevated TSH levels. After 2 months, L-thyroxine was added and titrated up to 100 µg/day, until a relatively normal thyroid state was reached (FT<sub>4</sub> 20.6 pmol/l, T<sub>3</sub> 2.0 nmol/l, TSH 5.6 mU/l). At 1 year, BW increased 2 kg and behaviour had somewhat normalized. Temporary replacement of PTU by MMI resulted in a decrease in FT<sub>4</sub> and an increase in T<sub>3</sub> and TSH levels. The present data demonstrate that serum TH levels slowly decrease after blocking synthesis. This may be explained by an accumulation of TH due to an impaired release in thyroids lacking functional MCT8. PTU appears more effective than MMI in reversing serum TH abnormalities. Since PTU also inhibits D1 activity, D1 likely plays a role in the biochemical phenotype.

**P425****Changes in urinary iodine status following 10-year voluntary salt iodization in Latvia**I Konrade<sup>1,5</sup>, M Makrecka<sup>2</sup>, M Dambrova<sup>2</sup>, L Neimane<sup>1</sup>, I Strele<sup>1</sup>, U Gruntmanis<sup>3</sup>, A Lejnicks<sup>1,5</sup> & V Pirags<sup>4</sup><sup>1</sup>Riga Stradins University, Riga, Latvia; <sup>2</sup>Latvian Institute of Organic Synthesis, Riga, Latvia; <sup>3</sup>Southwestern Medical Center, University of Texas, Dallas, Texas, USA; <sup>4</sup>Faculty of Medicine, University of Latvia, Riga, Latvia; <sup>5</sup>Riga East Clinical Hospital, Riga, Latvia.**Background**

Previous nation-wide survey on iodine deficiency disorders (IDD) in Latvia showed a mild iodine deficiency in 587 schoolchildren (median UI – 59 µg/l). At the time less than 1% of all edible salt was iodized. Despite of the results of National IDD Elimination Committee study, all preventive measures were limited to production of leaflets promoting voluntary iodine salt consumption and convincing industry to use iodized salt. In order to perform a 10-year follow-up study we performed a survey with similar design in the same regions of Latvia. Study design and methods

We conducted a cross-sectional school-based 20-cluster survey of children aged 9–12 in randomly selected 24 schools in all regions of Latvia. In total 508 samples of urine and questionnaires on diet, consumption of iodized salt were collected. UIE was measured by ammonium persulphate method.

**Results**

Self-reported prevalence of regular iodized salt consumption was 52.5%. The median creatinine-standardized UI concentration in Latvian schoolchildren was 129.7 (IQR 90.76) µg/g Cr. Frequency distribution of UI values showed that 68.5% (95% CI: 64.3–72.4) of samples had normal levels ( $\geq 100$  µg/g) of iodine in urine, while 27.8% (95% CI: 24.0–31.8) of schoolchildren had mild (50–99 µg/g), 3.3% (95% CI: 2.1–5.3) moderate (20–49 µg/g) and 0.4% severe (< 20 µg/g) decrease in urinary iodine concentrations. Socioeconomic status of parents or self-reported iodized salt consumption was not associated with iodine status.

**Conclusion**

Ten-year follow-up indicated an impressive increase in self-reported prevalence of regular iodized salt consumption and urinary iodine excretion among schoolchildren. It shows that iodine deficiency could be effectively eliminated even without Universal Salt Iodization programme by gradual changes in population eating habits, spreading of supermarket chains or increased iodized salt usage.

**P426****Triiodothyronine stimulates cystatin C production and glucose transport in bone cells**Ch Schmid, C Ghirlanda & C Zwimpfer  
University Hospital of Zurich, Zurich, Switzerland.**Background**

Thyroid hormones increase both serum levels of cystatin C and renal glomerular filtration rate (reflected by lower serum creatinine); the latter is considered the main determinant of cystatin C levels. A potential explanation for this apparently discrepant finding is an increased production, rather than a decreased clearance, of cystatin C. To study whether 3,3',5'-triiodo-L-thyronine (T<sub>3</sub>) increases the production of cystatin C in a well defined *in vitro* system.

**Methods**

Rat bone-derived osteoblastic (PyMS) cells were kept in long term serum-free culture and treated for different time periods and at different concentrations with T<sub>3</sub>, and cystatin C mRNA was assessed by Northern analysis and cystatin C protein in the medium of the cells by western analysis and by ELISA. [<sup>14</sup>C]-2-deoxy-D-glucose (2DG) uptake was also measured.

**Results**

T<sub>3</sub> increased 2DG uptake by the cells as well as cystatin C mRNA expression and cystatin C accumulation in culture media, in a dose (0.01 to 1 nM)- and time (more markedly after 4 days than after 1 day)-dependent manner, up to 2.5-fold (2DG uptake), 2.8-fold (cystatin C mRNA) and 1.5-fold (cystatin C release) at 1 nM after 4 days. Dexamethasone (100 nM) also increased cystatin C production but decreased 2DG uptake whereas 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>, 1 nM) also increased 2 DG uptake but decreased cystatin C production. T<sub>3</sub> did not stimulate cell replication.

**Conclusions**

T<sub>3</sub> stimulates glucose uptake and the production of cystatin C; the findings may be related to an increased metabolism and energy as well as proteolysis control

demand induced by thyroid hormones and help to explain (via spillover of locally increased cystatin C at multiple sites into the circulation) the dependency of cystatin C serum levels of T<sub>3</sub> *in vivo*.

**P427****Acoustic radiation force impulse elastography (ARFI) in patients with autoimmune thyroid disease**M Vlad<sup>1</sup>, I Zosin<sup>1</sup>, M Balas<sup>1</sup>, I Sporea<sup>2</sup>, S Bota<sup>2</sup>, A Popescu<sup>2</sup>, A Popa<sup>1</sup> & S Radu<sup>1</sup><sup>1</sup>Clinic of Endocrinology, 'Victor Babes' University of Medicine and Pharmacy, Timisoara, Romania; <sup>2</sup>Department of Gastroenterology and Hepatology, 'Victor Babes' University of Medicine and Pharmacy, Timisoara, Romania.**Introduction**

Elastography is a new dynamic technique that uses ultrasonography for the assessment of tissue stiffness. The principle of ultrasound-elastography is that compression of the examined tissue induces a smaller strain in hard tissues than in soft ones. Until now elastography has been applied to study the hardness and elasticity of thyroid nodules, aiming to differentiate malignant from benign ones. Aim

We decided to study if ultrasound elastography by Acoustic Radiation Force Impulse (ARFI) can offer information about the stiffness of thyroid gland parenchyma in patients with chronic thyroid pathology, Graves' disease and chronic autoimmune thyroiditis (CAT).

**Patients and methods**

We studied 51 patients, 29 with Graves' disease and 22 with CAT (diagnosed by specific tests), and 23 healthy volunteers. For all subjects, 10 elastography determinations were performed in the right thyroid lobe (RTL) and 10 in the left thyroid lobe (LTL). The measurements were performed with a Siemens Acuson S2000 ultrasound system, using a convex probe 2–6 MHz. The values were expressed in meters/second (m/s) and the median was calculated. We calculated a mean value between ARFI from LTL and RTL. The *t*-test was used to compare the ARFI values.

**Results**

We found a statistically significant difference between subjects without thyroid pathology (healthy volunteers) and those with autoimmune thyroid diseases (2.07 ± 0.44 vs 2.68 ± 0.50 m/s) ( $P < 0.001$ ). Thyroid stiffness was statistically significant higher in patients with Graves' disease versus those with CAT (2.82 ± 0.47 vs 2.49 ± 0.48 m/s) ( $P = 0.02$ ). In cases with CAT, we obtained a significant difference between ARFI values in the two thyroid lobes.

Explanations for this difference would be the distribution of fibrosis in the thyroid gland and the differences regarding vascularization and thyroid volume.

**Conclusion**

ARFI seems to be a useful method to predict accurately enough the presence of autoimmune thyroid diseases (AUROC = 0.80).

**P428****Proliferative potential of normo- and hyperfunctioning thyroid nodules**Marioara Cornianu, Alis Dema, Ioana Golu, Sorina Taban, Mihaela Vlad, Elena Lazar, Codruta Lazureanu & Mirela Grigoras  
UMF 'Victor Babes', Timisoara, Timis, Romania.**Introduction**

Thyroid follicular adenomas (FA) and adenomatous nodules (AN) – lesions frequently found in areas with iodine deficiency, can be normo-/hypofunctioning (scintigraphically cold – SCN) or hyperfunctioning (scintigraphically hot – SHN) nodules.

**Aim**

Evaluation of proliferation potential in thyroid nodules on tissue samples obtained at surgery from euthyroid patients clinically diagnosed with SCN and from patients with thyroid hyperfunction and SHN.

**Material and methods**

We investigated the proliferation activity estimated by assessing PCNA and Ki-67 proliferation markers in 20 SCN (8 FA, 12 AN) and 16 toxic nodules (6 hyperfunctioning FA, 10 toxic multinodular goiters), on formalin-fixed, paraffin-embedded tissue samples, 4–5 µ thick; we used the LSAB immunohistochemical technique (DAB visualization) with anti-PCNA (PC10) and anti-Ki-67 (MIB-1) monoclonal antibodies. We calculated the proliferation index PI-PCNA and PI-Ki67. The data were statistically evaluated using the *t*-unpaired test.

**Results**

PI-PCNA was higher in thyroid nodules than in normal surrounding thyroid tissue, with statistically significant values for FA (14.3 vs 3.8%;  $P < 0.029$ ) and for AN (8.3 vs 1.24%;  $P < 0.001$ ). Mean PI-Ki67 in nodules versus surrounding tissue was 1.64 vs 1.10% in FA ( $P < 0.35$ ) and 1.07 vs 0.51% in AN ( $P > 0.05$ ). We also noted: (1) significantly higher PI-PCNA values ( $P < 0.01$ ) in FA (14.03%) than in AN (8.36%), as compared to statistically insignificant values for Ki-67 (1.64 vs 1.07%;  $P > 0.05$ ); (2) increased proliferation rate ( $P < 0.01$ ) in thyroid nodules with aspects of lymphocytic thyroiditis (LT) (PI-Ki67 was 1.21%) as compared to nodules without LT (PI-Ki67 was 0.12%); (3) a mean PI-PCNA of 8.5% and PI-Ki-67 of 4.61% in toxic thyroid nodules (TTN) versus 3.01 and 1.5% in normal surrounding thyroid, respectively.

**Conclusions**

The clinical expression of SCN is the consequence of increased thyrocyte proliferation in the nodules; the increased proliferative potential of TTN thyrocytes is a common feature of nodules, independent of their histopathological characteristics.

**P429****The effect of age on thyroid function and diagnostic significance of TRH test in elderly people**

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The effect of age on thyroid function and hypothalamic-pituitary-thyroid axis have been studied. The objective of the study was to examine: correlation between triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) hormone levels, thyroid antibodies and thyroglobulin levels and old age; capability of TSH and prolactin (PRL) response to TRH, in TRH test depending on age and gender of the examinees. We have tested 125 subjects, classified in 4 group. First, control group comprised of 42 health adults aged 20–40 years, 22 females and 20 males. The next three groups comprised of 83 healthy elderly persons, aged 40–70 years, 34 males and 49 females. All the patients had total T<sub>3</sub> and T<sub>4</sub>, free T<sub>3</sub> and T<sub>4</sub>, thyroid antibodies and thyroglobulin, TSH and PRL basal and after TRH stimulation determined. The results showed that with ageing, there was significant decrease of total and free T<sub>4</sub> values and slightly lesser intensity of T<sub>3</sub> in the male group, while in the female group, it was noted a mild increase of the total hormones values, but within the normal euthyrosis values with no changes in free hormone values. A significant fall of thyroglobulin antibodies with ageing was observed in the group of males and a considerable increase of thyroglobulin was shown in the group of females. Basal values of TSH and PRL remained within their normal values with ageing in both groups of examinees. Diminished sensibility in TSH response to the negative recurrent relation with the thyroid hormones, i.e. certain resistance of adenohipophysitis to thyroid hormones was noted with ageing. TSH and PRL response in TRH test was diminished with ageing in the female group of examinees. It may be concluded that, despite of euthyroid status, TSH and PRL response has been changed in elderly examinees, more in women than man. The results also show difficulties in evaluating thyroid gland function in elderly examinees and TRH test limitations as diagnostic tool. In interpreting of the result of thyroid gland function tests, it is certainly necessary to take into consideration the age and gender of examinee. There is a need for a review of our strategy in thyroid gland function testing in elderly population.

**P430****Improved prognosis in myxedema coma: happy ending of an old story: case report**

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**Introduction**

Myxedema coma (MC) is a rare life-threatening form of hypothyroidism most often seen in patients with incompletely treated or unknown hypothyroidism exposed to stressful conditions (surgery, infections, hypothermia, trauma).

**Case report**

We report the case of a 58-years-old woman hospitalized for coma in a peripheral hospital after a severe respiratory infection. Two days after, patient's condition

was worsening and she was transferred to a University Hospital, where myxedema coma was suspected (history of untreated autoimmune hypothyroidism, apparently normal temperature – 36 °C despite the infection, bradycardia, hypotension, hypoventilation, hypercapnia, generalized edema). Biological findings showed respiratory acidosis without hyponatremia or other abnormalities. Very low FT<sub>4</sub> (<1 pmol/l) with high TSH (32 µIU/ml) confirmed MC. Intensive therapy (fluid replacement, dopamine, orotracheal intubation with oxygenotherapy) and hormonal therapy: 400 µg thyroxine by nasogastric tube, concomitant with hydrocortisone (HHC) 100 mg/6 h, improved patient's status. Coma became superficial and 4 days after, having spontaneous respiration, normal gasometria and hemodynamic data, she was transferred in the Department of Endocrinology. Treatment included oral L-thyroxine (100 µg/day), HHC, antibiotics. Edematous syndrome and cognitive function improved, although some confusional features persisted (Hashimoto's encephalopathy).

**Discussion**

Owing to the rarity of myxedema coma there are very few studies evaluating the efficacy of treatment and the evolution. Knowing its severe prognosis, it is important to promptly treat the patients with myxedema coma, even when the diagnostic is not certain. Nowadays the mortality rate decreased from 80% to 20–40% thanks to improved diagnostic testing, advances in intensive care, and increased awareness of physicians. Our happy ending story illustrates this improvement.

**P431****The immunohistochemical demonstration of parafollicular cells and evaluation of calcium-phosphate balance in patients with thyroid hemigenesis**

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**Introduction**

Thyroid hemigenesis (TH) is characterised by congenital absence of one thyroid lobe.

**Aim**

To assess possible association between TH and concomitant alterations of other structures derived from pharyngeal apparatus, which might potentially impact calcium-phosphate balance.

**Materials and methods**

Studied group: 20 patients with TH. Control group: 20 subjects with bilobed thyroid, matched for age and gender. Serum concentrations of total calcium, parathormon and calcitonin were measured. Additionally, an immunohistochemical expression of calcitonin, chromogranin A (chA), somatostatin, neuron-specific enolase (NSE) and calcitonin gene-related peptide (CGRP) was evaluated in surgical specimens from patients with TH and subjects with normal thyroid.

**Results**

There were no significant differences between concentrations of total calcium, parathormone and calcitonin between patients with TH and subjects with bilobed thyroid (total calcium:  $9.62 \pm 0.8$  mg/dl in TH vs  $9.89 \pm 0.6$  mg/dl in controls,  $P = 0.331$ ; parathormon:  $40.99 \pm 16.8$  pg/ml in TH vs  $42.96 \pm 14.4$  pg/ml in controls,  $P = 0.978$ ; calcitonin:  $6.84 \pm 3.8$  pg/ml in TH vs  $8.54 \pm 6.3$  pg/ml in controls,  $P = 0.408$ ). Positive staining for calcitonin was demonstrated in 3/8 (37.5%) of thyroid sections from patients with TH, while only in 2/33 (6.06%) of sections from control patients ( $P < 0.005$ ). All sections from patients with TH positive for calcitonin, also expressed chA, NSE and CGRP. Two sections from control subjects, positive for calcitonin, presented additionally positive reaction for chA and one of them also for NSE. None of them however, presented positive staining for CGRP and somatostatin. Out of three TH sections, in one hyperplasia of parafollicular cells of medium grade and of high grade in another one could be detected, in comparison to control sections, where hyperplasia of C-cells of low and medium grade was observed.

**Conclusions**

The study provides first immunohistochemical demonstration of the presence of parafollicular cells in subjects with TH. TH was associated with slightly enhanced C-cells hyperplasia in comparison to control specimens, what might indicate compensative proliferation of parafollicular cells. However, calcium-phosphate balance seems not significantly affected.

**P432****The influence of radioiodine therapy on ophthalmologic symptoms and their relation to urine cotinine level in patients with Graves' disease**

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**Introduction**

Radioiodine therapy (RIT) in patients with Graves' disease (GD) and orbitopathy (GO) is still controversial, especially in smokers, due to the risk of exacerbation of ophthalmologic symptoms.

**Aim**

To evaluate the effect of RIT in smoking and non-smoking patients with mild GO in a 6-month follow-up.

**Patients and methods**

We examined 26 smoking and 25 non-smoking patients, matched for age and gender, presenting hyperthyroidism and mild GO. In all patients, 22 mCi of radioiodine was administered, with subsequent protective 6-week oral prednisone therapy. The diagnostic procedures, performed at baseline and during follow-up included assessment of serum TSH, FT<sub>4</sub> and anti-TSH receptor autoantibodies (TRAb) concentrations, urinary level of cotinine (marker of tobacco smoking) and thyroid ultrasound examination. Additionally, severity of GO was evaluated according to NOSPECS scale and Clinical Activity Scale (CAS). Patients presenting  $\leq 4$  points in NOSPECS scale and  $\leq 3$  in CAS were enrolled.

**Results**

TSH concentrations in both groups were undetectable. In smokers, FT<sub>4</sub> levels before RIT was  $22.2 \pm 3.9$  and  $18.6 \pm 6.1$  pmol/l after 6 months, while in non-smokers,  $22.9 \pm 3.1$  and  $18.8 \pm 4.1$  pmol/l, respectively ( $P > 0.05$ ). TRAb concentration was higher in smokers ( $P < 0.05$ ) and increased from  $22.9 \pm 7.2$  IU/l before therapy to  $28.9 \pm 10.6$  IU/l after 6 months. In non-smokers, initial TRAb concentration was  $15.3 \pm 6.2$  and  $15.2 \pm 6.8$  IU/l after 6 months ( $P > 0.05$  for both groups). The CAS value in smokers before RIT increased from  $2.8 \pm 0.2$  points at baseline to  $4.0 \pm 0.5$  after 6 months, while in non-smokers was  $1.4 \pm 0.2$  and  $2.2 \pm 0.2$ , respectively ( $P > 0.05$ ). The level of urine cotinine correlated positively with CAS and TRAb in smokers ( $P < 0.05$ ) at baseline and during the follow-up.

**Conclusions**

RIT with ablative dose of radioiodine and complementary oral prednisone therapy is safe in both smoking and non-smoking patients with mild GO. However, nicotine was associated with increased severity of GO and TRAb at baseline and during follow-up.

**P433****TSH receptor antibody measurement in the diagnosis of Graves' disease**

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**Introduction**

Graves' hyperthyroidism is an autoimmune disease sustained by auto antibodies binding to and activating the TSH receptor located on the thyroid follicular cell. The diagnosis of Graves' hyperthyroidism is based upon clinical symptoms, signs and laboratory tests confirming the presence of thyrotoxicosis, positive thyroid peroxidase (TPO) and less commonly thyroglobulin (Tg) antibodies, and finally, positive serum TSH receptor antibodies (TRab). Recently study reported the positivity for TRab in 95.7% untreated Graves' patients.

The aim of our study is to determine the serum TSH receptor antibodies measured by RIA (CIS-Bio) in patients with Grave's in our endocrine clinic in Tirana.

**Material and methods**

During the years 2009–2010 in our clinic 265 patients were consulted for thyroid disorder and 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  $\pm 16.2$  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 by RIA (Cisbio) and the cut off for positivity was  $> 1.0$  U/ml. As control we took a group of normal subject and with Hashimoto hypothyroidism.

**Results**

With a cut-off point of TRAb  $\geq 1$  U/l the TRAb antibodies were positive in 90.7% of patients with Graves' disease before treatment with an average level  $8.89$  U/l  $\pm 13.71$  s.d. with a range 1.1–105. In those without Graves' disease, TRAb were positive in 1.6% one patient with autoimmune thyroiditis and undetectable in euthyroid and multinodular toxic goiter and normal controls.

**Conclusion**

In this study, we conclude that TRAb assay is of great interest in confirming the diagnosis. Using these assays in clinical routine is helpful in the differential diagnosis of hyperthyroidism, since the presence of autoantibodies confirms GD, while their absence indicates a non autoimmune origin of hyperthyroidism.

**P434****Autoimmune hyperthyroidism triggered by <sup>131</sup>I treatment for toxic nodular goitre: treatment with a second dose of <sup>131</sup>I versus thyrostatics**

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The transformation of toxic nodular goitre into autoimmune hyperthyroidism with development of thyrotropin receptor antibodies (TSHRab) as effect of <sup>131</sup>I therapy is rarely investigated.

The aim of the study is to present cases of patients with toxic nodular goitre who developed autoimmune hyperthyroidism after initial <sup>131</sup>I dose, and results of treatment with second <sup>131</sup>I dose or thyrostatics.

Four patients with toxic nodular goitre received therapeutic dose of <sup>131</sup>I. Thyroid function was assessed by measuring FT<sub>3</sub>, FT<sub>4</sub>, TSH, TSHRab levels.

Male aged 44 referred to <sup>131</sup>I treatment (after 6 months) again due to hyperthyroidism. TSHRab was increased to 3.3 U/l. Three months after administering second <sup>131</sup>I dose, hypothyroidism was diagnosed (TSH 46.74 mIU/l) and substitute treatment was added.

Female aged 40 (TSHRab  $< 0.3$  U/l before <sup>131</sup>I): euthyroidism after 3 months of <sup>131</sup>I therapy; recurrence of hyperthyroidism and an increase in TSHRab to 5.8 U/l after another 3 months. After administering thyrostatics for 6 months, euthyroidism was achieved. TSHRab decreased to 0.9 U/l.

Female aged 57 (TSHRab  $< 0.3$  U/l before <sup>131</sup>I): increase in FT<sub>3</sub>, FT<sub>4</sub> and TSHRab to 5.8 U/l after 3 months of <sup>131</sup>I therapy. After administering thyrostatics for 3 months, euthyroidism was achieved. TSHRab decreased to  $< 0.03$  U/l.

Female aged 53 (TSHRab  $< 0.3$  U/l), after 3 months of <sup>131</sup>I therapy, thyroid function tended to be normal (TSH = 0.34 mIU/l); after 6 months there was recurrence of hyperthyroidism with increase of TSHRab to 1.9 and to 4.0 U/l after another 3 months. When patient was treated with thyrostatics, her thyroid function tended to be normal.

**Conclusion**

Although insignificant number of patients makes it impossible to analyse results of treatment of autoimmune hyperthyroidism (second <sup>131</sup>I dose versus thyrostatics), it seems that treating patients with thyrostatics before administering possible second dose of radioiodine is justified.

**P435****The usefulness of human TSH-receptor antibody determination in the assessment of clinical activity of Graves' orbitopathy**

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**Introduction**

Graves' disease (GD) is a common autoimmune endocrinopathy where autoantibodies that activate TSH receptor (TRAb) cause clinical hyperthyroidism. Graves' Orbitopathy (GO) is one of the major complications of GD that results from the inflammation of retro-orbital tissues. Although the clinical usefulness of TRAb determination in the diagnosis of GD is well established, recent studies have shown that TRAb levels may also be helpful in assessing the activity and the clinical course of GO.



#### Aim

The aim of this study was to define the diagnostic cut-off point of TRAb at which we could identify patients with active GO.

#### Methods

We studied 124 females and 46 males with GD who were on treatment with antithyroid drugs and were euthyroid for at least 3 months. Disease duration varied from 6 to 72 months. According to the Clinical Activity Score (CAS), 50 patients had active and 120 non-active GO. Serum FT<sub>4</sub>, T<sub>3</sub> (AxSYM Abbott), TSH (electrochemiluminescence (ECL) method on Elecsys 2010 Roche), TRAb (DYNOTest TRAb Human kit Brahms Ria kit), anti-TPO and anti-Tg (ECL Elecsys 2010 Roche) were measured in all patients.

#### Results

Patients with active GO had significantly higher TRAb levels compared to patients with non-active GO ( $P < 0.001$ ) while no difference was found for anti-TPO and anti-Tg levels. Area under the sensitivity (true positives) versus 1-specificity (false positives) curve for TRAb  $\pm$  s.e.m. was  $0.85 \pm 0.032$  and the corresponding 95% confidence interval ranged from 0.79 to 0.91 indicating that TRAb is a useful marker in GO activity assessment. TRAb values higher than 4.7 IU/l (sensitivity 74%, specificity 74.2% and efficiency 74%) can discriminate patients with active GO.

#### Conclusion

In patients with GO, a TRAb cut-off value of 4.7 IU/l could be applied for anti-inflammatory treatment decisions during the course of the disease.

### P436

#### Postpartum follow-up of women positively screened for thyroid disease in pregnancy: the necessity of a better care

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#### Introduction

The utility of screening for antibodies against thyroperoxidase (TPOAb) in early pregnancy remains controversial. The aim of our retrospective follow-up study was to examine the thyroid function of initially euthyroid TPOAb+ women at a longer period after delivery.

#### Methods

We invited 822 women screened positive for thyroid disorders in the first trimester of pregnancy (9th–12th gestational week) for follow-up. This included measurement of TSH, free thyroxine (FT<sub>4</sub>), TPOAb and filling of a detailed questionnaire regarding family and personal history.

#### Results

One hundred eighty-nine (23%) of invited women joined the study. Of these, 100 were TPOAb+ and initially euthyroid in pregnancy. Only 49% of all TPOAb+ women had a positive family history for thyroid disorders. Median time between delivery and follow-up reached 22 months. At follow-up, 13% of the initially euthyroid TPOAb+ women had TSH below 0.37 mU/l, 15% had TSH between 4 and 10 mU/l; and 8% had TSH above 10 mU/l. Serum levels of TPOAb in pregnancy were not linked to the development of thyroid dysfunction. Thirty-five percent of those women who were never treated for thyroid disorders developed thyroid dysfunction at the follow-up. Surprisingly, 29% of women who were currently treated for thyroid disease had TSH outside of the normal range at the time of follow-up.

#### Conclusion

Thirty-six percent of TPOAb+ women euthyroid in early pregnancy have TSH outside of the normal range at 22 months after delivery. The follow-up and treatment of these women is insufficient. TPOAb+ women after delivery should be closely monitored even if they are euthyroid during pregnancy.

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### P437

#### CTLA4 exon1 A49G polymorphism in Slovak patients with rheumatoid arthritis and autoimmune thyroid disease

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Autoimmune thyroid diseases (AITD), such as Hashimoto's thyroiditis (HT) and Grave's disease (GD) frequently form overlap syndromes with rheumatoid

arthritis (RA). Among genetic factors, the role of the HLA antigens and CTLA4 gene polymorphisms in the formation of such overlap syndromes has been suggested.

#### Objective

Aim of this study was to investigate the allelic and genotypes frequency of the CTLA4 exon1 A49G polymorphism in Slovak patients with RA, HT and both (RA+HT) and to compare it with healthy controls.

#### Subjects and methods

Homogeneous groups of 57 unrelated adults with RA, 57 patients with HT, 34 patients with both RA and HT, and 51 randomly selected normal adults without any history of autoimmune disease were studied. All of them were ethnic Slovak living in the same geographical area. The CTLA4 exon1 A49G polymorphism was genotyped by using small amplicon melting analysis after real-time PCR.

#### Results

The CTLA4 49 GG genotype and G allele frequency in the group with RA was not significantly higher in comparison with controls (10.53 vs 9.8%, OR 1.39,  $P = 0.62$  for GG genotype, 39.47 vs 34.31% OR 1.25 for G allele). The frequency of GG genotype was slightly but not significantly higher in patients with HT as compared with control group (19.3 vs 9.8%, OR 2.27,  $P = 0.17$ ). However the frequency of GG genotype and G allele in patients with the coexistence of RA and HT was significantly higher than that in control group (29.41 vs 9.8%, OR 4.49,  $P = 0.02$  for GG genotype, 51.47 vs 34.31%, OR 2.02 for G allele,  $P = 0.03$ ).

#### Conclusion

Our results show that patients with RA who are carriers of GG genotype of CTLA4 A49G gene polymorphism may be susceptible to develop HT.

### P438

#### An unusual organ involvement in Werner syndrome: thyroid atrophy

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#### Introduction

Werner syndrome (WS) is an autosomal recessive disease characterized by premature aging, skin changes, gray hair, alopecia, muscle atrophy, osteoporosis, and cataracts and has a high frequency of association with rare neoplasms. In addition, some endocrinological abnormalities were manifested in this rare disease, such as hypogonadism, diabetes mellitus, hyperlipidemia. Atrophic changes of organs and systems such as skin, brain and genital organs were reported. However, as our knowledge, thyroid atrophy has not been reported in literature, yet. Here, we present a case with thyroid atrophy in WS.

#### Case

Nineteen-year-old female patient had been diagnosed with WS for 2 years. She had type 2 diabetes mellitus, osteopenia, hyperlipidemia, cataract, gray hair, and skin atrophy. She had been treated with insulin aspart and glargine, metformin, gemfibrozil, calcium and vitamin D. At the time of routine outpatient clinic control, her glucose was unregulated and she was hospitalized for regulation of the blood glucose. Her laboratory tests were as follows; glucose 245 mg/dl, HbA1c 7.9%, TSH: 2.3  $\mu$ IU/ml (0.6–4.8), fT<sub>3</sub> 3 pg/ml (2.3–4.2), fT<sub>4</sub> 0.71 ng/dl (0.74–1.52), anti-T 50.7 (0–64) U/ml, anti-TPO 33.7 U/ml (0–57), TG 7.3 (1.6–59.9) ng/ml. Thereafter, thyroid ultrasonography was performed and bilateral parenchymal heterogeneity and very low thyroid volume were detected (right lobe dimensions were 26  $\times$  10  $\times$  8 mm, volume was 1 cm<sup>3</sup> and left lobe dimensions were 19  $\times$  10  $\times$  7 mm, volume was 0.6 cm<sup>3</sup>). FNA biopsy of both lobes was performed. Decreased follicular epithelium cells were detected in specimens and there was not any thyroiditis signs. Because of having no symptoms and signs of hypothyroidism she was followed up without treatment.

#### Conclusion

In conclusion, we suggest that thyroid tests and ultrasonography should be performed in all patients with WS.

### P439

#### Thyroid pathology in patients with type 1 diabetes mellitus

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#### Introduction

The association of type 1 diabetes mellitus (DM1) with thyroid diseases is well known, especially in the field of polyglandular autoimmune diseases. Thyroid

status can influence the necessary of insulin – decreased in hypothyroidism and higher in hyperthyroidism. We wanted to evaluate the prevalence of thyroid pathology in patient with DM1.

#### Patients and methods

Cross-sectional study including 66 patients with DM1 (23 men, 43 women) aged between 20 and 65 years and 50 healthy volunteers age and sex matched (group B), evaluated biologically (TSH, fT<sub>4</sub>, anti-thyroidperoxidase antibody – TPO) and morphologically (thyroid ultrasonography).

#### Results

Autoimmune thyroiditis (AIT) was significantly more frequent in DM1 group (56 vs 16%,  $P=0.006$ ), as well as thyroid hypoechoogenicity (48.5 vs 26%,  $P=0.017$ ). We found a good correlation between thyroid hypoechoogenicity and autoimmunity ( $r^2=0.417$ ,  $P=0.006$ ). Hypothyroidism was confirmed in 12 cases (18.2%; 9 overt and 3 subclinical), more frequent in those with AIT (27 vs 6.9%,  $P=0.001$ ), and in only 3 cases (6%) in control group. None of our subjects had hyperthyroidism.

#### Discussion

Thyroid autoimmunity and dysfunctions are more frequent in DM1 patients than in general population. Hypothyroidism is associated with diminished insulin necessary, with higher requirement once TSH normalised, which impose rigorous glycaemic control. Owing to the good correlation between thyroid hypoechoogenicity and ATPO levels, thyroid us may be a useful tool in assessment thyroid status.

#### Conclusion

Screening of thyroid diseases should be systematic in DM1 patients in order to detect unknown thyroid dysfunctions.

## P440

### Goiter and epidemiology of autoimmune thyroiditis

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Thyroid disease epidemiology has changed recently. This change may be related to changes in the iodine status of the environment. Iodine deficiency was observed in earlier years in Greece. The progression of multinodular goiter to hyperthyroidism was frequently observed. Nowadays, the development of autoimmune thyroiditis is frequently observed in patients with simple or nodular goiter. This development may be related to the proinflammatory action of iodine, iodine being nowadays adequate in the environment.

The aim was the long term follow up of patients with simple or micronodular goiter in the area of Athens.

We studied 87 patients, 80 female and 7 male, aged 40–70 years, with simple goiter, micronodular goiter or nodular goiter. All of them had undetectable antithyroid antibodies at presentation. We followed up these patients for 5–8 years. During follow up 24 patients developed detectable antithyroid antibodies, in 2 of them only antithyroglobulin antibodies being detected. In 18 of them a progressive increase of the antibodies was observed, 1 patient female developing hypothyroidism due to autoimmune thyroiditis. Within the group of the patients with detectable antithyroid antibodies 5 had a positive family history for autoimmune thyroid disease.

During long term follow up patients with goiter appear to develop antithyroid antibodies, progression to autoimmune hypothyroidism being however infrequent. This development may be related to the iodine sufficiency currently observed in modern Greece, particularly in the area of Athens.

## P441

### Thyroid-associated ophthalmopathy (TAO) in association with another disorders

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Thyroid-associated ophthalmopathy (TAO) is an organ-specific autoimmune process that is strongly associated with thyroid dysfunction. Patients with TAO

may present eyelid retraction, proptosis, chemosis, periorbital oedema and altered ocular motility with significant functional and cosmetic consequences. There is the possibility of coexistence of two or more autoimmune diseases affecting the eye due to the cross-reactivity to common antigens from the eye muscles.

The aim of this study was to evaluate association of TAO with other autoimmune and non-autoimmune diseases and reveal some problems in differential diagnosis of TAO. This study was retrospective and performed on patients diagnosed with TAO in Endocrinology Clinic from Tg-Mures, Romania (January 2000–December 2010). We found 238 patients with TAO with ages between 14 and 75 years, with a peak for women at 40–49 years and 50–59 years for men. Female/male ratio was 4.4/1. 83.19% (meaning 198 patients) were found with hyperthyroidism, 10.09% (24 patients) presented hypothyroidism and 6.72% (16 patients) presented euthyroidism. One hundred and seventy-two patients presented asymmetric exophthalmos and 56 of them – symmetric. One hundred and fifty-eight patients (66.4%) presented associated pathology. We found TAO associated with: multiple sclerosis (2 cases), rheumatoid arthritis (2 cases), psoriasis (2 cases), Marine–Lenhart Syndrome (4 cases), glaucoma (3 cases), ovarian cysts (2 cases), premature ovarian failure (2 cases), systemic lupus erythematosus (4 cases), miastenia gravis (3 cases), vitiligo (6 cases), breast tumor (4 cases), obesity (7 cases), chronic hepatitis (7 cases), diabetes mellitus (8 cases), dysrhythmia (18 cases), anaemia (34 cases), hypertension (36 cases).

Also we found TAO after Amiodarone treatment (6 cases), TAO during early pregnancy (8 cases). Patients with TAO might have higher risk of developing another diseases, especially autoimmune diseases and this possibility may be due to immunological cross-reactivity against common autoimmune targets, as well as to a common genetic background.

## P442

### Evaluation of thyroid function test remission and relapse after low dose anti-thyroid drug therapy of Graves' disease, interim report of a long term retrospective study

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#### Introduction

Optimal anti-thyroid regimen for management of Graves' disease is still a matter of controversy. The aim of this study is determination of remission rate and long-term relapse rate after low-dose anti-thyroid drug therapy.

#### Method

This study is a long-term retrospective study. We selected 100 patients with the diagnosis of Graves' disease that admitted to our endocrinology clinic from January 2000 until December 2010. All of patients were treated with anti-thyroid drugs at the initial dose of 20 mg/day of Methimazole then, doses were gradually decreased or changed 25 mg/day and discontinued when the patients were able to maintain euthyroid (normal fT<sub>4</sub>, fT<sub>3</sub> and TSH). We recorded fT<sub>4</sub>, fT<sub>3</sub>, TSH measurements before starting drugs and then at 1, 2, 3, 6, 9, 12 months and 1, 2 and 5 years after starting of treatment to evaluate the remission and relapse rates.

#### Results

From all 100 Graves' patients 85% were female and mean age was 53.5 years. 79% had diffuse goiter Graves' disease and 21% had additional thyroid nodule. Percentage of patients with normal TSH was 20, 26.4, 51.6, 51.8, 65.4, and 81.3% after 3 months, 6 months, 1 year, 2 years, 5 years and 10 years respectively. Percentage of patients with normal free T<sub>3</sub> was 79, 82.8, 88.9, 89.7, 90 and 98.2% after mentioned time intervals respectively. Also 72.1, 84, 90.1, 82.8, 88.5, 93.1% of patients have normal free T<sub>4</sub> after mentioned time intervals respectively. We had 60.6 and 94.4% of complete remission after 1 and 2 years anti-thyroid drug therapy. TSH relapse rate after discontinuation of drug therapy was 2, 2, and 1% after 1, 2 and 5 years respectively.

#### Conclusion

Our study shows the effectiveness of low-dose anti-thyroid drug therapy in patients with Graves' disease. So regarding lower side effects, we recommend low-dose anti-thyroid regimen for initial treatment of Graves' hyperthyroidism.

#### P443

**Long-term follow-up of thyroid autoimmunity biomarkers in Graves' disease treated with low dose anti-thyroid medications (interim report)**  
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##### Background

There are remarkable debates on the pathogenic mechanisms of Graves' disease (GD). We prospectively followed up the serum levels of different autoimmunity indices in patients with GD in order to elucidate their pattern of alteration with time.

##### Methods

A total of 119 consecutive patients with GD were treated using low dose anti-thyroid medications at the initial dose of 20 mg/day of methimazole (MMI). Then, the dose was gradually decreased, when the patients were able to the minimum maintenance dosage of 5 mg MMI or 25 mg PTU and finally discontinued in euthyroid patients after one year. During the treatment and also after the discontinuation of the drugs, the thyroid hormones (FT<sub>3</sub>, FT<sub>4</sub>, and TSH) and thyroid auto-antibodies (anti-TPO, anti-TSHRab and anti-Tg) were measured routinely every 4 weeks for the first 3 months and every 3 months. Follow-up period was up to 10 years.

##### Results

The mean reductions of anti-TPO, anti-TSHRab and anti-Tg during the follow-up period were 6, 35.8 and 59.6%, respectively. Euthyroid status was achieved in 25.2, 26.1, 37, 45.4, 51.3, 52.9 and 47.9% in 3rd, 6th, 9th, 12th, 24th, 60th and 120th month follow-ups, respectively. No significant correlation was found between the reductions in serum concentrations of anti-TSHRab and anti-TPO as well as anti-TSHRab and anti-Tg and also anti-TPO and anti-Tg.

##### Conclusion

Low dose anti-thyroid medications reduce the serum concentrations of thyroid autoimmunity biomarkers during years, which can be considered as an indirect evidence for their immunosuppressive effects. Unparallel pattern of reduction of these thyroid auto-antibodies suggest the presence of different pathologic mechanisms and independent autoimmunity routes for GD.

#### P444

**Regulation of visceral adipose tissue-derived serin protease inhibitor in overt and subclinical hypothyroid patients**  
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##### Introduction

Visceral adipose tissue-derived serine protease inhibitor (vaspin) is a novel adipocytokine which might exert an insulin-sensitizing effect. It is thought that vaspin mRNA expression may be a compensatory mechanism associated with obesity and insulin resistance. Given the fact that subclinical and overt hypothyroidism are known risk factors for insulin resistance, we aimed to assess the regulation of vaspin by thyroid hormones in humans.

##### Methods

We enrolled 32 overt hypothyroid, 42 subclinical hypothyroid and 37 euthyroid patients in the study. We evaluated the body mass index (BMI), fasting glucose, lipid profile, TSH, free T<sub>3</sub>, free T<sub>4</sub>, fasting insulin and vaspin levels. Vaspin levels were determined by ELISA (ng/ml). We calculated the HOMA-IR index. We evaluated the change in vaspin levels in 16 overt hypothyroid patients after replacement of thyroid hormones.

##### Results

Overt hypothyroid patients were significantly older ( $P < 0.05$ ) and had higher BMI values ( $P < 0.05$ ) than subclinical and euthyroid patients. A significant difference in fasting glucose, triglyceride, HDL, LDL levels was found between the groups ( $P < 0.05$ ). However there was no significant difference in total cholesterol, insulin levels and HOMA-IR ( $P > 0.05$ ). Vaspin levels were not different between the groups (mean of vaspin levels in overt, subclinical and euthyroid patients respectively  $1.13 \pm 0.8$ ,  $1.32 \pm 1.08$ ,  $1.03 \pm 0.76$  ng/ml  $P > 0.05$ ). No significant association was observed between vaspin levels and BMI, fasting glucose, insulin and HOMA-IR ( $P > 0.05$ ). After replacement of thyroid hormones in overt thyroid patients, there was no significant change in serum vaspin levels (before and after treatment respectively,  $1.23 \pm 0.94$ ;  $1.23 \pm 0.07$  ng/ml  $P > 0.05$ ).

##### Conclusion

Although, in animal studies vaspin mRNA levels were shown to be down-regulated in hyperthyroid rats and increased in hypothyroid rats, we could not replicate these findings in humans. It's likely that thyroid hormones have no considerable effect on the regulation of vaspin in humans.

#### P445

**Thyroid function and mortality in euthyroid subjects**  
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##### Objective

Many studies suggest that thyroid function modifies the process of healthy aging in humans. However, both a progressive increase and decrease of TSH with advancing age have been reported. The discrepancy may lie in the iodine intake in the population under, since low iodine intake results in an inverse association of TSH with age, while high iodine intake results in a positive association. We aimed to investigate whether FT<sub>4</sub>, FT<sub>3</sub> and FT<sub>3</sub>/FT<sub>4</sub>-ratio are markers of healthy aging by prospectively investigating their association with mortality in euthyroid subjects.

##### Design

Prospective case-cohort study within the PREVENT study.

##### Subjects

A random subcohort of 2703 subjects was drawn from the PREVENT cohort (all aged 28 to 75 years). A total of 260 subjects were excluded from analyses, mainly because of TSH outside the reference range (0.35–4.94 mIU/l;  $n = 115$ ). In this subcohort, 69 subjects died during median follow-up of 5.4 years. During follow-up, 164 subjects died in the remaining part of the entire cohort. Of these, 141 remained for analyses after the exclusion criteria were applied. These subjects constituted the 'external' cases.

##### Results

After adjustment for age and sex, FT<sub>4</sub> was positively and FT<sub>3</sub>/FT<sub>4</sub>-ratio was inversely associated with cardiovascular mortality (HR, 95% CI: 1.38, 1.06–1.78,  $P = 0.02$  and 0.65, 0.48–0.89,  $P = 0.006$  respectively). Both FT<sub>3</sub> and FT<sub>3</sub>/FT<sub>4</sub>-ratio were inversely associated with all-cause mortality (HR, 95% CI: 0.66, 0.57–0.77,  $P < 0.0001$  and 0.67, 0.57–0.79,  $P < 0.0001$  respectively) and non-cardiovascular mortality (HR, 95% CI: 0.61, 0.51–0.73,  $P < 0.0001$  and 0.65, 0.54–0.79,  $P < 0.0001$  respectively).

##### Conclusions

We prospectively found that FT<sub>4</sub>, FT<sub>3</sub> and FT<sub>3</sub>/FT<sub>4</sub>-ratio predicted mortality in euthyroid subjects: high FT<sub>4</sub> and low FT<sub>3</sub>/FT<sub>4</sub>-ratio were associated with cardiovascular mortality and low FT<sub>3</sub> and low FT<sub>3</sub>/FT<sub>4</sub>-ratio were associated with all cause mortality and non-cardiovascular mortality.

#### P446

**Vitamin D levels are associated with serum TSH levels but not with thyroid autoantibodies**  
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##### Introduction

Hashimoto's thyroiditis (HT) is common autoimmune thyroid disorder and is predominantly the clinical expression of cell-mediated immunity leading to destruction of thyroid cells. Vitamin D plays a role in the regulation of immunity and cell proliferation. So, do the serum levels of vitamin D reduced in HT?

##### Methods

We examined the medical records of the 'Thyroid policlinic' patients with or without HT. Vitamin D, thyroid autoantibodies and TSH levels that evaluated in winter month's interval (January–March 2010), were assessed.

##### Results

Totally, 319 patients (196 with HT, 123 without HT and 25 male) medical records were reviewed. Their demographic and clinical characteristics were shown in Table 1. There were no differences between ages, vitamin D and TSH levels except for the autoantibodies. But there was a significant negative correlation between the vitamin D levels and TSH levels ( $P < 0.001$ ).

**Conclusion**

The serum levels of vitamin D were not reduced in patients with HT. Levels of vitamin D are associated with serum TSH levels (negatively) but not with thyroid autoantibodies.

**Table 1** Demographic and clinical characteristics.

Parameters	Patients with HT	Patients without HT	P
Age	44.8 ± 11.2	43.6 ± 12.9	NS
Vitamin D (ng/ml)	14.9 ± 9.9	14.9 ± 11.6	NS
Anti-TG (U/ml)	814.9 ± 1909	34.3 ± 10.9	0.001
Anti-TPO (U/ml)	897.1 ± 2150.2	38.0 ± 11.7	0.001
TSH (µIU/ml)	2.78 ± 3.2	3.07 ± 7.9	NS

**P447****Evaluation of thyroid surgery: 10 years of experience in a Military Hospital**

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**Introduction**

Thyroid surgery is nowadays associated with low morbidity and extremely low mortality. A consistent association has been observed between high surgical volume and better outcomes. Patients who undergo surgery by a skilled surgeon have fewer complications. On the other hand, different surgical techniques have been associated with different outcomes. Since 1997, thyroid diseases are treated in our department by a multidisciplinary team.

**Objective**

To access the results of thyroid surgery in our hospital, over the last 10 years.

**Methods**

We studied retrospectively the patient's medical files that underwent thyroid surgery from 1999 to 2010. Statistic tests were used to analyze the different variables.

**Results**

A total of 240 patients underwent thyroid surgery over the 10-year study period. The mean age of the patients was 54 years and 76% were females. Pre-operative diagnosis was thyroid nodular disease in 83.3% (13.9% toxic multinodular goiter and 12.5% substernal goiter), papillary carcinoma in 6.7% and follicular neoplasm in 5.8%. 4.2% of the patients had relapsed Graves disease.

We performed lobectomy in 42.2% of cases and total thyroidectomy in 57.6%. Eight percent of patients had permanent complications (total thyroidectomy 6.4% and lobectomy 1.6%). Permanent laryngeal recurrent nerve lesions occurred in 3.4% of patients. Permanent hypoparathyroidism occurred in 4.2% of cases, always associated with total thyroidectomy.

**Conclusions**

Benign thyroid disease is the main surgical diagnostic indication in our hospital. Lobectomy is associated with better outcomes. Our complications rates are slightly higher than international reference centres.

**P448****Fine needle aspiration biopsy in thyroid nodular disease: 10 years of experience in a Military University Hospital**

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**Introduction**

Fine needle aspiration biopsy (FNAB) plays an essential role in the evaluation of thyroid nodular disease (TND). It reduces the rate of unnecessary thyroid surgery for patients with benign nodules, and allows the choice of the appropriate surgery. In our department, 2124 FNAB were performed in 10 years. 60% of them are now ultrasound guided (UGFNAB).

**Objective**

To access the accuracy of FNAB in our hospital.

**Methods**

Retrospective study of 204 patient's medical files who underwent FNAB and who were submitted to surgery, between 1999 and 2010. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of FNAB

(without UG and UG) were examined based on cytohistologic correlation. All statistical evaluation was made using an SPSS software. A *P* value <5% was considered statically significant. Classification of cytological results were adapted from Bethesda system.

**Results**

Cytological results were benign in 78% of the cases. From the remaining, 8.6% were malignant and 13.4% were follicular neoplasms. Histological results were benign in 86.7% and malignant in 13.3%, being 9.1% papillary carcinomas. After the evaluation of cytohistologic correlation, there were 79.4% of true-negatives, 15.7% of true-positives, 3.4% of false-negatives and 1.5% of false-positives. All the false-negatives (7) correlates with large nodules (6) or with non dominant nodule in histology (not analyzed by FNAB). The sensitivity was 95.9%, the specificity was 91.4%, the negative predictive value was 82.1% and the positive predictive value was 98.2%. The comparison of FNAB without UG or UGFNAB demonstrates a false predictive value of 64.7 vs 100% and a positive predictive value of 100 vs 88.9% There were no unsatisfactory samples.

**Conclusions**

The results show a good accuracy of FNA in our hospital, with a high sensibility and specificity. Our results are similar to the published data.

**Thyroid cancer****P449****Routine serum calcitonin measurement in screening of medullary thyroid carcinoma (MTC) in Gohierri county**

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**Introduction**

The utility of serum calcitonin has been evaluated in a series of prospective, nonrandomized studies suggest that the use of routine serum calcitonin for screening may detect C-cell hyperplasia and medullary thyroid cancer at an earlier stage and overall survival may be improved. An earlier study estimated that routine measurement of plasma calcitonin in thyroid nodule patients would yield an additional 2.2 life-years per positive patient.

**Aim**

To determine the usefulness in our county of the determination of calcitonin in patients with thyroid nodules disease, to exclude the presence of MTC.

**Patients and methods**

The study included 434 patients seen in our Department between 2006 and 2009 for nodular thyroid disease, who were screened for MTC by serum CT measurement. The clinical diagnosis, as assessed by clinical examination, thyroid ultrasound, and thyroid function tests, included single nodules, non-toxic multinodular goiter, autonomous functioning thyroid nodules. Fine needle aspiration was performed as a routine procedure. The cost of calcitonin determination was € 15, per sample. Patients with impaired renal function, protons pump inhibitor treatment were been excluded. None of patient had had family with MCT.

**Results**

We studied 434 patients, 71 men and 361 women (60.29 ± 15.3 years), with multinodular goiter (76.3%) and uninodular (22.7%). They were analyzed a 595 samples of calcitonin, which was normal in all patients (< 8 pg/ml). The global cost of calcitonin determination for 4 year was 8925 euros. Surgical proceeding was been performed in 76 patients (compressive goiter, cytology suspicious of malignancy), the diagnosis was 4 papillary carcinoma and benign nodular lesion to the rest of patients, no MCT was found.

**Conclusion**

In our study, the analysis of calcitonin was normal in all patients, despite in our study we have not found any MCT we believe that calcitonin measurement is an useful test with low cost in early diagnosis of MCT.

**P450****Coexistence of hyperparathyroidism with papillary thyroid microcarcinoma**

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**Background**

Concomitant occurrence of primary (PHPt) or renal (RHPT) hyperparathyroidism and papillary thyroid microcarcinoma (PTMc) is rarely described in literature.

**Patients and methods**

Between 1986 and 2010 sixtythree subjects underwent operations for PHTP ( $n=20$ ) or RHTp ( $n=43$ ) in our clinic. In three cases (4.7%) a synchronous PTMc was intraoperatively discovered.

**Results**

First case, female – 56 years old presented with recurrent hyperparathyroidism 6 years after an 'extra muros' extirpation of a right sided inferior adenoma. At operation multiple gray-tan micronodules of 2–5 mm was discovered on the thyroid surface. Biopsy revealed benign parathyroid tissue leading to a diagnosis of parathyromatosis. A right thyroid lobectomy was done. At the paraffine examination a minute foci of PTMc was identified. Any postoperative therapy was recommended. At two years postoperatively she presents a symptomless course.

In two cases of RHTp, one male and one female of 48 and 50 years respectively, in both of whom a near-total parathyroidectomy together with a Dunhill operation was done due to the presence of a bilateral nodular goitre and also of a hard nodule of 5 mm in diameter.

Pathology precised existence of a PTMc classified as PT1.

The patients was guided to complementary therapy and both are alive without recurrences at 7 and 4 years respectively.

**Discussions and conclusions**

Coexistence between PHPT or RHTp with PTMc is coincidental but can be encountered in endemic goitre areas. The diagnosis of these lesions is rarely affirmed before surgery being established by serendipity or better by a careful intra- or postoperative exploration. Surgical treatment must be adapted to the gravely lesion: thyroid carcinoma.

**P451****Recombinant tsh-stimulated thyroglobulin measurements in patients with differentiated thyroid carcinoma with basal thyroglobulin <1 ng/ml**

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**Introduction**

A value of thyroglobulin (Tg) undetectable under treatment with levothyroxine (LT) does not exclude residual/metastatic disease; this can be found in 18–35% of patients after recombinant TSH-stimulated Tg (sTg) measurements.

**Objective**

Evaluating the role of recombinant TSH-stimulated thyroglobulin (sTg) measurements in patients with differentiated thyroid carcinoma, treated with suppressive LT doses, with basal Tg (bTg) <1 ng/ml.

**Methods**

Retrospective evaluation of patients with differentiated thyroid carcinoma with bTg <1 ng/ml submitted to sTg measurements between 01/01/2008–31/12/2009. Patients were divided into two groups: bTg <0.2 ng/ml (group 1); bTg between 0.2–1 ng/ml (group 2). Patients with anti-Tg were excluded. Limit of detectable Tg in our institution 0.2 ng/ml.

**Results**

$n=96$ ; 80.2% ♀; diagnosis age =  $41.8 \pm 13.4$  year; 78.1% papillary (10.4% microcarcinomas), 11.5% follicular. Ablative radioiodine dose =  $97.1 \pm 29.3$  mCi. 73 patients belonged to group 1 and 23 to group 2. There was no statistically significant relationship between these groups with respect to gender, diagnosis age, presence of nodal metastasis, stage or radioiodine dose. All patients of group 2 had papillary carcinoma.

**Table 1**

	Patients number with sTg > 2 ng/ml	P	Mean sTg (ng/ml)	P
Group 1	6	0.000	0.61	0.024
Group 2	10		2.57	

There was no statistically significant relationship between sTg > 2 ng/ml and gender, diagnosis age, histology, presence of nodal metastases or radioiodine dose. 31.3% of patients with sTg > 2 ng belong to stage IV while in the group with sTg < 2 ng/ml only 9.3% belong to this stage ( $P=0.050$ ). There was a positive correlation between bTg and sTg ( $r=0.329$ ,  $P=0.001$ ). There was no statistically significant relationship between sTg and gender, diagnosis age, histology, presence of nodal metastasis, stage or iodine dose.

**Conclusions**

In Group 1, 91.9% with bTg < 0.2 ng/ml kept sTg within normal range. In this group, 83.3% with sTg > 2 ng/ml had multifocal disease. In Group 2, 56.5% had

sTg < 2 ng/ml; 60% of this group with sTg > 2 ng/ml showed capsule's invasion. In 50% of patients with sTg > 2 ng/ml was detected locoregional disease. There was no distant metastasis.

**P452****Thyroid disease in patients with type-1 neurofibromatosis: an underestimated issue?**

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**Introduction**

In type-1 neurofibromatosis (NF-1) there is an increased risk of endocrine tumors, especially pheochromocytomas, whereas thyroid carcinomas seem to be extremely rare, with few cases reported in literature.

**Subjects and methods**

In order to investigate the frequency of hypercalcaemia and medullary thyroid cancer (MTC) in patients with NF-1, we evaluated the thyroid gland morphology and function in 17 patients with NF-1 by i) neck US, ii) Fine Needle Aspiration (FNA) biopsy of the nodules detected at US and the measurement of calcitonin (CT) in wash-out fluid from FNA (CT-FNA), iii) serum CT at basal level and after stimulation with pentagastrin (CT-Pg) and iv) thyroid function.

**Results**

i) 10/17 (58.8%) had nodular goiter and ii) on the basis of cytology at FNA, 3/10 underwent total thyroidectomy with histological confirmation of malignancy in 2/10 patients (1 papillary thyroid cancer and 1 infiltrative squamous carcinoma); iii) only 1/17 presented an increase in basal CT with pathologically increased CT-Pg (> 100 pg/ml) with an histological finding of C-cell Hyperplasia after thyroidectomy; iv) of the 16 remaining patients, CT-Pg was normal in 10 patients (< 50 pg/ml), while 6 patients had a borderline response (50 < CT-Pg < 100 pg/ml) (Elisei *et al. Journal of Clinical Endocrinology and Metabolism* 2004); v) of the 6 patients with 50 < CT-Pg < 100 pg/ml, 5 had thyroid nodules and 2 had notably increased CT-FNA levels (745 and 789 pg/ml, respectively); vi) thyroid function was normal in all patients.

**Discussion**

The finding of goiter in 60% of NF-1 patients, of thyroid cancer in 11 and 40% of borderline/high CT-Pg suggest that an accurate study of thyroid gland in patients with NF-1 is mandatory and that thyroid diseases may be underestimated in the context of NF-1.

**P453****Papillary thyroid microcarcinoma: size and metastatic behavior**

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**Introduction**

Papillary thyroid microcarcinoma (PTMC) is defined as a papillary thyroid cancer measuring 10 mm or less in greatest dimension. Diagnosis of PTMC could be established during the surgery for benign thyroid lesions and after fine needle aspiration biopsy. Despite the small size, PTMC could be highly aggressive tumor showing metastases and elevated invasiveness. However, correlation between tumor size and risk of lymph-node metastases remains unclear.

The aim of the study was to investigate and correlate local lymph-node metastases and multifocality as factors of tumor aggressiveness in patients with different sizes of PTMC.

**Materials and methods**

1408 patients operated on for PTMC during 1986–2009 in Kyiv City Teaching Endocrinological Center were identified for study. Three groups were established according to size of primary tumor. There were patients with size of PTMC less than 3 mm in maximum diameter in group 1 ( $n=197$ ), 3–5 mm in group 2 ( $n=297$ ), and 5–10 mm in group 3 ( $n=914$ ) respectively. Such clinicopathological features as local lymph-node metastases and multifocality were compared between groups.

**Results**

The statistically significant frequency of local lymph-node metastases was higher in group 3 (9.0%) compared to group 2 (3.0%) and group 1 (0%),  $P<0.01$ . Multifocality was higher in group 3 (24.5%) and group 2 (21.5%) compared to group 1 (10.7%),  $P<0.01$ . There was no statistical significant difference for multifocality in group 3 compared to group 2.

## Conclusions

Our finding suggest that patients with size of PTMC between 5 to 10 mm (group 3) may have a higher risk of lymph-node metastases compared to those who have smaller sizes of PTMC. Long-time follow-up study is ongoing in order to determine consequences for patients' outcome.

## P454

### Estrogen receptors and progesterone receptor expression and their relationship to types of thyroid cancer

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The role of hormones and reproductive factors in thyroid carcinoma is suggested by its higher incidence in women. The association with estrogens or other specific hormonal factors remains unclear. We assessed the estrogen receptor alpha (ESRa), estrogen receptor beta (ESRb) and progesterone receptor (PRR) expression in follicular cell-derived tumours (FDT).

Cross-sectional study of 50 (25%) males, median age 50.5 (interquartile range, IR, 34), and 149 (75%) females, median age 51.5 (IR, 30). The immunexpression (ESRa), (ESRb), and (PRR) was analyzed in tissue microarrays containing 199 FDT. We used the 'Allred Score'. A total score (TS) was calculated from the sum of a proportion score and intensity score (ranging from 0–5, and 0–3) with positive results when TS ≥ 3. Cross-tabulation significance levels were based on Pearson's  $\chi^2$  test to analyze histological type-adjusted receptor expression levels. Non-parametric tests were employed for comparison of quantitative variables. All statistical analyses were performed using SPSS software (v. 18.1).

50 follicular adenomas (FA), 73 papillary carcinomas (PC), 48 follicular carcinomas (FC), 15 poorly differentiated carcinomas (PDC) and 13 undifferentiated carcinomas (UC) were studied. Percentage of positive TS: ESRa. – 10.2 (FA), 50.8 (PC), 13 (FC), 0 (PDC), 30 (UC); ESRb. – 64.6 (FA), 65.1 (PC), 22.2 (FC), 60 (PDC), 45.5 (UC); and PRR. – 18.2 (FA), 85.2 (PC), 47.8 (FC), 26.7 (PDC), 0 (UC). We found the highest positive TS values for PC ( $P < 0.001$ ), with maximum scores in the cribriform-morular variant of PC.

The highest expression of hormone receptors in PC fits with its higher female prevalence and suggests such receptors play a pathogenetic role. We also provide the first standardized 'Allred Score' evaluation of FDT, offering a target for future therapy strategies. Grant P509/02050-FEDER from the ISCIII, Spain.

## P455

### The effects of four different tyrosine kinase inhibitors on medullary and papillary thyroid cancer cells

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## Introduction

Medullary and papillary thyroid carcinoma (MTC and PTC) are two types of thyroid cancer that can originate from activating mutations or rearrangements in the RET gene. Therapeutic options are limited in recurrent disease but since RET is a tyrosine kinase (TK) receptor involved in cellular growth and proliferation, treatment with a TK-inhibitor might be promising. Several TK-inhibitors have been tested in clinical trials, but it is unknown which inhibitor is most effective and if there is any specificity for particular RET mutations. We aimed to compare the effect of four TK inhibitors (axitinib, sunitinib, vandetanib and XL184) on cell proliferation, RET expression and autophosphorylation, and ERK activation in cell lines expressing a MEN2A (MTC-TT), a MEN2B (MZ-CRC-1) mutation, and a RET/PTC (TPC-1) rearrangement.

## Design

Three cell lines, MTC-TT, MZ-CRC-1 and TPC-1 were cultured and treated with different concentrations of the four TK inhibitors. Effects on cell proliferation, RET and ERK expression and activation were determined.

## Results

XL184 and vandetanib, most effectively inhibited cell proliferation, RET autophosphorylation in combination with a reduction of RET expression, and ERK phosphorylation in MTC-TT and MZ-CRC-1 respectively. TPC-1 cells showed a decrease in RET autophosphorylation after treatment with XL184, but no effect was observed on ERK activation.

## Conclusion

There is indeed specificity for different RET mutations, with XL184 being the most potent inhibitor in MEN2A and PTC, and vandetanib the most effective in MEN2B *in vitro*. No TK inhibitor was superior for all the cell lines tested, indicating that mutation-specific therapies could be beneficial in treating MTC and PTC.

## P456

### Thr300Ala ATG16L1 polymorphism influences susceptibility to and the prognosis of epithelial cell derived thyroid carcinoma

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## Background

Loss of autophagy protein ATG16L1 enhances endotoxin-induced interleukin 1 $\beta$  (IL1 $\beta$ ) production. IL1 $\beta$  has been found to have antineoplastic effects in thyroid malignancies. We hypothesized that a missense polymorphism in ATG16L1 autophagy gene (Thr300Ala) results in changes in the function of the molecule and influences susceptibility to and prognosis of epithelial cell derived thyroid carcinoma (TC).

## Objective

i) To assess the functional differences between the 300Thr (A) and 300Ala (G) allele of ATG16L1; and ii) to assess whether ATG16L1 Thr300Ala polymorphism influences the susceptibility to and the prognosis of TC.

## Patients and methods

The ATG16L1 genotype was analyzed by PCR in 139 TC patients and 136 healthy controls. In the control group peripheral blood mononuclear cells were stimulated with the NOD2 ligand muramyl dipeptide (MDP). The ATG16L1 function was assessed by measuring IL1 $\beta$  24 h later. Within the patients group we analyzed the genotype-phenotype associations.

## Results

MDP-stimulated IL1 $\beta$  production was significantly higher in individuals bearing the GG/GA allele compared to those bearing AA allele, supporting a gene-dosage or a dominant effect of the 300Ala variant on the function of the molecule. The difference in genotype frequencies between the patients and control groups were analyzed in a gene dosage dependent model using logistic regression and in a dominant model using  $\chi^2$  analysis. The G allele of ATG16L1 is associated with decreased risk for TC (OR=0.46,  $P=0.041$  in the gene dose dependent model, and OR=0.51,  $P=0.025$  in a dominant model). Furthermore, patients carrying the AG/GG genotype had a higher chance of successful I<sup>131</sup> ablation ( $P=0.017$ ) and required a lower cumulative I<sup>131</sup> dose to achieve remission ( $P=0.014$ ) than patients carrying the AA genotype.

## Conclusions

The Thr300Ala ATG16L1 polymorphism has important functional consequences, with significantly higher IL1 $\beta$  release in individuals bearing the 300Ala variant. The 300Ala allele was associated with both a lower susceptibility for TC and lower I<sup>131</sup> dose required to achieve remission.

## P457

### Reversine, an anti-cancer candidate, induces cell cycle arrest and apoptosis in human thyroid cancer

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Thyroid cancer is the most prevalent cancer among endocrine malignancies. Surgical resection combined with radioactive iodine therapy has been proved

effective in treating differentiated thyroid cancer, including papillary and follicular thyroid cancers. However, regarding metastatic poorly differentiated or anaplastic thyroid cancers, there have been no available effective treatment modalities. The genomic instability, such as chromosome aneuploidy, represents a hallmark of solid cancers including thyroid carcinomas, and it has been suggested to underlie the progression to more aggressive phenotypes. Aurora kinase is now thought to play a role in tumor genetic instability and has been found to be altered in human cancer tissues. Several studies disclosed that aurora kinases were overexpressed in thyroid cancers and increased with the dedifferentiation extent.

Reversine, a purine analogue, has reported to inhibit aurora kinases and the phosphorylation of histone H3. Through this mechanism, reversine was shown inhibiting colony formation of leukemic cells from patients with acute myeloid leukemia. However, up to date, no reports aimed to thyroid cancer treatment by using reversine. Besides, there has been no study to investigate its cancer cell death mechanism.

In this study, we revealed that reversine could inhibit thyroid cancer tumor growth and inducing apoptosis. Firstly, we determined the cellular viability of reversine in human thyroid cancer cell lines. Furthermore, we found that reversine could induce cell cycle arrest in these cells. In addition, apoptosis was also revealed in reversine treated cells. Moreover, the extrinsic pathway was demonstrated to involve in reversine mediated apoptosis. Finally, the xenograft nude mice model was used to prove reversine's anti-tumor effect *in vivo*. In the present study, we demonstrated that reversine could be a chemo-therapeutic candidate for human thyroid cancers.

#### P458

##### Intraindividual comparison of serum calcitonin levels after calcium versus pentagastrin stimulation

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##### Objectives

The aim of the study was to compare serum calcitonin levels after calcium versus pentagastrin stimulation in healthy individuals and in patients with medullary thyroid carcinoma (MTC).

##### Methods

The study enrolled 44 healthy individuals and 27 patients with MTC.

Time of measurement: baseline, 2, 5 and 10 min after injection of:

– Calcium 2.0 mg/kg body mass i.v. (10% Calcium Gluconate, Braun Melsungen AG, Germany). Injection speed 10 ml/min.

– Pentagastrin 0.5 µg/kg body mass i.v. (Pentagastrin Injection BP, Cambridge Laboratories, Wallsend, UK). Injection speed 10 s.

After calcium stimulation there was a washout period of at least two weeks before the pentagastrin stimulation.

Calcitonin assay: solid phase chemiluminescent enzyme immunoassay (IMMULITE 2000, Siemens Healthcare Diagnostics).

##### Results

Mean calcitonin levels (pg/ml) in healthy individuals after calcium injection: 1.2/19.5\*/16.4\*/10.2\*. After pentagastrin injection: 1.3/4.5/4.0/2.4 (\**P*<0.05). Nonresponse rate after calcium stimulation 18.2%, after pentagastrin stimulation 56.7%. Mean peak calcitonin levels in each group were significantly different between men and females (after calcium: 53.7\* vs 11.8 / after pentagastrin: 18.3\* vs 1.8 (\**P*<0.05)).

Mean calcitonin levels (pg/ml) in MTC patients after calcium injection: 113/1557/1467/1397. After pentagastrin injection: 114/3549/3252/2053 (n.s.). Mean peak values of calcitonin in each group between men and females were not significantly different.

##### Conclusions

The calcium stimulation is more sensitive than the pentagastrin stimulation in healthy individuals. In untreated or non-cured MTC patients the calcium stimulation is not inferior to the pentagastrin stimulation. Moreover we suppose an earlier detection of MTC recurrence by calcium stimulation than by pentagastrin stimulation.

#### P459

##### Classical and follicular variant papillary thyroid carcinoma: comparison of clinical, ultrasonographical, cytological and histopathological features in 402 patients

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##### Introduction

Follicular variant papillary thyroid carcinoma (FVPTC) is the most common variant of papillary thyroid carcinoma (PTC) after classical PTC (CPTC). In this study, we aimed to compare functional status, ultrasonographical features, cytological results and histopathological characteristics of patients with CPTC and FVPTC.

##### Methods

Patients diagnosed with CPTC and FVPTC in our clinic were reviewed retrospectively. Data of preoperative thyroid functional status, thyroid autoantibodies and ultrasonographical features were obtained from the records. Sensitivity of fine needle aspiration biopsy (FNAB) was calculated and histopathological features were compared in two variants.

##### Results

There were 322 (80.1%) CPTC and 80 (19.9%) FVPTC patients and sex distribution, mean age, thyroid functions and autoantibody positivity rates were similar. 228 CPTC and 62 FVPTC patients had preoperative US examination. Echogenicity, texture, presence of microcalcifications and macrocalcifications were similar in two groups. A hypoechoic halo was observed more frequently (15.4 vs 29%, *P*=0.012) and marginal irregularity was observed less commonly (81.6 vs 67.7%, *P*=0.018) in FVPTC lesions. Among 261 CPTC and 73 FVPTC patients with preoperative FNAB results, rate of malignant cytology was significantly higher in CPTC (37.2 vs 19.2%, *P*=0.004) and rate of suspicious cytology was significantly higher in FVPTC (35.6 vs 53.4%, *P*=0.006). When only malignant cytology was treated as true positive, sensitivity of FNAB in CPTC and FVPTC was 37.2 and 19.2%, respectively (*P*=0.004). Mean tumor diameter was markedly higher in FVPTC compared to CPTC (16.04±13.44 vs 10.88±9.88 mm, *P*<0.001). There was no difference in terms of multicentricity, capsular and vascular invasion, extrathyroidal involvement and lymph node invasion.

##### Conclusion

FVPTC tends to have more benign features in US and less malignant results in cytology. Higher tumor size in FVPTC compared to CPTC might be explained by the recognition of clinical importance of these lesions after reaching particular sizes due to benign US features.

#### P460

##### Serum 25-OH Vitamin D levels in patients with benign and malignant thyroid nodules

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##### Introduction

Increased exposure to sunlight and high serum levels of Vitamin D were reported to relate with decreased risk of various cancer types, such as prostate, breast and colon. Vitamin D receptor and 1α-hydroxylase expression were shown in papillary thyroid carcinoma. In this study, we aimed to evaluate serum 25-OH vitamin D levels in patients with benign and malignant thyroid nodules

##### Methods

Patients admitted to our thyroid clinic were recruited for the study. Thyroid ultrasonography and serum 25-OH vitamin D measurement were done in all patients and controls. Because serum 25-OH vitamin D levels may change with seasons, blood samples were taken in April–May 2009 and 2010. Results were compared in patients with histopathologically proven thyroid papillary cancer,

patients with histopathologically or cytologically proven benign thyroid nodules and patients without thyroid nodules. Patients with diseases related to bone metabolism and thyroid dysfunctions were excluded.

#### Results

There were 31 patients with histopathologically proven papillary thyroid cancer, 128 patients with cytologically or histopathologically proven benign nodules and 269 patients without any nodule. Of 428 subjects, 360 (84.1%) were female and 68 (15.9%) were male and sex distribution were similar in three groups ( $P=0.079$ ). Mean serum 25-OH vitamin D levels were  $16.91 \pm 10.42$ ,  $16.35 \pm 9.02$  and  $17.19 \pm 10.74 \mu\text{l}$  in malignant, benign and control groups, respectively ( $P=0.746$ ). Including all subjects, although mean serum vitamin D levels were higher in males compared to females, the difference did not reach statistical significance.

#### Conclusion

Serum 25-OH vitamin D levels, here in this study, were shown not to differ in patients with benign nodules, malignant nodules and patients without nodules. Possible effect of vitamin D metabolism and receptors on thyroid papillary cancer that was suggested in previous *in vitro* and *in vivo* studies seems to be independent from serum 25-OH vitamin D levels.

## P461

### Role of $^{99\text{mTc}}$ HYNIC Octreotide in the follow-up of thyroid cancer patients that after surgery and $^{131\text{I}}$ therapy present raised thyroglobulin levels and negative $^{131\text{I}}$ whole body scan

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#### Introduction

Patients bearing thyroid cancer that have already gone into surgery and  $^{131\text{I}}$  therapy that in the follow-up have high thyroglobulin levels and negative  $^{131\text{I}}$  whole body scan represent a diagnostic and therapeutic challenge.  $^{99\text{mTc}}$  HYNIC Octreotide recognizes somatostatin receptors that are expressed in thyroid cancer. The objective of this paper is to evaluate these patients by means of  $^{99\text{mTc}}$  HYNIC Octreotide and to correlate the results with other imaging methods.

#### Materials and methods

$^{99\text{mTc}}$  HYNIC Octreotide scintigraphy was performed to 20 patients bearing differentiated thyroid cancer (16 papillary and 4 follicular) postthyroidectomized with raised thyroglobulins (2.5 to 114 ng/ml mean 15.7 ng/ml) and negative whole body scans (18 women and 2 men, age 18–72 years). Studies were performed after obtaining informed consent. Images started 2, 4 and 24 h after the injection of 900 MBq of  $^{99\text{mTc}}$ -HYNIC Octreotide, performing a whole-body scan followed by a SPECT acquisition of the region of interest. A double detector gamma camera equipped with LEHR collimators (Mediso Nucline Spirit DH -V, Hungary) was used. Three-dimensional images were co-registered with CT scans in order to define better the abnormal  $^{99\text{mTc}}$  HYNIC Octreotide uptake using software fusion imaging.

#### Results

$^{99\text{mTc}}$  HYNIC Octreotide allowed us to detect abnormal areas in 17 patients, being 3 negatives. We could correlate these results with computed tomography in 12 of them. We found tomographic alterations that corresponded to the abnormal  $^{99\text{mTc}}$  HYNIC Octreotide uptake in 7. In the remaining 5 we didn't find an anatomic alteration. In all cases, SPECT-CT fusion images allowed a better diagnostic confidence by providing anatomic correlation.

#### Conclusions

These preliminary results highlight the role of  $^{99\text{mTc}}$  HYNIC Octreotide as a potential radiopharmaceutical method that could aid in the diagnosis of these thyroid cancer patients. Also this information can be used to plan radioguided surgery or radionuclide therapy with  $^{177\text{Lu}}$  somatostatin analogues.

#### Conclusions

These preliminary results highlight the role of  $^{99\text{mTc}}$  HYNIC Octreotide as a potential radiopharmaceutical that could aid in the diagnosis of these thyroid cancer patients. Also this information can be used to plan radioguided surgery or radionuclide therapy with  $^{177\text{Lu}}$  somatostatin analogues.

## P462

### A case of calcitonin and CEA negative medullary thyroid carcinoma

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#### Background

The most reliable biochemical markers in the follow-up of medullary thyroid carcinoma (MTC) are serum calcitonin (CAL) and carcinoembryonal antigen (CEA).

#### Case

A 82 years male presented with rapidly growing neck tumour in April 2008. The patient underwent total thyroidectomy with lymph node dissection (June 2008). The pathology revealed MTC localized only in the left lobe (pT3NoMx), the immunohistochemistry showed CAL-, synaptophysin, chromogranin- and p53 positivity. His CEA 1.7 ng/ml and CAL 2.19 pg/ml levels were normal (CEA 05, CAL 3–15). The peak CAL after calcium infusion test was 278 pg/ml (normal <244 pg/ml). January 2009: the postoperative octreoscan and CT showed no recurrent tumour or metastasis. February 2009: CAL (5.3 pg/ml) and CEA 1.7 ng/ml levels were normal. June 2009: acute myocardial infarct (with intraarterial stenting). August 2009: a recurrent tumour presented in the right upper side of the neck with multiple intrapulmonary metastases, but the CAL (7.48 pg/ml) and CEA (1.7 ng/ml) (levels were normal again.). September 2009: severe anemia with occult gastrointestinal haemorrhage presented. Adenocarcinoma of the colon was diagnosed. October 2009: hemicolectomy and neck lymphnode metastases dissection were carried out. Adenocarcinoma of the prostate was also detected. December 2009: a recurrent tumour of the neck was found but despite of the positive metaiodobenzylguanidine (MIBG) scan, the MIBG treatment was unsuccessful. External irradiation of the neck was done, followed by capecitabine (Xeloda) therapy, only with partial and transient response. After almost 3 years of the diagnosis the patient is still in satisfactory condition.

#### Conclusions

The unusual case highlights the importance the detection of possible early metastases despite of normal CAL and CEA levels. Our case confirms that MTC sometimes does not secrete CT or CEA. The disease progression can be delayed with capecitabine and irradiation.

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## P463

### Comparison of clinical and histopathological characteristics of thyroid papillary microcarcinoma and papillary thyroid carcinoma

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Papillary thyroid carcinoma (PTC) is most frequent endocrine cancer on the other hand papillary microcarcinoma can be seen increasing frequency in recent years. In this study we compare clinical and histopathological characteristics of thyroid papillary microcarcinoma and papillary thyroid carcinoma retrospectively.

Eighty one PTC and 38 PMC total 119 patients followed between 2005 and 2009 were enrolled study. Average follow-up time is 35 months in PTC and 36 months in PMC. Age, sex, background, tumour variants, pathological features, surgical procedure and postoperative surgical complications were collected and analysed from archive records.

There were no significant difference between PTC and PMC patients for prognostic factors like capsul invasion, vascular invasion, lymph node metastasis, distant metastasis, bilaterality, multicentricity, age, sex and tumour variants ( $P>0.05$ ). Lymph node metastasis was more frequent in bilateral and multicentric tumours ( $P<0.05$ ).

As a result we found no significant difference between PTC and PMC for poor prognostic factors like capsul invasion, vascular invasion, lymph node metastasis, distant metastasis, bilaterality and multicentricity. According to these findings follow-up and therapy for PMC is processed like PTC.



#### P464

##### **Both Follicular and Papillary neoplasms in a patient with Hashimoto's thyroiditis: complete coincidence?**

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Even though it is unusual, many cases presenting with combinations of thyroid carcinomas have been reported. Concurrent occurrences of medullary – papillary carcinoma, follicular – papillary – medullary carcinoma, follicular – anaplastic – papillary carcinoma, papillary – follicular carcinoma have been presented. The cases reported up to now had no thyroid autoimmunity. We present a case with known Hashimoto's thyroiditis having a combination of papillary and follicular carcinoma.

A 49 year old female patient presented to the Endocrinology department with known type 2 diabetes and hypothyroidism. She was on levothyroxine 50 µg/day and glimepiride 3 mg/day. Physical examination of the thyroid gland revealed suspicious nodules on both lobes and ultrasonography was performed at the Endocrinology department. Multiple nodules were viewed in both lobes coalescent in character with maximum diameters ranging from 15 to 40 mm together with a heterogenous parenchyme compatible with Hashimoto's thyroiditis. Total thyroidectomy was performed. The pathology report revealed a 4.5 cm follicular neoplasm on the right lobe, 1 mm papillary neoplasm on the left lobe and lymphocytic infiltration compatible with Hashimoto's thyroiditis. Surgical boundaries were clean. She received 100 millicuries of radioactive iodide treatment two months after the surgery. Posttreatment scan showed no pathological activity or residual tissue. She is still under levothyroxine suppression therapy and is under remission for two years with normal thyroglobulin levels and negative whole body iodide scans.

Patients with combinations of thyroid carcinomas have been discussed in the literature. Complex genetic mechanisms theories have been proposed like 'stem cell theory', 'divergent differentiation theory', 'field effect theory', 'hostage theory' and 'collision theory'. The last theory states that these events are of complete coincidence. Whether Hashimoto's thyroiditis contributes to our case is of complete controversy. More cases are needed to be able to explain the exact pathogenesis.

#### P465

##### **Association between thyroid autoimmunity and papillary thyroid carcinoma: recent confirmations from a prospective study**

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##### **Introduction**

The association between autoimmune thyroid diseases and papillary thyroid carcinoma (PTC) is still controversial and it is based only on retrospective analyses.

##### **Objective**

Aim of the present research was to evaluate this association in a prospective study of unselected consecutive thyroid nodules (TN) submitted to fine-needle aspiration cytology (FNAC), anti-thyroid autoantibodies (ATA) measurement and histological analyses of surgical samples.

##### **Subjects and methods**

One hundred and ninety-six patients (252 nodules) with TN were studied. 106 patients (142 TN) had undetectable ATA (ATA-), while 90 (110 TN) were ATA positive (ATA+), including 78 (93 TN) with definite autoimmune thyroid disease (AITD) with hyper or hypothyroidism and/or extranodular thyroid hypoechoogenicity on ultrasound. Cytology was classified as benign (TIR 2), indeterminate (TIR 3), suspect (TIR 4) and malignant (TIR 5). Of 61 patients (75 TN) submitted to total thyroidectomy, histological diagnosis and lymphocytic thyroid infiltration (LTI) description were available for the present report.

##### **Results**

A significantly higher prevalence of classes TIR 4–5 (20 vs 9.8%,  $P < 0.05$ ) and lower prevalence of class TIR 2 (59.1 vs 63.4%,  $P < 0.05$ ) were found in ATA+ vs ATA- nodules, respectively. These differences were even more evident when only TN with coexistent AITD were compared to ATA- group (TIR 4–5, 22.6 vs 9.8%,  $P < 0.01$ ; TIR 2, 53.8 vs 63.4%,  $P < 0.01$ ). Histological analysis confirmed a significantly increased prevalence of thyroid cancer, mostly PTC, (68.8 vs 44.2%,  $P < 0.05$ ) in AITD versus ATA- group, while a higher prevalence of LTI was also observed in malignant versus benign nodules (66 vs 34%,  $P < 0.05$ ).

##### **Conclusions**

To our knowledge this is the first prospective study, carried out on a unselected series of consecutive thyroid nodules, confirming the significant association between AITD and PTC, described only in retrospective analyses.

#### P466

##### **Primary malignant thyroid teratoma combined with papillary thyroid carcinoma in a female patient with Hashimoto thyroiditis**

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##### **Introduction**

Primary malignant thyroid teratoma is a rare neoplasm. Even rarer is the concomitance of the mentioned tumor and papillary thyroid cancer.

##### **Case report**

We report a case of a 44-year old woman diagnosed with Hashimoto thyroiditis and multinodular, non-toxic goiter since 2004. The two larger, initially non palpable nodules were located in right thyroid lobe, 16 and 12 mm in diameter respectively. Under suppressive thyroxine therapy the diameter of the two nodules constantly increased (2005–20 and 12 mm, 2006–23 and 12.6 mm, 2007–25 and 15 mm, 2008–30 and 22 mm). The nodules were palpable in physical examination since 2007. Two FNA biopsies of the described nodules on 1/2005 and 12/2006 revealed benign/reactive cytopathology, without any indication of malignancy. Due to continuous nodule enlargement, thyroidectomy was suggested and performed on 12/2008. Histopathological evaluation of the resected thyroid gland (subtotal thyroidectomy) revealed chronic thyroiditis and i) lesion of malignant teratoma (~2.5 cm) with primitive mesenchymal, neuroectodermal and epithelial tissue from grade 1 to grade 3 and ii) lesion (~1 cm) of papillary thyroid carcinoma (collision tumors) of follicular variant cells, evading thyroid capsule. After thorough whole body radiological evaluation, no site of possible primary neoplasm was recognized and the diagnosis of primary malignant thyroid teratoma was set. Three sessions of adjuvant chemotherapy, based on cis-platinum, followed the surgical resection for the stage I teratoma and RAI therapy with 70 mCi <sup>131</sup>I for the stage I papillary thyroid cancer. Further periodic laboratory and radiologic evaluation support disease remission. Two years after subtotal thyroidectomy and 1.5 years after adjuvant therapy completion, there is no sign of regional or distal tumor relapse.

##### **Conclusion**

Concomitant primary malignant thyroid teratoma with papillary thyroid cancer is an extremely rare, if not the unique, case. Early diagnosis is critical for successful therapy. Complex cancer cases like this demand multidisciplinary approach, in order to achieve maximal patient survival.

#### P467

##### **Differences between incidental and preoperatively diagnosed papillary thyroid microcarcinomas. Review of the last decade**

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Increasing incidence of papillary thyroid microcarcinoma (PTMC) is due to accurate histological study of surgical specimens (incidental, INC) or to improved preoperative diagnostic techniques (preoperatively diagnosed, PD). We evaluate the differences between both groups of PTMC from 2000 to 2009.

We reviewed data of patients diagnosed of PTMC (<10 mm) during the last decade with complete follow-up since surgery. Demographic data, tumour size, histological features (variant, extra capsular involvement (ECI), number of foci), lymph node disease (LND), treatment and clinical course were recorded.

Eighty-seven patients included 50 INC and 37 PD; 29 between 2000 and 2004 (18 INC, 62%) and 58 between 2005 and 2009 (32 INC, 55%). Main features are showed:

	Size (mm)	Age (years)	≥ 2 foci (%)	ECl (%)	NX/NO/ N1 LND	High risk <sup>a</sup>	ReQx <sup>b</sup>	I131 (%)	Cure <sup>c</sup>
INC	4.2	52.5	26	14	35/10/5	5/50	12/3	76	43/50
PD	7.7	49.6	38	27	17/9/11	3/37	0/1	100	35/37

<sup>a</sup>High risk – patients with stage ≥ III (pT3 and/or N+ over 45 years).

<sup>b</sup>Reoperation – thyroidectomy completion/compartamental dissection (LND). Two cases of 12 INC, showed new PTMC foci.

<sup>c</sup>Cure, undetectable thyroglobulin (Tg) levels and normal cervical sonography in last evaluation. Seven not cured INC comprised 4 without radioiodine and minimal residual stable Tg levels (3–7 years after surgery), one treated with I131 and stimulated Tg:2.6 ng/ml without foci (9 years after surgery), plus two patients with positive antiTg Ab not radiated. In PD there was one metastatic patient, died of AMI 4 years after surgery, and other with ECl and LND in surgery, with stimulated level of Tg:2.8 ng/ml and normal sonography two years after surgery.

The incidence of PTMC shows an increasing trend in our series. INC tumours occur in older patients, are more frequently unifocal, without ECl and without LND, but they can display a high risk stage and require treatment as PD and larger tumours.

## P468

### A diagnostic value of elastasonographically determined strain index in the differential diagnosis of benign and malignant thyroid nodules

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#### Aim

In this study, we aimed to determine the strain index (SI) as well as the ES scoring of thyroid nodules, and establish the role for these parameters in the differential diagnosis of thyroid nodules using histopathological analysis as a reference standart.

#### Materials and method

Real-time ES in transverse axis (TA) and longitudinal axis (LA) was performed in 391 nodules of 292 patients. ES scoring was made for all the nodules. SI in TA and LA was calculated for 4 times in each nodule and mean values were determined. The results were compared with final histopathological diagnosis.

#### Results

In histopathological examination, 125 (31.97%) of 391 nodules were malignant and 266 (68.03%) were benign. Of these histopathologically benign nodules, 189 (71.05%) were also in probably benign group according to elastasonographic scoring (score 1,2,3), while 77 (28.95%) were in probably malignant group (score 4,5). Among 125 histopathologically malignant nodules, 52 (41.60%) were in probably benign and 73 (58.40%) in probably malignant group according to elastasonographic scoring. There was a significant relation between scoring and histopathological findings ( $\chi^2=36.513$ ;  $P<0.001$ ). Accordingly, sensitivity and specificity of ES scoring were 58.4 and 71.0%, respectively. ROC analysis value obtained for strain ratios in LA (AUC: 75.5%;  $P<0.001$ ) had a higher significance compared to ROC analysis value obtained for strain ratios in TA (AUC:66.0%). Thus, ROC analysis evaluation was applied only for SI in LA. The optimal SI cut-off value in LA for all the nodules was found to be 16.709 (sensitivity: 73.4%, specificity:70.0%) (AUC: 75.4±0.03%; 70.2–80.5%). SI cut-off value corresponding to 90% sensitivity in this axis was 4.516 (specificity: 35.7%).

#### Conclusion

This study indicates that measurement of SI with ES as a non-invasive procedure may be used as an adjunctive method to the conventional methods for the differential diagnosis of thyroid nodules.

## P469

### Diagnostic value of elastasonographically determined strain indexes in differentiated thyroid carcinomas

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#### Aim

We aimed to determine strain index (SI) values using Elastasonography (ES) in histopathologically confirmed differentiated thyroid carcinomas (DTC) and investigate the role of these values for the differential diagnosis of nodular thyroid diseases.

#### Material and method

ES in longitudinal axis (LA) was performed in 391 thyroid nodules of 292 patients. ES scores of thyroid nodules were determined and SI in LA for each nodule was calculated. The findings were compared with histopathological results.

#### Results

Histopathologically, of 391 nodules, 125 (31.97%) were malignant and 266 (68.03%) were benign. Among 125 malignant nodules, 100 were papillary thyroid carcinoma (PTC) (70 classical, 24 follicular variant, 3 solid trabecular variant, 2 columnar variant, 1 oncocytic variant), 15 were follicular carcinoma, 9 were hurthle cell carcinoma and 1 was medullary carcinoma. When we compared SI of 100 nodules with PTC/PTC variants and 266 benign nodules, we found that SI cut-off value for 90% sensitivity (Sn) was 5.885 (AUC 77.6±0.03%;  $P=0.014$ ). In hypoechoic nodules with ES score of 4–5, irregular margins and type 1 vascularization pattern, SI cut-off value for 90% Sn was 17.020 (Sp: 50%, AUC 72.4±0.08%;  $P=0.011$ ). Data of 70 nodules with classical PTC was compared with data of benign nodules and SI cut-off value for 90% Sn was calculated as 7.885 (AUC 79.2±0.03%;  $P<0.001$ ). The optimal SI cut-off value in 30 nodules with variant PTC was 14.785 (Sn:73.3%, sp:66.2%, PPV:19.6%, NPV:95.7%). In these nodules, SI cut-off value for 90% Sn in LA was 4.480 (Sp:35.7%). Since sample sizes of follicular and hurthle cell carcinomas were small, we did not make any analysis for these nodules.

#### Conclusion

SI measurement using ES may be a valuable method with high sensitivity in PTC and PTC variants, however further studies with larger sample sizes are required to conclude about follicular and hurthle cell carcinomas.

## P470

### An uncommon thyroid tumor: hyalinizing trabecular adenoma

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#### Introduction

Hyalinizing trabecular adenomas (HTA) of the thyroid is an unusual disorder. HTA can be present as a thyroid nodule or incidental finding in a thyroidectomy specimen. Most behave as benign neoplasms, but their cytological features can be similar to those of papillary thyroid carcinoma.

#### Case report

A 68 year old woman admitted to our institution with a solitary thyroid nodule. Her serum free thyroxine (fT<sub>4</sub>), free triiodothyronine (fT<sub>3</sub>) and TSH levels are 1.03 ng/dl, 2.79 pg/ml, and 0.364 µIU/ml. Ultrasonography of the thyroid gland showed a 1.7 cm sized, regularly marginated, hypoechoic, calcified thyroid nodule. Fine needle aspiration biopsy of the thyroid nodule revealed suspicious for papillary carcinoma (PTC). Thyroid scan is showed a cold thyroid nodule in the right lobe. Total thyroidectomy were performed. The final pathology was 1.1 cm sized hyalinizing trabecular adenoma. Histologically, the tumor showed tumor cells arranged in trabeculae and a prominent hyaline stroma. The neoplastic cells were focally immunoreactive for thyroglobulin and negative for calcitonin and chromogranin. After surgery L-thyroxine replacement therapy was started. Two years after thyroidectomy, the patient is alive and free of disease.

#### Conclusion

Ultrasonographic features of HTA similar to malign thyroid lesions and frequently misdiagnosed as papillary carcinoma on fine-needle aspiration cytology. The most recent and controversial debate surrounding HTA concerns its potentially malignant behavior and the possible relationship to PTC. Although cases of malignant HTT have been recorded, HTT should be considered a benign neoplasm or, at most, a neoplasm of extremely low malignant potential. In this report we present and discuss an unusual case of a patient who had a hyalinizing trabecular adenoma.

## P471

### Differentiated thyroid cancer: primary tumor size and clinicopathologic features

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#### Introduction

Several recent studies have reported an increase in the incidence of thyroid cancer during the last decades. The delineation between low-risk and high-risk tumor

size is not well defined. The purpose of this study was to evaluate the relationship between the primary tumor size of differentiated thyroid cancer and clinicopathologic data.

#### Methods

We have analyzed all records of all patients submitted to thyroidectomy at our hospital between 2005 and 2010, with the histologic result of differentiated thyroid cancer. We studied 64 case reports considered and considered medical data such as age, gender, tumor size, solitary/multiple nodularity, presence/absence of chronic thyroiditis, of compressive symptoms and of microcalcifications, cytological, histologic and neoplastic stage classification.

#### Results

The studied population (58 females, 8 males) was characterized by a mean age of  $49.2 \pm 15.9$  years old. Mean tumor size was  $2.7 \pm 1.3$  cm; it was significantly associated with the presence of compressive symptoms ( $P=0.011$ ). Solitary tumoral nodules were significantly larger than neoplastic nodules associated with multinodules ( $P=0.037$ ). In addition, the tumor size was significantly smaller in patients with previous malignant cytological result ( $P=0.002$ ). There was a significant association between tumor size and the neoplastic stage of the disease ( $P=0.014$ ).

#### Conclusion

Lesion size of differentiated thyroid cancer influences the occurrence of compressive symptoms. Tumor size of differentiated thyroid cancer is greater in solitary nodules, in patients with previous non malignant cytological result, and in more aggressive stages of the disease.

### P472

#### Incidental papillary thyroid microcarcinoma: a retrospective study

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#### Introduction

Recent data reveal that in the last 25 years it has been a 2.4 fold increase in thyroid cancer, with concomitant rise in thyroid microcarcinoma cases. The main purpose of this study was to analyse the surgical indications that leads to incidentally noted papillary microcarcinoma diagnosis.

#### Methods

We analysed clinicopathologic data of nineteen cases of incidentally found papillary thyroid microcarcinoma, from 1244 thyroid surgeries realized in our institution between 2005 and 2010.

#### Results

The mean age was  $53.8 \pm 9.2$  years old. Female to male ratio was 3:1. Mean tumor size was  $5.7 \pm 3.01$  mm. Hashimoto thyroiditis was detected in 4 cases. Multinodular nontoxic goiter was the endocrinological reason of consultation in 63%. The surgical indication was the increase nodule volume in 43% of the cases, indeterminate cytology report in 21%, nodule diameter  $> 4$  cm in 21%, Graves disease and multinodular toxic goiter in 5% each. Total thyroidectomy was primary realized in 13 cases and completed in 2 with multifocal disease.

#### Conclusions

The main surgical indication behind the incidental papillary microcarcinoma is the increase of nodule volume.

### P473

#### Clinicopathologic significance of BRAF mutation in papillary thyroid carcinoma

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#### Background

Thyroid carcinoma is the most common endocrine malignancy. BRAF (V600E) mutation is the most frequent detected genetic change in papillary thyroid carcinomas (PTC). There are some discrepancies regarding the overall frequency of the mutation, its prevalence in PTC-variants and its relationship with clinicopathological parameters of poor outcome.

#### Aims

To investigate the relationship of clinicopathological features of PTC with BRAF mutation with particular reference to histological subtype.

#### Patients and methods

We analysed 97 patients (77.3% female, mean follow up  $33.9 \pm 26.7$  months) with pathological diagnosis of PTC. DNA was extracted from tissues blocks or fresh

tissue and BRAF (V600E) mutations were detected by PCR and sequencing. Univariate ( $\chi^2$  and Student's *t* test) and multivariate analysis were realised.

#### Results

BRAF (V600E) was detected in 45.4% of the study group. The prevalence of mutation was found in 54.4% of classical PTC, 22.2% of follicular variant and 30.8% of microcarcinomas ( $P < 0.05$ ).

BRAF (V600E) mutation was associated with male sex ( $P < 0.05$ ), extrathyroidal extension ( $P < 0.05$ ) and with tumours of greater size or locally invasive (T1 + T2 versus T3) ( $P < 0.05$ ). There were no associations with age, tumour size, multicentricity or lymph node metastasis. BRAF (V600E) was negatively associated with the presence of distant metastasis in our group ( $P < 0.05$ ). By multivariate analysis, there was not any parameter independently associated with the presence of BRAF (V600E) mutation.

#### Conclusions

Our data suggest that BRAF (V600E) mutation is associated with some histological subtypes of PTC and with cancers with locally invasive growth. The small number of cases in this report could explain the insufficient statistical significance in multivariate analysis.

### P474

#### Diagnostic performance and concordance analysis between ultrasonographic findings suggestive of malignancy and fine-needle aspiration (FNA) in eufunctional thyroid nodule

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#### Introduction

In population, thyroid nodule incidence is 1 to 30%; malignancy incidence is 1–20% up to different series, which make us to realize diagnostic methods with variable sensibility and specificity, taking as point of reference, the cytological analysis.

#### Materials and methods

Fifty consecutive patients (44 women, 6 men) with thyroid nodule, performed neck ultrasonography (ALOKA SSD3500 equipment); material of FNA was processed according to international protocols (Koss-Papanicolaou), the evaluated ultrasonographic findings were: solitary nodule, microcalcifications, solid structure, hyperechogenicity, irregular margins, perinodular halo and intranodular vascular spots; it was analyzed Sensibility (S), Specificity (E), Predictive Values (PPV, NPV), Likelihood Ratio (LR+) and concordance (kappa index), with cytological report as gold standard which was categorized as positive or negative for malignancy, for the samples reported as 'unsatisfactory or undetermined', the FNA was performed until the result was positive or negative (3 × maximum).

#### Results

The mayor performance for malignancy diagnostic was the presence of microcalcifications, S: 85.7% (95% CI: 42.01–99.25); E: 91.7% (95% CI: 71.53–98.54%); PPV: 75%; NPV: 95.7%; LR(+): 10.29%, kappa: 0.737. The presence of solitary nodule showed S: 100%; E: 66.67% (95% CI: 44.69–83.57%), NPV: 100%; PPV: 46.7% (95% CI: 22.28–72.58%); LR(+): 3.0; kappa: 0.475. When unite this ultrasonographic variables: uninodular, solid structure, hyperechogenic, microcalcifications, irregular margins, perinodular halo, intranodular vascular spots, all together show S: 100%, E: 70.83% (95% CI: 48.75–86.56%); PPV: 50% (95% CI: 24.04–75.96%), NPV: 100%, LR(+): 3.43, kappa: 0.737.

#### Conclusion

Ultrasonographic findings such as microcalcifications and solitary nodule have the most diagnostic performance for malignancy in thyroid nodule, however with other findings like solid structure, hyperechogenic, irregular margins, perinodular halo, intranodular vascular spots, all together increase the diagnostic performance for malignancy in patients with thyroid nodules.

### P475

#### Prevalence and features of incidental thyroid carcinoma in 1955 patients surgically treated for benign lesions

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Incidental thyroid carcinoma (IC) is defined as a tumor diagnosed at histology after surgery for benign diseases and without a preoperative diagnosis of

malignancy. Since the prevalence of IC reported in the literature is extremely variable (4.6–17%), mostly due to the limited number of cases, we aimed to study the features of IC in our large series. We retrospectively evaluated 1955 patients who consecutively underwent total thyroidectomy for non malignant diseases from 1998 to 2010 in our Endocrine Surgery Centre. The pre-surgical diagnoses were of uni-multinodular goiter, toxic adenoma or Graves' disease. Moreover, 211 patients had a preoperative cytological diagnosis of micro-follicular lesion. Overall, an IC was found in 251 (12.8%) patients, and the diagnosis was of papillary or follicular carcinoma in 90 and 9% of cases, respectively. Poorly differentiated carcinoma or lymphoma were found in two patients. The prevalence was higher in patients with a preoperative diagnosis of micro-follicular lesion (64/211, 30%) and toxic adenoma (4/17, 23%), compared to patients with Graves' disease (25/258, 9.7%), with uni- or multinodular non-toxic goiter (142/123, 11.5%) or with toxic multinodular goiter (16/231, 7%). The TNM staging was pT1NX-N0 in 75% of cases (81% were microcarcinomas  $\leq 1$  cm) and pT2NX-N0 in 6.8% of patients. An extra-thyroidal invasion (pT3-T4NX-N0) was found in 10.7% of tumors, and lymph node metastases (pT1-T4N1) were diagnosed in 6.3% of patients. In conclusion, in a large series of patients submitted to thyroidectomy for benign diseases, a high prevalence (12.8%) of IC was found. Although most of the tumors were small and intra-thyroidal, a significant proportion of carcinomas showed extra-thyroidal invasion or lymph node involvement. Thus, present data indicate the need to perform an accurate preoperative evaluation in patients with benign diseases regardless of the association with thyroid autoimmunity or hyperfunction.

#### P476

##### Failure to suppress TSH level in a thyroid cancer patient; a case report

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TSH suppression therapy with thyroid hormone is a mainstay of thyroid cancer treatment. Effectiveness of thyroid hormone therapy may be disrupted by nonadherence of the patient, interfering drugs, foods and intestinal disorders. We here present a case of papillary thyroid cancer with unsuccessful suppression therapy.

##### Case

A 38 year old woman, underwent total thyroidectomy for euthyroid multinodular goitre, with a pathological diagnosis of papillary thyroid carcinoma was referred to our clinic for further evaluation. She received radioiodine ablative treatment and was started TSH suppression therapy. Her past medical and family history were unremarkable. On physical examination, a blood pressure of 100/60 mmHg, heart rate of 72 beats/min, thyroidectomy scar, normal reflexes were recorded. Laboratory evaluation revealed; hemoglobin:11.6 g/dl (12–16), MCV:79.9 FL (80–95), calcium:8.8 mg/dl (8.4–10.2), albumin: 4.6 g/l (3.5–5), TSH: >75 IU/ml (0.4–5), free T4: 0.6 ng/dl (0.8–1.9), anti-thyroid peroxidase antibody: <10 IU/ml (0–50), anti-thyroglobulin antibody:<20 IU/ml (0–50). During follow up, the optimum TSH suppression could not be achieved despite the progressive increments in thyroid hormone dose (Levothyroxine sodium, 350 µ/day; levotriiodothyronine sodium, 150 µ/day). She had no compliance problems and told about mild diarrheal episodes when asked for malabsorptive symptoms. Antidromysium and anti gliadin antibodies were negative. Endoscopic biopsy of the small intestine revealed intraepithelial lymphocytes and focal villous atrophy characteristic for celiac disease. Gluten free diet was started, her diarrheal episodes were relieved and her TSH level fell to target suppression levels.

##### Conclusion

Oral thyroid hormone treatment is a relatively vulnerable therapy which is easily affected by ingested substances or medications. In a patient with unsuccessful thyroid hormone replacement or suppression therapy, with no adherence problems and interfering substances or drugs, malabsorption should come into mind and be evaluated.

#### P477

##### Long term follow-up and outcome in thyroid papillary microcarcinoma

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##### Background and patients

The clinical significance and management of papillary thyroid microcarcinoma (PTMC) is widely debated. The objective of this study was to assess the clinical presentation, tumor characteristics and the outcome of the disease in patients with PTMC. In this retrospective study we evaluated 286 patients who were followed for a minimum of three years.

##### Results

Of the 286 patients with a mean age 48.9 years, 226 (79%) were females. Two hundred and sixty one patients (91.2%) underwent a near total and 25 patients an incomplete thyroidectomy. PTMC was discovered incidentally in 248 (86.7%) patients undergoing surgery for other pathology not related to thyroid malignancy, while 38 patients were operated for suspicious FNAB or positive lymph nodes. Unifocal disease was detected in 182 (63.6%) patients; with 92 having a focus of 1–5 mm and the remaining a focus of 6–10 mm. Multifocal disease was detected in 104 (36.3%) patients. From these patients, none had distant metastases; however, loco-regional extension and lymph-node involvement were detected in 13 and 29 patients respectively. Two hundred twenty six (79%) patients underwent adjuvant radioiodine ablation for worrisome histologic subtypes, thyroid capsule invasion, multifocality or thyroid remnants. The mean follow-up was 8.19 years. Disease recurrence was observed in only one patient (with lobectomy and radioiodine ablation), 20 years after the initial diagnosis. Six patients had persistent disease during the follow-up period, all of whom had undergone near-total thyroidectomy and remnant ablation. The remaining 279 (97.5%) patients were disease free.

##### Conclusions

PTMC appears to have a benign course in the majority of patients initially treated with near-total thyroidectomy and therefore, according to the revised ATA thyroid cancer guidelines, additional radioiodine ablation is not justified. However, an aggressive subtype of PTMC seems to exist. More prospective studies or probably molecular markers are needed, in order to identify this subtype.

#### P478

##### Incidentally discovered thyroid carcinomas. An experience of a centre through 18 years

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Incidental thyroid carcinoma includes thyroid carcinomas detected after surgery in patients whose thyroid has been correctly studied with no data of malignancy. Lately, the widely spread use of image techniques and thorough search of thyroid nodule complains, have lead to a growing number of thyroid carcinomas. But still there are some of these incidentally discovered after thyroidectomy.

We report 98 cases of thyroid carcinomas incidentally found from a series of 360 thyroid carcinomas (27.2%), diagnosed since 1992. Demographic data, thyroidectomy criteria, histopathology, tumour size, multicentric cases, extension, radioiodine treatment and follow up data were collected.

Our results show a mainly female cohort (81.6%), with ages ranging from 21 to 81 years (mean 51.29 and median 31). First 3 cases detected in 1994 and ever since the number has been increasing (14 cases in 2008, 11 in 2009 and 11 in 2010). The criteria for thyroidectomy was nodule size with benign cytology (87.7%), associated hyperparathyroidism (6.2%) and hyperthyroidism (3%). Histopathology result was Papillary thyroid carcinomas (PTC) in 96%. Mean tumour size was 13.5 mm and median of 7 mm, ranging from 1 to 75 mm. Multicentric cases were found in 27.5%. Extra thyroid extension was recorded in 15.3% cases. Radioiodine treatment (<sup>131</sup>I) was used in 80.8% of PTC. During the years of follow up there has been only one death.

Incidental tumours are more common in women of young mean age. PTC of small size, not multicentric and not extended out of the thyroid is the main outcome after thyroidectomy. Most of them underwent <sup>131</sup>I treatment. Due to the described features the general prognosis of these cases is good. Careful histopathologic analysis and standard treatment and follow up is our basic approach. Probably <sup>131</sup>I shows overused in our series since retrospectively it was more used in the past.

#### P479

##### Evaluation of safety and tolerability of calcitonin stimulation using high-dose calcium in patients with medullary thyroid carcinoma (MTC)

Aldona Kowalska, Iwona Palyga, Danuta Gasior-Periczak, Anna Sluszniaik & Stanislaw Gozdz  
Holycross Cancer Centre, Kielce, Poland.

The pentagastrin-stimulated calcitonin test is known and widely used in monitoring patients with MTC. Given the problems of pentagastrin availability other diagnostic tests are being tried.

The goal of the study was the evaluation of safety and tolerance of high-dose calcium to stimulate calcitonin in MTC patients.

##### Materials

The study included 41 patients after surgery for medullary thyroid cancer who were under observation. Patients underwent calcitonin stimulation using high-dose calcium while observing tolerance and undesired side effects. Six months earlier the same patients underwent calcitonin stimulation using pentagastrin. Patients compared the tolerability of the two tests.

##### Results

Undesired side effects occurred in 37 (90%) of 41 patients. The most frequently reported side effects by patients were: feeling heat 35/37, metallic taste 10/37, flushing of face 8/37, sweating 5/37, nausea 5/37, facial paresthesia 4/37, dizziness 1/37, and weakness 1/37. Symptoms occurs in the first or second minute of administering of calcium. Symptoms resolved after 1 to 5 min until the completion of injection. Patients assessment comparing tests using pentagastrin and calcium rated the calcium test: more tolerable 30/41, equally tolerated 10/41, worse tolerated 1/41.

##### Conclusions

The calcitonin stimulation test using high-dose calcium is safe and well tolerated. Although side effects occurred frequently (90% of patients), most were mild and transient.

#### P480

##### Comparison of calcitonin stimulation tests with pentagastrin (Pg) and high-dose calcium in patients with medullary thyroid carcinoma (MTC)

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##### Introduction

The Pg calcitonin stimulation test is widely used in monitoring patients with MTC. In view of accessibility problems of Pg it is necessary to use high doses of calcium as the calcitonin stimulant.

##### Objective

The aim of the study was the comparison of calcitonin concentrations after Pg and calcium stimulation, respectively.

##### Materials

The study was performed in 31 patients under observation after deemed surgically cured of MTC.

##### Method

In patients calcitonin secretion stimulation tests were performed first with Pg, and after 6 months with high-dose calcium. Recorded data were analyzed.

##### Results

i) Baseline calcitonin concentration using Pg was 6,187 ( $\pm$ 1,0468), and using calcium was 6,389 ( $\pm$ 1,3148).

ii) Pg stimulated calcitonin concentration

- Mean calcitonin concentration after 3 min was 7,217 ( $\pm$ 3,2741).
- Mean calcitonin concentration after 5 min was 6,992 ( $\pm$ 2,4282). No statistically significant increase in calcitonin concentration was found after Pg.

iii) Calcium stimulated calcitonin concentration

- Mean calcitonin concentration after 3 min was 9,247 ( $\pm$ 10,4396).
- Mean calcitonin concentration after 5 min was 8,122 ( $\pm$ 7,7745). No statistically significant increase in calcitonin concentration was found after calcium.

iv) Comparison of calcitonin concentrations stimulated with Pg and calcium. No statistically significant difference was observed at 3 and 5 min between Pg and calcium stimulation of calcitonin.

##### Conclusions

Calcitonin stimulation tests with Pg and calcium were comparable in patients with deemed surgically cured MTC.

#### P481

##### The value of CK19, Ki-67 and p53 expression in the diagnosis of thyroid follicular neoplasms

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##### Introduction

Cytokeratin-19 (CK19), p53 and Ki-67 are some of the proposed markers for differentiating benign from malignant thyroid tumors.

##### Material and method

We evaluated the tissue expression of the three markers in 60 thyroid lesions including: 8 cases of nodular hyperplasia (NH), 16 follicular adenomas (FA), 26 papillary carcinomas (PC) and 10 follicular carcinomas (FC). Tissue sections fixed in formalin and embedded in paraffin were immunohistochemically (IHC) stained (LSAB technique, visualization with DAB) using monoclonal antibodies anti-CK19 (clone RCK 108), anti-Ki67 (clone MIB-1) and anti-p53 (clone DO-7); the data were statistically analyzed using the unpaired *T*-test.

##### Results

CK19 was significantly expressed only in PC  $\geq$  20 ( $P < 0.001$ ). For p53 and Ki-67 the results of statistical analysis were not significant for the compared pairs.

##### Conclusions

CK19 is useful in differentiating the follicular variant of PC from FC and PC from the papillary aspects in NH. Immunoreactivity of p53 and Ki-67 was not proven to be useful in differentiating PC from NH, the results being statistically insignificant.

#### P482

##### Does preoperative fine-needle aspiration decrease the thyroidectomy rates?

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##### Introduction

Fine-needle aspiration (FNA) biopsy of thyroid nodules is minimally invasive and safe. The clinical importance of the thyroid nodule evaluation is primarily related to the need to exclude thyroid cancer. We aimed to investigate the relationship between preoperative FNA cytology and results of thyroidectomy in this study.

##### Materials and Methods

Histopathologic examination reports of 302 patients undergoing thyroidectomy and preoperative FNA cytological reports of 125 patients were included in the study. The results of FNAs were classified as benign, suspicious, malignant and inadequate, postoperative histopathologic results were classified as benign or malignant. Suspicious and malignant cytology were evaluated in same group in terms of surgical indication. The results were compared in patients with and without preoperative FNA.

##### Results

FNA cytology results were found benign 55.2%, suspicious 19.2%, malignant 10.4% and inadequate 15.2%. After the removal inadequate group, malignant-suspicious and benign results were 34.9 and 65.1% respectively. Malign disease frequencies was 24.6 and 17.5% for patients with and without preoperative FNA. Benign and malignant disease rates were not statistically significant in both groups. Malignant disease was found 84.6% of patients with malignant cytology, those with suspicious in 37.5 and 8.7% of benign ones. Malignant disease rates was observed 54 and 17.5% as a result of suspicious/malignant cytology and without FNA patients respectively ( $P < 0.01$ ).

##### Conclusion

The main surgical indication of thyroid nodules is evidence or suspicion of malignancy. We concluded that preoperative FNA significantly reduced (82.5 and 46%) operations performed for benign disease, when patients with malign or suspicious cytology compared to without FNA group. We think that, FNA decreases the risk of unnecessary surgery when performed to determine the indications for thyroidectomy.

**P483****Malignancy rate is not different in dominant nodule and non-dominant nodule by fine needle thyroid aspiration**Omer Yazgan<sup>1</sup>, Fahri Halid Besir<sup>1</sup>, Yusuf Aydın<sup>2</sup>, Sibel Yazgan<sup>1</sup>, Melih Engin Erkan<sup>3</sup>, Burhan Yazici<sup>1</sup>, Ramazan Büyükkaya<sup>1</sup> & Omer Onbas<sup>1</sup><sup>1</sup>Faculty of Medicine, Radiodiagnostic Department, Düzce University, Duzce, Turkey; <sup>2</sup>Faculty of Medicine, Endocrinology and Metabolism Department, Duzce University, Duzce, Turkey, <sup>3</sup>Faculty of Medicine, Nuclear Medicine Department, Duzce University, Duzce, Turkey.**Objective**

Thyroid fine-needle aspiration biopsy (FNAB) is the golden standard for approaching thyroid nodule's malignancy. It is recommended to carry out an FNAB from the dominant nodule in multi nodular goiter. In our study, we compared the FNAB results that we carried out from dominant nodule and any other nodule which have features that may be malignant to US. Besides, the relationship between the results of cytology and US features of nodules has been analyzed.

**Material and methods**

One hundred and ninety-seven cases diagnosed MNG were analyzed between 2009–2010. These cases followed by applying to endocrinology polyclinic were carried out FNAB from both dominant nodule and non-dominant nodule. Whole cystic nodules, nodules with peripheral calcification and nodules that its FNAB result has poor material were not used in the study and 171 cases were used. 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**

While malignancy rate was found 1.8% in dominant nodules, in non-dominant nodules malignancy rate was found 0.6% (total malignancy rate: 2.4%) and statistically it was not observed significant difference ( $P=0.53$ ). In the cytological analyze of non-dominant nodules, malignancy rate was found significantly high ( $P < 0.001$ ) which those including micro calcification. In nodules not having peripheral halo, malignancy rate was also found significantly high ( $P=0.048$ ). It was not determined statistically significant relation between malignancy and, hypo echoic pattern, solid structure as US features of nodules.

**Conclusions**

According to our data, the last UICC/TNM classification did not provide better prognostic information for low and intermediate risk patients, however further studies with a longer follow-up and a larger number of patients are needed to confirm these findings.

**P485****Very low-risk papillary thyroid carcinoma: where to set the limits?**

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Currently there is no widely accepted definition of very low-risk papillary thyroid carcinoma (PTC). Although unifocal microcarcinomas without extrathyroidal extension and no local or distant metastases have been recognized as having an excellent overall prognosis the threshold for such a favourable clinical behaviour remains controversial. The study aim was to identify the clinicopathological characteristics of T1 tumours ( $\leq 2$  cm) presenting a very low-risk of mortality or recurrence. We retrospectively reviewed the medical data of 258 patients with PTC presenting the following characteristics: T1N0M0 disease, no history of head-neck irradiation, all macroscopic tumour had been resected and the tumour did not have aggressive histological subtype. Periodic follow-up included clinical and ultrasound examination and serum Tg, TSH and antithyroglobulin antibodies measurements. Other imaging modalities were used as required. There were 226 (87.6%) female patients. Mean age at diagnosis was 48.0 years (range, 22–79 years). One hundred ten patients were classified as T1b. The majority of the patients (93.4%) were treated with total or near-total thyroidectomy. Forty-nine patients (18.9%) underwent a variety of cervical lymph node dissections. Radioiodine ablation therapy was administered to 111 patients (43.0%). Patients were followed for a median of 8.2 years after thyroidectomy. Residual/recurrent disease in regional lymph nodes was identified in six patients (2.3%). There was no disease-specific mortality and none of the patients developed distant metastases during follow-up. All patients were considered free of disease at the last follow-up. This study shows that it is possible with a simple set of criteria to accurately identify a subgroup of T1N0M0 PTC patients with very low-risk of recurrence. Our results may suggest that these very low-risk patients may be effectively treated with a conservative management strategy in terms of surgical treatment (extent of thyroidectomy and prophylactic lymph node dissections), radioiodine ablation therapy and levothyroxine therapy.

**P484****Seventh UICC/TNM staging criteria for medullary thyroid carcinoma: is there any prognostic advantage?**M Martinho<sup>1</sup>, T Azevedo<sup>1</sup>, F Carrilho<sup>2</sup>, T Martins<sup>1</sup>, C Ribeiro<sup>2</sup>, M Melo<sup>2</sup>, N Cunha<sup>1</sup>, M J Rascão<sup>1</sup>, A Neves<sup>1</sup>, P Gilde<sup>1</sup>, J L Pires<sup>1</sup>, C Cruz<sup>1</sup>, C Oliveira<sup>1</sup>, J Neto<sup>1</sup>, S Oliveira<sup>1</sup>, F Valido<sup>1</sup>, I Gomes<sup>1</sup>, M Carvalho<sup>2</sup> & F Rodrigues<sup>1</sup><sup>1</sup>Instituto Português de Oncologia de Coimbra FG EPE, Coimbra, Portugal; <sup>2</sup>Hospitais da Universidade de Coimbra EPE, Coimbra, Portugal.**Background and aims**

The value of the revised (2009) UICC/TNM criteria for medullary thyroid cancer (MTC) is not established. We aimed to evaluate its predictive value, analyzing the consequences of the inclusion of T3N0M0 patients previously included in stage III of the 6th edition, in stage II of the 7th.

**Patients and methods**

We retrospectively analysed the clinical records of 85 patients operated for MTC and followed in two tertiary hospitals in the central region of Portugal between 1978 and 2009; staging was done according to both the 6th and 7th UICC/TNM criteria.

**Results**

Mean age at first operation was  $52.1 \pm$  years (range: 6–86 years); 52 patients (61.2%) were female. Fourteen patients (16.5%) were MEN2A carriers and 2 were MEN2B (2.4%). Mean follow-up time was 5.9 years. There were 26 (30.6%), 11 (12.9%), 17 (20%) and 31 (36.5%) patients in 6th edition stage I, II, III and IV, and 26 (30.6%), 22 (25.9%), 6 (7.1%), in 7th stages I, II, III and IV, respectively. Eleven patients (12.9%) were reclassified according to the 7th edition, changing from stage III to stage II. Five-year survival for stage I, II, III and IV according to the 7th was 100, 87, 100 and 52%, respectively. The two patients who died in stage II would have been classified as stage III according to the 6th edition. Five-year survival rate in the new classification for stage II was 100 and 86% for stage III.

**P486****mTOR inhibition hampers cell viability in selected human medullary thyroid carcinoma primary cultures**C Filieri<sup>1</sup>, M C Zatelli<sup>1</sup>, M Minoia<sup>1</sup>, F Tagliati<sup>1</sup>, M Buratto<sup>1</sup>, M R Ambrosio<sup>1</sup>, M Pelizzo<sup>2</sup> & E C degli Uberti<sup>1</sup><sup>1</sup>Section of Endocrinology, Department of Biomedical Sciences and Advanced Therapies, University of Ferrara, Ferrara, Italy; <sup>2</sup>Department of Medical and Surgical Science, General Surgery III, University of Padova, Padova, Italy.

It has been demonstrated that everolimus, an mTOR inhibitor, has potent anti-proliferative effect in a human Medullary Thyroid Carcinoma (MTC) cell line, TT, and in two human MTC primary cultures. We aimed at evaluating the possible antiproliferative effects of everolimus in a group of 20 human MTC in primary culture. To this purpose, 20 MTCs were dispersed in primary cultures, treated without or with 1 nM – 1  $\mu$ M everolimus, 10 nM SOM230, and/or 50 nM IGF1. Cell viability and apoptosis were evaluated after 48 h and Calcitonin (CT) secretion was assessed after a 8 h incubation. Somatostatin receptor expression was investigated by quantitative PCR. We found that in 14 cultures everolimus reduced cell viability (~40%), promoted apoptosis (+30%), inhibited p70S6K activity (~20%) and blocked IGF1 proliferative and anti-apoptotic effects. In selected tissues co-treatment with SOM230 had additive effects. It did not affect CT secretion, but blocked the stimulatory effects of IGF1. In conclusion, everolimus reduced MTCs cell viability by inducing apoptosis, with a mechanism likely involving IGF1 signalling but not CT secretion, suggesting that it might represent a possible medical treatment for persistent/recurrent MTCs.

#### P487

##### Synchronous thyroid and gastric diffuse large B cell lymphoma

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##### Introduction

Thyroid lymphoma is a rare disease. Furthermore, coincidence of extrathyroidal lymphoma is very rare.

##### Case

A 69 years-old woman patient was admitted to the hospital with dyspnea, hoarse voice and swelling of throat for a month. In her history, she had been diagnosed as gastric lymphoma 6 years ago, while she was being investigated for upper gastrointestinal bleeding. Six cures of chemotherapy has been applied. She was not on follow up. In physical examination, she looked cachectic and dyspneic. Thyroid was enlarged diffusely and multiple nodules were palpated. Trachea was shifted and pressed by the thyroid. Laboratory tests were as follows: Hgb: 10.1 g/dl, TSH: 0.8  $\mu$ IU/ml (0.6–4.8), fT<sub>4</sub>: 1.1 ng/dl (0.74–1.52), fT<sub>3</sub>: 1.3 pg/ml (2.3–4.2), anti-TPO: 133 U/ml (0–57), anti-T: 130 U/ml (0–64), TG: 155 (1.6–59.9) ng/ml. Thyroid ultrasonography revealed chronic thyroiditis and multinodular goitre. FNA biopsy was performed to the nodules and diffuse large B cell lymphoma (CD 20 was positive, CD3 and CD30 were negative) was detected. Also, diffuse large B cell lymphoma was revealed at the endoscopic gastric biopsy specimen, too (CD 20 and bcl-6 were positive and Ki-67 proliferation index was 99%). At radiological evaluation for the staging of disease, subcarinal and paratracheal lymphadenopathies and multiple nodules in each of the lungs were detected. Because her performans status was poor, bronchoscopy could not be applied. After several days, her performance status worsened and she died.

##### Conclusion

In this case report, we purposed to remind that thyroid nodules which were developed on the base of chronic thyroiditis should be immediately evaluated for lymphoma, especially in cases which gastric or another organ lymphoma had been detected synchronously.

#### P488

##### Radiodine therapy and age at menopause in women with differentiated thyroid cancer

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##### Introduction

Radiodine (<sup>131</sup>I) is widely used in the treatment of patients with thyroid cancer. This therapy is associated with some adverse effects including possible impairment of ovarian function and earlier menopause.

##### Objective

The aim of this study was to evaluate female fertility after treatment with <sup>131</sup>I of differentiated thyroid cancer (DTC).

##### Methods

We retrospectively analysed 250 women with DTC treated with <sup>131</sup>I. We selected those who had received the first <sup>131</sup>I before or at the age of 45 and who still presented regular menses on this occasion. Hysterectomized patients and those submitted to external pelvic radiotherapy or chemotherapy before menopause were excluded. Only 33 women filled these criteria. The control group consist of 64 women with multinodular goiter (MNG) in whom we applied the same criteria. Age at menopause was defined by the absence of menses for 6 months. We also investigated smoking habit and thyroid autoimmunity. The t student, linear regression and Kruskal-Wallis test were used for statistical analysis. A *P* value of <0.05 was considered significant.

##### Results

The age at menopause was 49.3 ± 3.8 years in the group of women with DTC and 49.41 ± 3.8 years in the group with MNG (*P*=ns). We didn't find any difference between the age at menopause and the number of therapies. We found a marginally significant correlation (*R*<sup>2</sup>=0.116; *P*=0.05) between the cumulative dose of <sup>131</sup>I and the age at menopause.

##### Conclusion

In our study the age at menopause was similar between the 2 groups. We verified a correlation between the cumulative dose of <sup>131</sup>I and age at menopause: higher doses were associated with earlier menopause.

#### P489

##### Value of sonographic index point and resistivity index in the diagnosis of the malignant potential of nodular thyroid disease

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##### Objective

To assess the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of sonographic index point and resistivity index in predicting the risk for malignancy in thyroid nodules.

##### Methods

Fifty consecutive patients undergoing thyroid ultrasound for clinical nodular thyroid diseases prior to planned surgery were recruited for the study. Sonographic index point was calculated for each nodule using morphological characteristics (margins, shape, echogenicity, echostructure and nature of calcification) as a score. Spectral analysis was performed from the region showing maximum colour flow on 1 to 3 arteries of greater calibre in each nodule. Resistivity index (R.I) was recorded as an average of indices obtained. Sensitivity, specificity, PPV and NPV was obtained for sonographic index points and resistivity index for their ability to differentiate benign form malignant nodule using the post-operative histopathology report as the gold standard.

##### Results

There were 16 (5M/11F) malignant lesions and 34 benign lesions (4M/34F) among the study patients. The sonographic index points for malignant nodules were in the range of 3–8 and for benign lesions 1–7. The sonographic index point at a cut off value of > 2 had a sensitivity of 93.7%, specificity of 75.3%, PPV of 15.7% and NPV of 98.4% in differentiating malignant from benign nodule. Using resistivity index at a cut off value of 0.75 showed sensitivity of 81.5%, specificity 92.3%, PPV 95.6% and NPV of 70.5%. A combination of both the indices had a sensitivity of 89.2%, specificity of 92.3%, PPV of 97%, NPV of 75% and diagnostic accuracy of 90%.

##### Conclusions

Sonographic index point had high sensitivity and with a high negative predictive value for diagnosing malignant nodules despite poor specificity. In combination with the Resistivity index there was improvement in the specificity with some loss of sensitivity but with a diagnostic accuracy of over ninety percent.

#### P490

##### Retrospective analysis of predictive factors for recurrence during long term follow-up in papillary thyroid cancer patients who had remission after primary treatment

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The aim of our study is to detect parameters which may predict recurrences during long-term follow-up in papillary thyroid cancer patients who achieved remission after primary treatment. The data of 492 patients who achieved remission criteria after primary treatment were recorded. Remission criteria were for anti-thyroglobulin (antiTg)-negative patients: no uptake in first iodine-131 whole body scan (WBS) after primary treatment and stimulated thyroglobulin (Tg) level < 2 ng/ml when TSH > 30  $\mu$ U/ml. Recurrence was detected in 12 of 492 patients (2.4%). Tumor size, stimulated Tg concentrations and frequency of antiTg positivity at first WBS and duration of follow up were significantly higher in patients with recurrence compared with remaining patients (*P*: 0.024, 0.013, 0.01, 0.002 respectively). Patients with stimulated Tg  $\geq$  0.25 ng/ml at first WBS had significantly higher frequency of recurrence compared with patients having Tg < 0.25 (*P*: 0.009). Cox regression and Kaplan-Meier survival analyses indicated that lymph node metastasis at initial presentation was the only parameter significantly related to recurrences during follow-up (*P*: 0.011, 0.01 respectively). We conclude that patients with lymph node metastases at presentation and relatively higher (> 0.25 ng/ml) Tg and anti-Tg positivity at first WBS have higher risk for recurrence.

**P491****Factors connected with the female sex – association with differentiated thyroid cancer**

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Differentiated thyroid cancer (DTC) is more frequent in women than in men. The aim of the study was to analyze whether factors connected with the female sex could increase the risk of DTC.

**Material and methods**

Ninety nine patients with a mean age  $\pm$  s.d. of  $40.5 \pm 5.9$  years with DTC and 51 healthy women with a mean age of  $36.52 \pm 8.3$  years were examined. Gynecological and obstetric histories were taken and serum estradiol and progesterone levels were analyzed in all women.

**Results**

Patients with DTC had more frequent menstrual cycle disturbances, used hormone-containing medicines more frequently, were multiparous more frequently, had spontaneous miscarriages more frequently, and their duration of lactation was significantly shorter than in controls. The mean serum estradiol level  $\pm$  s.d. in women with DTC was significantly higher than in the controls, in the follicular phase  $193.74 \pm 66.31$  vs  $157.63 \pm 42.88$  pmol/l and in the luteal phase  $519 \pm 176.9$  vs  $369 \pm 71.49$  pmol/l. The mean serum progesterone level  $\pm$  s.d. was higher in the controls than in DTC patients in the follicular phase:  $2.11 \pm 0.70$  vs  $1.38 \pm 0.56$  nmol/l, in the luteal phase  $20.95 \pm 17.46$  vs  $17.31 \pm 12.28$  nmol/l.

**Conclusions**

The results of these studies imply that multiparity and gynecological or obstetric disorders connected with excessive activity in endogenous or exogenous estrogens were more frequently observed in patients with differentiated thyroid cancer than in healthy ones. The female sex hormones probably intensify the actions of other carcinogens as well.

**Bone/calcium/Vitamin D****P492****Does vitamin D replacement therapy improve insulin sensitivity in elderly?**Ilkin Naharci<sup>1</sup>, Nuri Karadurmus<sup>2</sup>, Ergun Bozoglu<sup>1</sup>, Turker Turker<sup>3</sup>, Huseyin Doruk<sup>1</sup> & Muhittin Serdar<sup>4</sup>

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**Introduction**

Vitamin D has a potentially beneficial role in insulin resistance, but there is a lack of evidence in elderly. We report preliminary results of an ongoing study that investigated the effect vitamin D replacement treatment (12 weeks) on insulin resistance and metabolic parameters in elderly people with normal glucose tolerance.

**Methods/design**

The study's eligibility criteria were body mass index  $< 30.0$  kg/m<sup>2</sup>, creatinine levels  $< 1.2$  mg/dl, no evidence of prediabetes and diabetes, no self-reported use of hypoglycemic drugs, and fasting serum glucose  $< 100$  mg/dl. Eighty-six participants (aged  $\geq 65$  years) were eligible for the present investigation. Serum concentrations of 25(OH) D were categorized as deficient (group I,  $< 20$  ng/ml), insufficient (group II, 20 to 30 ng/ml), or normal (group III,  $> 30$  ng/ml) vitamin status, respectively. Group I was treated initially with 300 000 international units (IU) of vitamin D<sub>3</sub> ampoule orally once for six weeks, and then 880 IU of vitamin D<sub>3</sub> plus 1000 mg of ionized calcium tablet daily thereafter. Group II was treated with 880 IU of vitamin D<sub>3</sub> plus 1000 mg of ionized calcium tablet daily. Group III received with 400 IU of vitamin D<sub>3</sub> plus 600 mg of ionized calcium tablet as daily supplement.

**Results**

There were no significant differences in all baseline parameters except vitamin D levels among three groups. After 12 weeks of vitamin D replacement therapy, 95.3% of all subjects had 25(OH) D levels within the normal range with a mean level of  $53.4 \pm 21.3$  ng/ml. There was a significant reduction in HOMA-IR scores in group I ( $P < 0.001$ ), but not in group II ( $P = 0.074$ ) and group III ( $P = 0.123$ ). There were no statistically significant differences in lipid parameters in all groups.

**Conclusion**

We found that supplementation in vitamin D deficient subjects may modify insulin sensitivity in elderly.

**P493****Increased circulating levels of FGF23 an adaptive response in primary hyperparathyroidism?**J E Witteveen, A H van Lierop, S E Papapoulos & N A T Hamdy  
Leiden University Medical Center, Leiden, The Netherlands.**Introduction**

Fibroblast growth factor 23 (FGF23) and PTH have been identified as major players in the bone-parathyroid-kidney axis controlling phosphate homeostasis. In patients with primary hyperparathyroidism (PHPT) data on the relationship between PTH and FGF23 are scarce and not always concordant.

**Objective**

The aim of our study was to evaluate whether the relationship between PTH and FGF23 was altered in patients with primary hyperparathyroidism and whether, if present, this alteration was sustained after cure following successful parathyroidectomy.

**Patients and methods**

We identified 22 patients with primary hyperparathyroidism (PHPT) and 24 patients with long-term cure after successful PTx (EuPTH). All patients underwent biochemical evaluation of renal function, parathyroid status, vitamin D status, bone turnover markers and serum intact FGF23 levels.

**Results**

Mean serum FGF23 concentration was significantly higher in PHPT than in EuPTH patients ( $50.4 \pm 27.2$  vs  $33.1 \pm 12.5$  pg/ml,  $P = 0.011$ ). FGF23 levels significantly correlated with PTH levels ( $r = 0.362$ ,  $P = 0.013$ ), even after correction for 1,25(OH)<sub>2</sub>D levels ( $r = 0.422$ ,  $P = 0.01$ ). FGF23 levels showed a significant negative correlation with 1,25(OH)<sub>2</sub>D, which was more pronounced in PHPT than in EuPTH patients ( $r = -0.780$ ,  $P = 0.000$  vs  $r = -0.519$ ,  $P = 0.023$ ).

**Conclusion**

Our findings suggest that in PHPT, increased PTH levels can directly stimulate FGF23 production and that this increase is reversible when euparathyroidism is achieved. Based on the greater negative relationship between FGF23 and 1,25(OH)<sub>2</sub>D in PHPT patients, we propose that the PTH-induced increase in FGF23 levels may be an adaptive mechanism to prevent the potential deleterious effects of 1,25(OH)<sub>2</sub>D levels on phosphate homeostasis.

**P494****Effects of ibandronate versus risedronate on hip bone density in the osteoporotic elderly**Ilkin Naharci<sup>1</sup>, Ahmet Turan Isik<sup>1</sup>, Ergun Bozoglu<sup>1</sup>, Ozdes Emer<sup>2</sup>, Turker Turker<sup>3</sup>, Metin Uyanik<sup>4</sup>, Muhittin Serdar<sup>4</sup> & Huseyin Doruk<sup>1</sup>

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**Introduction**

The aim of this study was to investigate whether monthly ibandronate improves hip bone mineral density (BMD) to the same degree as weekly risedronate in osteoporotic elderly.

**Material and methods**

Eighty-one older adults with osteoporosis, age ranging from 65 to 95 years (mean: 77.5 years) were completed the study. Patients were treated either ibandronate 150 mg once monthly ( $n = 56$ ) and risedronate 35 mg once weekly ( $n = 25$ ). Changes in BMD at the total hip, neck, and trochanter, changes in bone turnover markers, and safety were compared at 12 months.

**Results**

There were no significant differences in all baseline parameters for two groups. After treatment in the ibandronate group, BMD was significantly increased by 3.86, 3.31, and 3.71% at the total hip, neck, and trochanter, respectively, as compared with baseline ( $P < 0.001$ , for all comparisons). In the risedronate group, BMD was also significantly increased by 3.60, 3.24, and 3.78% at the total hip,



neck, and trochanter, respectively, as compared with baseline ( $P < 0.001$  for total hip and trochanter;  $P = 0.003$  for neck). No differences in BMD changes at total hip, neck, and trochanter were found between two groups, respectively ( $P = 0.323$ ,  $P = 0.523$ ,  $P = 0.985$ ). The changes in bone turnover markers (urine hydroxyproline and serum total alkaline phosphatase) were  $-3.93$  and  $-24.7\%$  for ibandronate group and  $-5.58\%$  and  $-22.4\%$  for risedronate group, respectively. No significant difference in the incidence of adverse events was found between two treatment groups.

#### Conclusion

Our findings indicate that monthly ibandronate was as effective as weekly risedronate at increasing hip BMD after 12 months in elderly with OP. Use of monthly ibandronate possibly could improve compliance and persistence as compared weekly therapies and could be good option to minimize polypharmacy in elderly people.

### P495

#### Overweight and risk for osteoporosis

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BMI over 30 kg/m<sup>2</sup> is associated with lowest risk for osteoporosis and hip fracture in both sex. Increased body fat mass is followed with greater hip fracture risk, independently of BMI.

#### Aim

Evaluate risk for osteoporosis in postmenopausal female with BMI 25–29.9 kg/m<sup>2</sup> and influence of body fat mass on mineral bone density in overweight women.

#### Material and methods

One hundred postmenopausal female, age 48–88 (62.8 ± 12.3), divided in two groups: group A, BMI 20–24.9 kg/m<sup>2</sup> ( $N = 19$ ) and group B, BMI 25–29.9 kg/m<sup>2</sup> ( $N = 81$ ) and according of body fat mass on group A1 with <30% fat mass ( $N = 4$ ), and B1 with more than 30% fat mass ( $N = 96$ ). Mineral bone density (femoral neck) percentage of body fat mass was measured by DEXA. Break point for osteoporosis was  $-2.5$  s.d.

#### Results

In group B bone mineral density was more than group A ( $\chi^2 = 10.17$ ,  $P = 0.0014$  with correction for continuation  $\chi^2 = 7.96$ ,  $P = 0.0048$ ). Between A1 and B1 there was no difference according osteoporosis appearance ( $\chi^2 = 0.42$ ,  $P = 0.5176$  with correction for continuation  $\chi^2 = 0.01$ ,  $P = 0.9297$ ).

#### Conclusion

Postmenopausal female with BMI from 25 to 30 kg/m<sup>2</sup> have more mineral bone density than normal weight female, and lower risk for osteoporosis. Percentage of body fat mass 30–51% in overweight post menopausal female has no influence on bone mineral density.

### P496

#### Hip and lumbar spine BMD in overweight postmenopausal women

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Obese subjects have lower risk for osteoporosis than normal weight people. Body mass index over 33 kg/m<sup>2</sup> is associated with lowest risk for hip fracture in both sexes.

#### Aim

Investigate overweight influence on bone mineral density of hip bone and lumbar spine in overweight postmenopausal women.

#### Material and methods

Postmenopausal women  $N = 100$ , without co-morbidity, 48–88 years of age, divided in two groups: group A, with BMI 20–24.9 kg/m<sup>2</sup> ( $N = 19$ ) and group B, BMI 25–29.9 kg/m<sup>2</sup> ( $N = 81$ ). Bone mineral density (femoral neck and L1-L4) were measured by DEXA. Break point of T score for osteoporosis was  $-2.5$  s.d.

#### Results

Group B have significantly higher hip bone mineral density, than group A ( $\chi^2 = 10.17$ ,  $P = 0.0014$ ,  $\chi^2 = 7.96$ ,  $P = 0.0048$ ). There was no significant differences between two groups in bone mineral density of lumbar spine  $\chi^2 = 0.03$ ,  $P = 0.8558$  with correction for continuity  $\chi^2 = 0.1$ ,  $P = 0.9186$ ).

#### Conclusion

Postmenopausal women with BMI 25–30 kg/m<sup>2</sup> have higher hip bone mineral density, also overweight has no influence on lumbar spine mineral density.

### P497

#### Incidence of osteoporosis study in premature ovarian failure

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At patients with premature ovarian failure, follicular endowment is very low and therefore is deficient ovarian hormonogenesis. As the number of follicles is lower, the lifetime of the ovary is reduced, representing a clinical spectrum from total or partial absence of pubertal sexualisation by early onset climacteric. The disruption of ovarian hormones that control bone homeostasis, is disturb-training report bone resorption with decreased bone mass and osteoporosis.

#### Method

The study was conducted on 48 patients whose age ranged between 20–38 years. Investigations were focused on the study hormonology FSH, LH, PRL, oestradiol, progesterone. Pelvic ultrasound was also performed utero-ovarian. At all patients the bone mineral density (BMD) was assessed by dual X-ray absorptiometry (DEXA). As biochemical markers of bone turnover were studied and serum Osteocalcine CrossLaps by ELISA.

#### Results

Hormonal dosages showed low levels of oestradiol and progesterone, but those of gonadotrophic hormones (LH, FSH) were between 210–385 mil/ml (upper limit of normal: 0.110 to 190 mil/ml). BMD measurement showed the presence of osteoporosis in 22 cases, representing 45.8% of all cases investigated. BMD is correlated with biochemical markers of bone turnover.

#### Conclusion

- i) A study of BMD and biochemical markers of bone turnover in premature ovarian failure, it must be conducted regularly to identify patients who rapidly lose bone mass and increased risk of osteoporosis.
- ii) The estrogen-progestive substitution represents the therapeutic attitude which is the first choice for premature ovarian failure to prevent osteoporosis, and metabolic complications of visceral.
- iii) Patients with osteoporosis will be associated the antiresorbition or proformation medication to prevent fragility fractures.

### P498

#### Bone markers of osteoblast/osteoclast activity in postmenopausal osteoporosis

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of Medicine and Pharmacy 'Victor Babes', Timisoara, Romania.

#### Aim

The aim of this study was to assess the implications of TRACP-5b (bone tartrate-resistant acid phosphatase izoform-5b), BAP (bone alkaline phosphatase) BGP (bone Gla protein) and E2(estradiol) in bone remodelling process, in osteoporotic postmenopausal women.

#### Material and method

The study comprised two groups of women with postmenopausal osteoporosis (with different degrees of estrogenic deprivation): group I ( $n = 48$ , estrogenic deprivation <15 years) and group II ( $n = 26$ , over 15 years of estrogenic deprivation), compared to a control group ( $n = 20$ , postmenopausal women

without osteoporosis). Serum levels of the above mentioned bone markers were measured by ELISA technique.

#### Results

The levels of TRACP-5b were significantly higher in postmenopausal osteoporosis (group I:  $4.21 \pm 1.18$  U/l,  $P < 0.001$ , group II:  $4.57 \pm 1.41$  U/l,  $P < 0.001$ ) versus control group ( $2.45 \pm 1.070$  U/l), demonstrating osteoclastogenesis activation.

BAP levels were increased in postmenopausal osteoporosis (group I:  $13.76 \pm 0.6$   $\mu$ g/l,  $P < 0.001$ , respectively group II:  $11.88 \pm 0.38$   $\mu$ g/l,  $P < 0.001$ ) versus control group ( $8.68 \pm 0.44$   $\mu$ g/l), showing osteoblastic activation.

Serum BGP levels in postmenopausal osteoporosis were increased in group I ( $20.12 \pm 0.87$  ng/ml,  $P < 0.03$ ), attesting osteoblastic activation and decreased in group II ( $15.12 \pm 1.55$  ng/ml,  $P < 0.05$ ) versus control group ( $16.22 \pm 1.62$  ng/ml), secondary to osteoblastic apoptosis stimulation.

Estradiol levels were significantly lower in both study groups as compared to control cases (group I:  $28.3 \pm 1.81$  pg/ml,  $P < 0.004$  and group II:  $19.66 \pm 1.23$  pg/ml,  $P < 0.002$ ). Estradiol levels correlated with low bone mineral density (in group I: spine BMD =  $0.639 \pm 0.051$  g/cm<sup>2</sup>,  $P < 0.0003$  and femoral BMD =  $0.653 \pm 0.042$  g/cm<sup>2</sup>,  $P < 0.0002$ ; in group II: spine BMD =  $0.563 \pm 0.012$  g/cm<sup>2</sup>,  $P < 0.0002$ , respectively femoral BMD =  $0.577 \pm 0.015$  g/cm<sup>2</sup>,  $P < 0.0001$ ) versus control group (spine BMD =  $0.773 \pm 0.141$  g/cm<sup>2</sup> and femoral BMD =  $0.785 \pm 0.128$  g/cm<sup>2</sup>).

#### Conclusion

This imbalance of bone markers reflects a decreased bone formation and an increased bone resorption. This will lead to bone demineralization and osteoporotic bone microfractures.

## P499

### Osteoporosis in the women with metabolic syndrome

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#### Background

It is recognized worldwide that the endocrine diseases lead among the causes of secondary osteoporosis. Based on many studies there was established the frequency of osteoporosis during the thyroid disorders, diabetes mellitus, hypercorticism. The most obscure in this line is the metabolic syndrome. The study of bone mineral density index during the metabolic syndrome is interesting from the aspect that until now the obesity has been considered to be the prevention of osteoporosis...

#### Objectives

The objective of the work was to study the bone mineral density in women with metabolic syndrome, detection of correlations between the bone metabolism and those criteria which accompany the metabolic syndrome.

#### Methods and materials

Ninety women with metabolic syndrome (MS) were studied, aged 25–50 (the average age 30.5–40.7). The diagnosis included the body mass index (BMI), fat distribution variants, obesity accompanying complications. All patients underwent the oral glucose meal, fasting blood sugar test, fasting immunoreactive insulin test and glucose loading insulin test. The patients were divided into 3 groups: 1st Group – 34 women with full MS diagnosis (30.7%), 2.27 patients with MS diagnosis without carbohydrate metabolism disorder (39%), 3.29 patients with MS diagnosis without dislipidemy. All patients underwent the densitometric study by the dual energy X-ray absorption densitometers (Hologic Q-1000); the bone mineral density was measured in lumbar vertebrae, in the femoral neck, distal forearm area, using  $T_{score}$  criterion.

#### Results

The results of the study appeared to be interesting not only for determination of the osteoporosis frequency during MS but for establishment of rhetoric relations between obesity and bone mineral density; out of 90 patients in 31 cases the low bone mineral density ( $T \leq -2.5$ ) was fixed mainly (35%) in the femoral neck – 11 patients, Ward's triangle – 10 patients and in the distal forearm area – 20 patients. The osteopenic syndrome ( $T \leq -2.0$ ) was revealed in 11 patients mainly in those skeleton fragments which are represented with the cortical bones. From the aspect of patient's age the frequency of low bone density index was very surprising.

#### Conclusion

The more frequent low bone mineral density was revealed during MS with hyperinsulinemia. No correlation between the BMD index and dislipidemia quality was revealed. Obesity does not exclude the osteoporosis during MS and correlated with the gravity of syndrome.

## P500

### Single nucleotide polymorphisms of the OPG/RANKL system genes in primary hyperparathyroidism and their relationship with bone mineral density

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#### Background

Primary hyperparathyroidism (PHPT) affects mainly cortical bone. It is thought that PTH indirectly regulates the activity of osteoclasts by means of the osteoprotegerin/ligand of the receptor activator of nuclear factor- $\kappa$ B (OPG/RANKL) system. Several studies have confirmed that OPG and RANKL loci are determinants of bone mineral density (BMD) in the general population. The aim of this study is to analyze the relationship between fractures and BMD and the 163 A/G, 245 T/G and 1181 G/C SNPs of the OPG gene and the rs2277438 SNP of the RANKL gene, in patients with sporadic PHPT.

#### Methods

We enrolled 298 Caucasian patients with PHPT and 328 healthy volunteers in a cross-sectional study. We analyzed anthropometric data, history of fractures or renal lithiasis, biochemical determinants including markers for bone remodelling, BMD measurements in the lumbar spine, total hip, femoral neck and distal radius, and genotyping for the SNPs to be studied.

#### Results

Significant lower BMD in the distal radius with similar PTH levels was found in the minor allele homozygotes (GG) compared to heterozygotes and major allele heterozygotes in both OPG 163 A/G and OPG 245 T/G SNPs in those with PHPT but not in control subjects. We found no difference between genotypes of the OPG 1181 G/C SNP with regard to BMD in the PHPT subjects. However, we did find higher BMD in the lumbar spine in the CC than in the GG genotype group in the control subjects.

#### Conclusions

Our study provides the first evaluation of the relationship between SNPs of the OPG/RANK system and sporadic PHPT. Subjects with PHPT and GG genotype for the OPG 163 A/G and 245 T/G SNPs have lower BMD in the distal radius, and this association does not appear to be mediated by differences in PTH serum levels.

## P501

### Vitamin D deficiency in Spain: a population-based cohort study

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#### Background

Vitamin D deficiency is common worldwide. No homogenous reference values have yet been established and no studies of values have been done in Spain involving a large number of participants.

#### Objective

To study the population concentrations of vitamin D in a representative sample of the Spanish population.

#### Subjects/methods

The study involved two cohorts from Spain, the Asturias Study and the Pizarra Study, which are two prospective, population-based studies involving 2260 participants. In 1262 subjects (age: 20–83 years) we studied 25-hydroxyvitamin D, intact parathyroid hormone (iPTH), calcium, phosphorus and creatinine.

#### Results

The median population values of 25-hydroxyvitamin D and iPTH were 22.46 ng/ml and 42.29 pg/ml respectively. The values of 25-hydroxyvitamin D were significantly higher in summer and correlated with age ( $\beta = -0.05 \pm 0.01$ ,  $P < 0.0001$ ), creatinine ( $\beta = 6.42 \pm 1.17$ ,  $P < 0.0001$ ), and iPTH ( $-0.07 \pm 0.01$ ,  $P < 0.0001$ ), but not with calcium, phosphorus or sex. The increase in iPTH with age was seen whatever the values of 25-hydroxyvitamin D, and was greater in the older persons. The concentration of iPTH rose continuously with effect from 25-hydroxyvitamin D values below  $\approx 30$  ng/ml. Values above  $\approx 35$  ng/ml were associated with a significantly lower concentration of iPTH.

#### Conclusions

One third (33.9%) of the Spanish population may be at risk for Vitamin D deficiency. 25-hydroxyvitamin D values above 30 ng/ml can safely discard 'hyper PTH'. The increase in iPTH concentration is greater in older persons for similar values of 25-hydroxyvitamin D.

#### P502

##### Serum testosterone and estradiol in relation to bone mineral density, muscle strength and body composition in elderly men

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#### Objective

Sex steroids play an important role in the maintenance of bone health. However, there is limited information on the association between sex hormones and age-related bone loss in men. Our objective was to study the relationship between sex steroid levels and the changes accompanying aging process, including bone mineral density (BMD), muscle strength and body composition in elderly Egyptian men.

#### Methods

Free testosterone (FT), estradiol (E<sub>2</sub>) and sex hormone-binding globulin (SHBG) were measured in thirty elderly men, age range 60–73 years, and 15 young men (age range 30–36 years). Patients receiving hormonal ablation for prostatic neoplasm and patients with chronic liver, renal disease or receiving corticosteroids were excluded. Sex steroid levels were correlated to BMD measured by DXA, lower limbs muscle strength calculated by isokinetic Biodex dynamometry and body composition assessed by body fat analyzer.

#### Results

FT and E<sub>2</sub> levels were significantly lower in elderly men compared to young group ( $P < 0.01$ ), whereas SHBG significantly increased with age ( $P < 0.01$ ). Peak torque values of both extensors and flexors were significantly lower in elderly men ( $P < 0.01$ ). Low BMD was found in 90% of elderly men; osteopenia (60.5%) and osteoporosis (29.5%). E<sub>2</sub> levels were associated positively with BMD at neck of femur in elderly men ( $P < 0.05$ ). FT correlated positively with lean mass and muscle strength ( $P < 0.01$ ), but didn't correlate with BMD. SHBG showed significant negative correlation with lean mass and muscle strength ( $r = -0.833$ ,  $P < 0.01$ ).

#### Conclusions

FT is not associated with BMD in elderly men, however, FT has strong relation with muscle strength and lean mass; so older men with lower FT could be more liable to falls. Elderly men with low E<sub>2</sub> are more likely to be osteoporotic as E<sub>2</sub> is an important determinant of bone density changes in old age.

#### P503

##### Differential gene expression in Runx2 and osterix double heterozygotes during skeletal development

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The transcription factors Runx2 and osterix (Osx), which are known as downstream genes in BMP2 pathway, are essential for osteoblast differentiation and bone formation. Runx2 expression is normal in Osx null mice, but Osx is not expressed in Runx2 null mice, indicating that Osx acts as a downstream of Runx2. Here, we questioned whether or not all bones could be formed by BMP2–Runx2–Osx pathway or unknown their own gene axis. To answer this question, we first analyzed bone phenotype of Runx2;Osx double heterozygotes which were generated by Runx2 and Osx heterozygous mice. Compared to each Runx2 and Osx heterozygous embryos, Runx2;Osx double heterozygotes showed a reduction of the length of long bones including humerus and femur. Maxillary and palatine

shelf, presphenoid bone, zygomatic bone, and tympanic ring were hypoplastic or missing. Severe inward bendings were observed in ribs and humerus. In histological analysis, the region of hypertrophic chondrocytes was expanded and the area of mineralized bones was reduced in Runx2;Osx double heterozygotes. To further elucidate the difference of Runx2 and Osx downstream target genes in skeletal development, DNA microarray analysis was conducted in calvaria of Runx2, Osx, and Runx2;Osx double heterozygotes. Many genes involved in cell cycle regulation, growth and apoptosis, immune response, extracellular matrix structure, skeletal development and morphogenesis were upregulated or down-regulated in Runx2;Osx double heterozygotes. Especially, among skeletal development related genes, matrilin 1 and 4, hyaluronan and proteoglycan link protein 1, aggrecan, epiphygan, Col2a1, Col9a1, and Sox9 were decreased, while JunD, peptidase inhibitor 16, elastin microfibril interfacer 2, mast cell protease, dynein, and angiotensin II receptor were remarkably increased in Runx2;Osx double heterozygotes. Taken together, differential gene expression profiles in Runx2;Osx double heterozygotes provide the understanding of the correlation between Runx2 and Osx in skeletal development (Grant support: RT104-01-01, KRF 2008-331-E00039).

#### P504

##### Sex-specific response of mammalian bone *in vivo* and *in vitro* to estrogens and to androgens

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Rat and mice bones *in vivo* and their derived cell cultures *in vitro* respond sex-specifically to gonadal steroids (GS) by stimulating DNA synthesis (DNA) and the specific activity of creatine kinase (CK; a hormone receptor response marker). Similar results were demonstrated in cultured human male or female-derived bone cells (Obs); female Obs responded only to estradiol-17 $\beta$  (E<sub>2</sub>) whereas male Obs responded only to dihydrotestosterone (DHT) by stimulating DNA, CK, 1 $\alpha$ ,25 vitamin D hydroxylase (1OHase) mRNA expression and production of 1,25(OH)<sub>2</sub>D<sub>3</sub>. Rat epiphyseal cartilage *in vivo* and its derived cell cultures *in vitro* responded to both hormones. The sex-specific response of Obs was modified by manipulating the endocrine environment in early development like gonadectomy, neonatally androgenization of female rats and androgen-receptor deficiency in male rats (Tfm). Rat bone marrow (BM) transplanted under the renal capsule formed bone ossicles responding to GS according to the donor gender. Rat demineralized tooth matrix particles (DTM) implanted under the skin induced bone formation responding to GS according to the host gender. Cultured femoral rat bone BM differentiated into Obs responding to GS according to the donor gender, losing sex-specificity after gonadectomy. Human Obs responded sex-specifically to GS via both genomic (DNA and CK) and non-genomic (intracellular Ca<sup>++</sup> concentration) binding sites. Male Obs although not responding to estrogens, expressed estrogen receptors suggesting involvement of post-receptor mechanism(s). The 'less differentiated' epiphyseal cartilage cells and the 'de-differentiated' Obs after gonadectomy lost sex-specific responses. In conclusion cultured Obs and bones *in vivo* respond sex-specifically to GS by different parameters.

#### P505

##### Insulin sensitivity and bone mineral density in primary hyperparathyroidism

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#### Introduction

Recent data suggest a reciprocal influence between bone and energy metabolism. Mediators have been identified as osteocalcin (OC), which stimulates insulin (IRI) secretion and activity and IRI as bone anabolic factor. Primary hyperparathyroidism (PHPT) stimulates bone turnover, induces osteoporosis

and is associated with increased IRI resistance. A positive relationship between OC and IRI sensitivity has been found in PHPT, but no data exist on the relationship between IRI and bone mineral density (BMD).

**Aim**

To evaluate in a series of PHPT patients without known diabetes mellitus (DM), the relationship between IRI levels or sensitivity and BMD.

**Subjects and methods**

We studied 267 patients with PHPT (age, mean  $\pm$  s.d., 58.5  $\pm$  13.8 years; F/M 198/69; BMI 25.2  $\pm$  4.6 kg/m<sup>2</sup>; PTH 228.4  $\pm$  269.3 pg/ml; calcium 11.2  $\pm$  1.2 mg/dl) without known DM. Fasting blood glucose and IRI as well as BMD at lumbar spine, femur and forearm were measured. IRI sensitivity was assessed by the quantitative insulin sensitivity check index (QUICKI).

**Results**

In univariate analysis a positive relationship between IRI levels and BMD ( $R=0.19$ ,  $P<0.03$ ) or  $T$  score ( $R=0.23$ ,  $P<0.005$ ) at femoral site was found, but not at lumbar spine nor at forearm. A negative relationship between QUICKI and BMD ( $R=-0.20$ ,  $P<0.015$ ) or  $T$  score ( $R=-0.23$ ,  $P<0.004$ ) at femoral site was found. In multivariate analysis, we found that age ( $\beta=-0.35$ ,  $P<0.000004$ ), BMI ( $\beta=0.39$ ,  $P<0.000001$ ), PTH ( $\beta=-0.18$ ,  $P<0.013$ ) and QUICKI ( $R=-0.15$ ,  $P<0.048$ ) exerted an independent effect ( $R^2=0.29$ ) on femoral  $T$  score.

**Conclusions**

Our data show a relationship between insulin levels and/or sensitivity and BMD at femur in PHPT, as found in other diseases associated with insulin resistance. This finding suggests a link between bone and energy metabolism in PHPT. However, the clinical influence of this relationship on the PHPT-related bone damage is to be established.

## P506

### Suspected parathyroid lesions and incidentally detected parathyroid adenomas during thyroid ultrasonography

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**Introduction**

Although incidentalomas in endocrine glands such as pituitary, adrenal or thyroid are well defined, parathyroid incidentaloma is a rare concept. In this study, we aimed to determine the prevalence of suspicious parathyroid lesions and true parathyroid incidentalomas in patients referred for thyroid ultrasonography (US) and investigate the possible factors that might cause inadvertent suspicion of a parathyroid adenoma.

**Methods**

Patients suspected to have parathyroid lesions during thyroid US were recorded prospectively between August 2009 and January 2010. Patients referred for parathyroid US and patients with known high serum calcium or parathyroid hormone (PTH) levels were excluded. Suspected parathyroid lesions were defined as hypoechoic homogeneous solid lesions with regular margins located outside the thyroid lobe, most commonly inferior to the thyroid gland.

**Results**

Thyroid US was performed in 6528 patients during the study period. There were 78 patients (1.19%) (73 female and 5 male) with suspected parathyroid lesion and the mean age was 45.32  $\pm$  12.59. The diagnosis of a true parathyroid adenoma was confirmed six (7.69%) patients. Mean serum calcium, phosphorus and PTH levels were 10.57  $\pm$  0.48 mg/dl, 3.03  $\pm$  0.52 mg/dl and 182.91  $\pm$  46.62 pg/ml respectively in patients with true adenoma. Among 72 patients with false positive parathyroid lesion, antithyroid peroxidase antibody was positive in 50 (69.4%), antithyroglobulin antibody was positive in 46 (63.9%) and one of these antibodies were positive in 59 (81.9%) patients. Also, 46 (63.9%) of 72 patients had thyroid dysfunctions (43 hypothyroidism and 3 hyperthyroidism) and 59 (81.9%) had chronic thyroiditis ultrasonographically.

**Conclusions**

Parathyroid incidentaloma was detected in 0.09% of patients referred for thyroid US. Presence of clinically or ultrasonographically chronic thyroiditis seems to be the major factors related to inadvertent interpretation of a hypoechoic lesion as a parathyroid pathology during thyroid US. Most of these lesions are probably lymphadenopathies that may occur commonly in patients with chronic thyroiditis.

## P507

### Challenges and pitfalls in the management of persistent hyperparathyroidism: a case series

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**Introduction**

Persistent hyperparathyroidism is reported to occur in 0–6% of cases after initial surgery for sporadic primary hyperparathyroidism (PHPT). The management of these cases is complex, however, with a poor cure rate despite repeated surgery. The aim of our study was to identify the challenges and pitfalls encountered in the management of a series of patients with persistent hyperparathyroidism after initial parathyroidectomy.

**Patients and methods**

Using the Leiden University Medical Center hospital records, we identified 20 patients with sporadic PHPT who had undergone 33 revision surgeries for persistent hyperparathyroidism. Clinical, operative and histology data were documented from the patients' notes.

**Results**

The most common cause of persistent HPTH was a missed ectopic gland at initial surgery (33%), followed by missed multiglandular disease (15%). The least common cause was parathyromatosis (9%), with its risk increasing with repeated surgery. Pre-operative localization studies had poor sensitivity: US 18%, MRI 20%, Tc99m-MIBI-SPECT 25%, CT scan 30%. In contrast, selective venous sampling for PTH had a sensitivity of 50% and a specificity of 89%. The decrease in intraoperative PTH was significantly less marked (63  $\pm$  26% vs 89  $\pm$  11,  $P=0.003$ ) and less rapid in patients in whom PHPT persisted compared to those who achieved cure. The risk of complications, particularly recurrent laryngeal nerve palsy, increased with each subsequent surgery: 20% after the first, 50% after the 2nd and 67% after the 3rd revision surgery.

**Conclusion**

Persistent PHPT represents a significant management challenge largely due to the poor predictive value of pre-operative localization studies and to increased risk of complications with each subsequent surgery. Intraoperative PTH decreases operating time and thus risk of complications. Cases of parathyromatosis are the most challenging, with decreased likelihood of cure after repeated surgery. The potential of calcimimetics in the management of these patients remains to be explored.

## P508

### The diseases which are associated with asymptomatic primary hyperparathyroidism as an aid and obstacle for early diagnosis of the disease, especially arterial hypertension and malignancy

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Best of all, the primary hyperparathyroidism (PHPT) should already be disclosed in the asymptomatic stage. The authors of this contribution are participating in a longitudinal study, which is focused on several aspects of asymptomatic PHPT. The study has been in progress in the Czech Republic at the two tertiary endocrinology centers. Previously we had attempted to verify the assumption that the presence of a chronic disease results in more frequent contact of the patient with the health-care establishment, and that more frequent visits to the consulting room increase the probability of a laboratory examination, and therefore also the earlier detection of PHPT. This time we were particularly interested in a 6-month period before diagnosis of asymptomatic PHPT was completed, wanted to know what the original disease was and whether the contact with the physician increased the likelihood of early diagnosis of PHPT in its asymptomatic stage. At the end of 2010 our cohort included 167 patients aged 20–87 years (median 63) who were followed-up for asymptomatic PHPT for 3–96 months (median 30). The most common concomitant illness was that of endocrine. Out of 118 probands 96 cases suffered from thyroid disease and 10 from pituitary respectively. Seventy-nine patients had cardiovascular disorders; out of them 61 were hypertensive. Twenty patients were followed-up because of malignancy. We were not able to confirm that repeated contact of patients with their physician resulted in earlier detection of PHPT. In 47 PHPT patients, the diagnosis was the result of the search for the cause of osteoporosis. Almost every endocrinologist checked the parathyroid hormone level as a part of endocrinological examination. Surprisingly late PHPT was diagnosed in patients with malignancies. Hyperparathyroidism has been probably a far more common disease than it was previously expected.

**P509****DT56a (Femarelle), contrary to estradiol-17 $\beta$ , has a positive stimulatory effect on human derived female cultured bone cells in hyperglycemic conditions**D Somjen<sup>1</sup>, S Katzburg<sup>1</sup>, O Sharon<sup>1</sup>, D Hendel<sup>2</sup> & I Yoless<sup>3</sup><sup>1</sup>Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center, Tel-Aviv 64239, Israel; <sup>2</sup>Department of Orthopedic Surgery, Shaarei-Zedek Medical Center, Jerusalem, Israel; <sup>3</sup>Secure Pharmaceuticals, Dalton, Israel.

We have previously reported, that cultured female- derived human Osteoblasts (hObs) responded to DT56a (Femarelle). Since the skeletal protective effects of estrogens are not discernible in diabetic women, we sought to test the effects of DT56a on hObs grown in high glucose concentration in the growth medium (HG) as compared to estradiol-17 $\beta$  (E<sub>2</sub>). We used the stimulation of creatine kinase specific activity (CK), and <sup>3</sup>[H] thymidine incorporation into DNA (DNA synthesis), in response to E<sub>2</sub> or DT56a as markers for treatment responsiveness. hObs were isolated from premenopausal and postmenopausal women. Cells were grown either in normal glucose (NG) (4.5 g/l; 22 mM) or HG (9.0 g/l; 44 mM) for 7 days. HG slightly increased DNA synthesis in hObs. In HG environment, the stimulation in response to E<sub>2</sub> was decreased but not to DT56a in both age groups. Growing hObs in HG resulted in increased expression of both ERs' (alpha and beta) mRNA in hObs from pre-menopausal but not in hObs from post-menopausal women. Pre-treatment of hObs with DT56a increased the expression of both ERs mRNA in hObs from both age groups. Pre-treatment of hObs with E<sub>2</sub> increased expression of ER $\alpha$  mRNA in hObs from pre-menopausal women but inhibited it in hObs from post-menopausal women. Pre-treatment with E<sub>2</sub> in hObs from both age groups inhibited ER $\beta$  mRNA expression. Growing hObs in HG led to decrease of intracellular binding of <sup>3</sup>[H] E<sub>2</sub> in cells from both age groups when competed by E<sub>2</sub> but not by DT56a. This study shows that DT56a, contrary to E<sub>2</sub>, is active on hObs even in HG environment and, contrary to E<sub>2</sub>, activates ER even in HG environment. These findings suggest that Femarelle (DT56a) may be used as an effective treatment for the hyperglycemic/diabetic postmenopausal women.

**P510****Calcium homeostasis in diabetic postmenopausal women with bone fracture**A A Gusova, M G Pavlova, G A Melnichenko, G M Kavalersky, L L Silin & N V Zaitseva  
I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation.**Background and aims**

The aim was to compare calcium homeostasis indexes in diabetic and non-diabetic patients with or without fractures.

**Materials and methods**

We examined 62 postmenopausal women with type 2 diabetes mellitus (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. Tests involved blood and urine calcium and phosphorus, serum parathyroid hormone (PTH), IGF1 and 25-hydroxyvitamin D, C-terminal telopeptide of type I collagen (sCTX) and osteocalcin levels.

**Results**

In patients with DM without fractures levels of total and ionized calcium were higher compared to women without DM ( $P < 0.01$ ). There also was a reduction of serum calcium in subgroups with fractures ( $P < 0.05$ ). Urine phosphorus excretion in diabetic fracture subgroup was decreased compared to non-fracture one ( $P = 0.002$ ). In both subgroups with fractures was marked reduction of PTH ( $P < 0.01$ ) and vitamin D ( $P < 0.05$ ) compared with women without fractures. Vitamin D level was lower in diabetic groups ( $P < 0.03$ ). No difference in level of IGF1 between all groups was detected. Higher sCTX concentration was registered in non-diabetic women compared to diabetic group ( $P < 0.01$ ). There was a significant decrease of osteocalcin level in both diabetic subgroups (with and without fractures) compared to non-diabetic ones ( $P < 0.01$ ). There was also decrease of osteocalcin concentration in fracture subgroups, but it was relevant only for diabetic group ( $P = 0.03$ ).

**Conclusion**

Our data indicate that diabetic patients have a lower rate of bone remodeling and predisposed to vitamin D deficiency development. Study results could give a clue to understand previously reported increased fracture risk in diabetic patients, who have higher bone mineral density compared to non-diabetic women.

**P511****Vitamin D in Southern Italy multiple sclerosis patients**C Di Somma<sup>1</sup>, L Vuolo<sup>1</sup>, R Lanzillo<sup>1</sup>, E Guerra<sup>1</sup>, P Contaldi<sup>1</sup>, M Rubino<sup>1</sup>, V Brescia Morra<sup>2</sup> & A Colao<sup>1</sup><sup>1</sup>Department of Molecular and Clinical Endocrinology and Oncology Federico II University, Napoli, Italy; <sup>2</sup>Department of Neurological Sciences Federico II University, Napoli, Italy.

To evaluate vitamin D levels in relapsing remitting (RR) and secondary progressive (SP) courses of multiple sclerosis (MS) and to evaluate correlations between vitamin D and clinical features of disease progression, in a southern Italy population. 63 (41 RR and 22 SP) 'naive' MS patients (47 females, 16 males aged 37.7  $\pm$  5.7 years) and 63 healthy controls were enrolled. Patients were assessed through clinical, magnetic resonance imaging (MRI) and laboratory investigations. A secondary progression index was calculated for SP patients at baseline. Serum 25 hydroxy vitamin D (25OHD) was measured on serum obtained before immunomodulator therapy. Serum 25 OHD vitamin D was lower in patients than in controls (18.8  $\pm$  9.9 vs 29.2  $\pm$  12.4 ng/ml,  $P < 0.001$ ). Vitamin D deficiency ( $< 10$  ng/ml) was observed in 28.6%, vitamin D insufficiency (10–30 ng/ml) in 57.1% and normal value of vitamin D (30–100 ng/ml) in 14.3% of MS patients. No difference in 25 OHD vitamin D was found between two MS patient groups (19.4  $\pm$  9.0 vs 17.7  $\pm$  11.6 ng/ml  $P = NS$ ). Secondary progression index was not related to 25 OHD vitamin D levels. The hypothesis that adequate vitamin D nutrition can contribute to the prevention of MS was proposed to explain the geographical distribution of MS. There are many concerns regarding the role of vitamin D in MS pathogenesis and disability accumulation and its relation with sun exposure. We found a high prevalence of vitamin D deficiency and insufficiency both in RR and SP patients respect to normal controls, without a high disability preventing normal outdoor activity and not related to secondary progression index, before therapy onset. If vitamin D deficiency could have a pathogenetic role in this autoimmune disease is not yet clear and needs further study.

**P512****rs219780 SNP of CLDN14 gene is not associated with kidney stones, fractures or bone mineral density in primary hyperparathyroidism**M Piedra<sup>1</sup>, A Berja<sup>1</sup>, M T García-Unzueta<sup>1</sup>, M T Gutiérrez-Hierro<sup>1</sup>, N García-Llana<sup>1</sup>, C Rentería<sup>1</sup>, B A Lavín<sup>1</sup> & J A Amado<sup>1,2</sup>  
<sup>1</sup>University Hospital 'Marqués de Valdecilla', Santander, Spain; <sup>2</sup>University of Cantabria, Santander, Spain.**Background**

*CLDN14* gene encodes a protein involved in regulation of paracellular permeability or ion transport at epithelial tight junctions as in the nephron. C allele of the rs219780 SNP of *CLDN14* gene has been associated with high levels of PTH and with low bone mineral density (BMD) in women. Our aim is to study the relationship between rs219780 SNP of *CLDN14* and fractures, renal lithiasis and bone mineral density in patients with primary hyperparathyroidism (PHPT).

**Methods**

We enrolled 298 Caucasian patients with PHPT and 328 healthy volunteers in a cross-sectional study. We analyzed anthropometric data, history of fractures or renal lithiasis, biochemical determinants including markers for bone remodelling, BMD measurements in the lumbar spine, total hip, femoral neck and distal radius, and genotyping for the SNPs to be studied.

**Results**

We did not find any difference in the frequency of fractures or renal lithiasis between the genotype groups in PHPT patients. We found a non-significant trend towards lower ionic calcium, PTH, bone alkaline phosphatase,  $\beta$  Crosslaps and osteocalcin levels in TT genotype group than in TC or CC groups in patients with PHPT. There was a non-significant trend towards lower levels of BMD in the TT genotype group in the three skeletal sites studied, especially in lumbar spine in the control subjects, but there was no difference in patients with PHPT.

**Conclusions**

rs219780 SNP of *CLDN14* does not appear to influence the expression of primary hyperparathyroidism in bone or kidney in contrast with previous studies in general population.

**P513****Influence of thyroid disease in diagnostic accuracy of Tc99m-sestamibi scintigraphy in patients with primary hyperparathyroidism (PHP)**

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Bilateral cervical exploration has been the gold standard in parathyroid surgery. Nowadays, the preoperative localization procedures have facilitated the minimally invasive surgery. This study aims to analyze the influence of thyroid disease in Tc99m-sestamibi scintigraphy (MIBI) results.

A series of 154 patients with PHP who underwent parathyroidectomy was reviewed. Patients were divided into two groups: group 1 (G1, 58 patients) with thyroid diseases, and group 2 (G2, 96 patients) without goiter or thyroid nodules. Predictive positive value (PPV) of the MIBI was defined as true positive results (when the pathologic gland was correctly localized)/true positive + false positive (when no pathologic gland was found).

**Results**

There was no difference between two groups in sex, age and most of the preoperative biochemical parameters.

**Table 1**

	Group 1	Group 2	P
Sex (male)	9 (15.5%)	16 (16.7%)	0.8
Sex (females)	49 (84.5%)	80 (83.3%)	0.7
Age	63.1±12	62.8±14	0.9
Serum calcium	11.8±1.2	11.6±0.9	0.2
PTH	279.7±265.5	236.7±148.6	0.2
25-OHvitD	21.3±15.8	22.2±14.7	0.8
Urinary calcium	394.6±158.4	401.6±224.5	0.8

Histopathology showed 143 (93%) single adenomas (G1=51, G2=92), 3 carcinomas (two in G1), 5 hyperplastic (three in G1), and 3 double adenomas (two in G1).

The PPV of MIBI in G1 was 65% (26/26+12) and in G2, 85% (69/69+12);  $P=0.003$ .

Since minimally invasive surgery is performed, the preoperative localization procedures have become very important, particularly MIBI. Because the presence of thyroid nodules reduces the sensitivity of MIBI, patients with HPT should ruled out coexistent thyroid disease.

**P514****Secondary hyperparathyroidism in a patient with multiple myeloma**

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Multiple myeloma is a hematologic malignancy characterized by osteolytic bone destruction which follows the increased osteoclastic resorption of bone that is not accompanied by increased bone formation. This can lead to bone pain, diffuse osteopenia, focal lytic lesions, pathologic fractures, spinal cord compression, and hypercalcemia, so it is evident that osteolytic bone disease is a major cause of morbidity and mortality in patients with multiple myeloma. Long-term monthly infusions of pamidronate as an adjunct to chemotherapy or as a maintenance therapy can reduce skeletal events and may improve the survival of patients. Hypercalcemia is mainly caused by interleukin-6 and tumor necrosis factor. Myeloma cells activate genes that both stimulate bone resorption via osteoclast activation and prevent new bone creation by inhibiting osteoblasts. Pamidronate is a second-generation bisphosphonate, which inhibits bone resorption. Bisphosphonate bind strongly to hydroxyapatite crystals in the bone matrix, preferentially at the sites of increased bone turnover and inhibit the formation and dissolution of the crystals. Pamidronate does not interfere with bone mineralization at therapeutic doses.

A standard daily infusion dose of pamidronate for the treatment of hypercalcemia in patients with normal renal function is 60–90 mg over 2–4 h every 3–4 weeks for 2 years. Pamidronate appears to reduce calcium levels for prolonged periods of time, and hypocalcemia, which would increase PTH levels, might be an adverse complication. Some studies showed that intravenous pamidronate can aggravate secondary hyperparathyroidism, but for 2-year period, pamidronate is a safe and effective treatment for the correction of hypercalcemia. Here we present a female patient with multiple myeloma who has been receiving pamidronate for more than 5 years and developed secondary hyperparathyroidism.

**P515****Bone mineral density and its contributing factors in type 1 diabetic patients**

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**Aim**

To investigate factors associated with low bone mineral density (BMD) in type-1 diabetic (T1D) patients.

**Patients and methods**

One hundred and seventy-five eugonadal T1D patients (age  $32.8 \pm 8.4$  years, diabetes duration  $13.0 \pm 8.4$  years) were studied. Body mass index (BMI), daily insulin dose (DID), age at diagnosis (AgeD), presence of diabetic complications, glomerular filtration rate (GFR), HbA1c, and BMD at lumbar spine (LS) and femur (F) by dual X-ray absorptiometry (as Z-score) were determined. Patients were categorized on the basis of LS- and F-BMD  $\leq$  (low BMD) or  $> -1.0$ .

**Results**

As compared with T1D patients without low LS-BMD, those with low LS-BMD ( $n=46$ , 26.3%) had lower BMI ( $22.3 \pm 3.4$  vs  $24.4 \pm 3.7$  kg/m<sup>2</sup>,  $P=0.005$ ) and higher DID ( $0.9 \pm 0.3$  vs  $0.7 \pm 0.2$  U/kg,  $P=0.0001$ ). As compared with T1D patients without low F-BMD, those with low F-BMD ( $n=58$ , 33.1%) had lower BMI ( $23.0 \pm 3.3$  vs  $24.5 \pm 3.8$  kg/m<sup>2</sup>,  $P=0.013$ ), AgeD ( $17.3 \pm 8.7$  vs  $21.0 \pm 9.2$  years,  $P=0.013$ ) and GFR ( $88.1 \pm 31.3$  vs  $101.8 \pm 31.3$  ml/min,  $P=0.007$ ), and higher DID ( $0.8 \pm 0.3$  vs  $0.7 \pm 0.2$  U/kg,  $P=0.006$ ) and HbA1c ( $8.6 \pm 1.1$  vs  $8.2 \pm 1.2\%$ ,  $P=0.05$ ). The diabetic complications were not associated with low BMD. The LS-BMD was independently associated with age ( $\beta=0.193$ ,  $P=0.049$ ), BMI ( $\beta=0.187$ ,  $P=0.022$ ), DID ( $\beta=-0.177$ ,  $P=0.025$ ), while F-BMD with GFR ( $\beta=0.170$ ,  $P=0.03$ ). The cut-off values of these factors for predicting low BMD at any site were determined (age 33.5 years, BMI 23.5 kg/m<sup>2</sup>, DID 0.67 U/kg, GFR 88.8 ml/min) and in each patient the presence/absence of each risk factor and a total risk factors' score for low BMD (RFSc) was calculated by summing the number of present risk factors. The RFSc of 0 showed a negative predictive value of 81.8% and the RFSc of 4 a positive predictive value of 63.4%, for low BMD respectively.

**Conclusion**

In T1D patients, low LS-BMD is associated with young age, low BMI and high DID, while low F-BMD with low GFR.

**P516****Hungry bone syndrome following parathyroidectomy**

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**Aim**

Hypocalcemia is a common problem after parathyroidectomy but is generally transient. In some cases the postoperative hypocalcemia is severe and prolonged despite normal or even elevated levels of parathyroid hormone (PTH). This phenomenon, called the hungry bone syndrome (HBS), occurs due to sudden withdrawal of PTH leading to a marked net increase in bone uptake of Ca, P, and Mg. We aimed to evaluate predictive factors for HBS development after parathyroidectomy.

**Material and method**

Forty-four patients who undergone surgical resection for parathyroid adenoma were evaluated retrospectively. Age, gender and preoperative serum levels of corrected Ca, P, iPTH, ALP, urea and creatinine were recorded. We accepted HBS diagnosis as the serum calcium concentration below normal limits for at least 1 week after parathyroidectomy.

**Results**

We divided the patients into two groups as HBS ( $n=13$ ) and non-HBS ( $n=31$ ). There was no difference between the groups in terms of age and gender. Serum Ca and ALP levels of groups were similar. Serum P, iPTH, urea and creatinine levels were higher in HBS group than non-HBS group ( $P<0.05$ ,  $P<0.01$ ,  $P<0.05$ ,  $P<0.05$  respectively). 6 of 13 (46.2%) patients with HBS had chronic renal failure (CRF), whereas CRF percentage was just 6% in non-HBS group ( $P<0.01$ ).

**Conclusion**

We suggest that CRF is an important risk factor for HBS development after parathyroidectomy.

**P517****Genome-wide association in the Rotterdam study implicates the 16q24 locus as determinant of osteoporotic vertebral fractures**

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Risk for vertebral fractures (VFX), the most common osteoporotic fractures, is a heritable complex trait. No genome-wide association studies (GWAS) searching for genetic susceptibility factors for VFX have been reported. We performed a GWAS for VFX in a population-based study of Dutch elderly. Thoracolumbar spine radiographs were scored for osteoporotic VFX using the McCloskey/Kanis method. Genetic data was available in 329 VFX cases and 2666 controls. We tested 2 543 887 imputed (HapMap CEU release 22, build 36) SNPs using a logistic regression model (MACH2DAT) adjusted for age, sex, and admixture principal components. At a genome-wide significant  $\alpha$ -level (GWS) of  $5 \times 10^{-8}$ , the design has 0.80 power to detect risk effect sizes (OR) of 1.7 to 2.4 for minor allele frequencies (MAF) of 0.20 to 0.05, respectively. A SNP on chromosome 16q24 (MAF=0.10) was associated at GWS level ( $P=4.6 \times 10^{-8}$ ) with an increased risk for VFX. Heterozygous carriers of the minor allele had 1.7-fold (95% CI: 1.3–2.2) and homozygous carriers 5.8-fold (95% CI: 2.6–12.6) increased risk compared to non-carriers. The VFX-associated SNP maps in a region previously found associated with lumbar spine BMD (LS-BMD) in a meta-analysis of 19 125 individuals, yet represents an independent signal from the reported BMD SNP ( $LD r^2=0.002$ ). The VFX-associated SNP was not associated with LS-BMD. FOXC2 is a strong candidate gene mapping ~200 kb upstream from the associated SNP. FOXC2 is a transcription factor essential for axial skeletogenesis in mice, highly expressed in human bone tissue and involved in osteoblast differentiation through activation of canonical Wnt- $\beta$ -catenin signals. Inactivating mutations affecting the FOX gene cluster cause severe vertebral malformations in humans. In conclusion, our findings implicate the 16q24 locus as a strong determinant for osteoporotic VFX. Pleiotropic effects of FOXC2 are potentially driving these associations with LS-BMD and VFX. Replication within the GEFOS/GENOMOS consortia is currently underway to confirm this finding.

**P518****Bone turnover markers and the metabolic syndrome**

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Obesity was considered to be a protective condition against osteoporosis. New studies suggest that visceral obesity is associated with low BMD and a higher risk for fracture. Patients with diabetes mellitus, although they do not have a lower BMD, have a higher fracture risk, probably due to the lower bone turn over.

**Aim**

The aim of the study was to evaluate bone metabolism in connection with hormonal profile in postmenopausal women with metabolic syndrome compared with matched controls without metabolic syndrome.

**Subjects and methods**

A number of 144 postmenopausal women were enrolled and 2 groups were formed (57 subjects with metabolic syndrome, 87 controls). Fasting blood samples were taken in order to determine the biochemical and hormonal status. SPSS version 15 was used for statistical analyze. Results were expressed as mean values  $\pm$  s.d. and were considered to be statistically significant if the *P* value was  $\leq 0.05$ .

**Results**

Significant higher mean values were found for BMI, waist circumference, blood pressure, glycemia, triglycerides and apolipoprotein B, white blood cells, lymphocytes, CRP (markers of the pro-inflammatory state), insulinemia and insulin resistance expressed by HOMA, GHBP ( $P < 0.001$ ) and PTH ( $P = 0.041$ ). Postmenopausal women with metabolic syndrome had lower HDL-cholesterol, SHBG, IGFBP1 ( $P = 0.027$ ) and 25OH vitamin D ( $P = 0.025$ ) levels. Osteocalcin correlated positively with crosslaps ( $P < 0.001$ ), PTH ( $P = 0.025$ ) and 25OH vitamin D ( $P = 0.005$ ); PTH correlated positively with glycemia, uric acid and negatively with IGFBP3 ( $P = 0.048$ ).

**Conclusions**

Postmenopausal women with metabolic syndrome have a modified hormonal status and different correlations between bone turn-over markers. Lower 25 OH vitamin levels, leading to higher PTH levels reinforce the importance of optimal vitamin D concentrations and its role in metabolic syndrome.

**P519****Trace elements and bone mineral density in pre- and postmenopausal women**

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**Introduction**

In postmenopausal women the rate of bone remodeling increases. Trace elements are known to influence bone metabolism. The aim of the study was to assess serum concentrations of some elements in pre- and postmenopausal women with and without osteopenia.

**Methods**

Serum concentrations of magnesium, copper, cadmium and vanadium were measured by inductively coupled plasma atomic emission spectroscopy in 51 postmenopausal women (19 women with osteopenia and 32 with normal bone mineral density) and 29 age- and BMI-matched premenopausal women (12 women with osteopenia and 17 with normal bone mineral density). The diagnosis of osteopenia was based on assessment of bone mineral density (BMD) at the spine and proximal femur by dual-emission X-ray absorptiometry.

**Results**

Mean concentration of vanadium was significantly higher in postmenopausal than in premenopausal women. In addition, magnesium, copper and cadmium concentrations in postmenopausal women were lower than in premenopausal women, but with no significant differences. There were no statistically significant differences observed between the osteopenic and normal pre- and postmenopausal women with respect to magnesium, copper, cadmium and vanadium levels. In premenopausal women the serum level of copper was positively correlated with the *T*-score at the femoral neck and with the BMI.

**Conclusion**

The consequences of changes in bone turnover can be detected by increased levels of bone biomedical markers. Vanadium accumulates in bone, though its role on bone quality is not clear. Serum vanadium level might be a marker of bone turnover and its serum level may reflect processes related to bone resorption. Copper plays an important role in bone development in part due to its role as cofactor for various enzymes (lysyl oxidase and superoxide dismutase) required for the synthesis or modification of bone matrix. The results of study provide more evidence of a correlation between copper status and osteopenia.

**P520****Fahr's syndrome in a patient with unknown hypoparathyroidism**

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**Introduction**

Fahr's syndrome is characterized by bilateral calcifications in basal ganglia, dentate nucleus and semioval center. Its clinical manifestations are a rigid hypokinetic syndrome, mood disorders and cognitive impairment. The most frequent aetiology of this syndrome is, together with idiopathic causes and congenital infections, calcium disorders, mainly primary hypoparathyroidism, accounting for up to 80% of all cases.

**Case report**

A 57-year-old woman was admitted to the Neurology Unit for study of parkinsonian features that had worsened over the last 6 months. She had a medical history of dyslipidemia, 6-year-history of depressive syndrome. She reported no family history of interest. She related a progressive 6-month self-limiting walking disorder along with cramps and stiffness in lower limbs. On physical exam, the most important findings were: cogwheel rigidity and absence of reflexes; Chvostek and Trousseau signs were positive. Laboratory findings were: calcium 3.8 mg/dl (reference range 8.4–10.5 mg/dl), phosphorus 6.8 mg/dl (2.5–4.5 mg/dl), CK 693 IU/l (20–135), 25-OH vitamin D 32 ng/ml (20–54), PTH 6.3 pg/ml (11–79.5), calcium urine 81.4 mg/24 h (100–300). The electromyogram showed spontaneous and continuous muscular activity in lower limbs. CT scan showed diffuse, symmetric parenchymal calcifications involving the dentate nuclei, basal ganglia and deep cerebral white matter. All these findings were ascribed to Fahr's syndrome due to a primary hypoparathyroidism. The patient was treated with intravenous calcium and after restoring normal plasmatic levels she continued with 1000 mg calcium carbonate orally and 0.5  $\mu$ g/day calcitriol, maintaining normocalcemia. Other endocrine disorders were excluded as well as

Huntington's disease and viral infections. Calcium sensing receptor and antiparathyroid tissue antibodies were also negative. Chvostek and Trousseau became negative. Stiffness and cramps disappeared and electromyogram became normal; however, no changes in walking were seen. Six months later, the patient continued normocalcemic. No improvements in parkinsonian walking were seen despite normocalcemia and treatment with levodopa.

#### Conclusions

Primary hypoparathyroidism should be ruled out when diagnosing a Fahr's syndrome. Restoring normocalcemia improves cramps and myoclonus; however, it has little effect on mood or parkinsonism.

## P521

### Antagonism between body weight and adipocytokines on bone mass acquisition in pre- and postmenopausal women

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#### Introduction

Body weight is positively correlated to bone mineral density. The exact mechanism is unclear but gravity may be one of the involved factors. Adipocytokines (leptin and adiponectin), secreted by the adipose tissue, are involved in bone turnover. We investigated the influence of body weight, body composition, and adipocytokines upon bone mass in pre and postmenopausal women.

#### Design

Cross-sectional study including six groups of 8 to 16 healthy female volunteers of different ages, pre and post menopausal, and weights (lean BMI < 25 kg/m<sup>2</sup>, overweight BMI 25–30 kg/m<sup>2</sup>, and obese BMI > 30 kg/m<sup>2</sup>). Excluding criteria were chronic disease and/or medication which could influence BMD. Lumbar BMD and BC were evaluated by DXA (Hologic). Serum leptin, adiponectin were evaluated by ELISA.

#### Results

Owing to the age heterogeneity, we have used Z score. Lumbar Z score was well correlated to body weight and body compartments. The best correlation was attained with lean mass, that was an independent predictor of bone mass irrespective of age ( $r^2=0.4671$ ) and weight ( $r^2=0.2918$ ).

Lean and postmenopausal women had lower BMD than premenopausal women ( $P<0.05$ ). Bone mass of obese postmenopausal women did not differ from that of premenopausal women ( $P>0.5$ ).

Bone mass was negatively correlated to leptin normalised to fat mass ( $r^2=0.2128$ ) and adiponectine ( $r^2=0.1975$ ).

#### Conclusions

Increased body weight prevents bone loss in postmenopausal women. Lean mass predicts bone mass independently of body weight, irrespective of age. The beneficial role of fat mass and total body weight on bone mass through gravitational stress seems to be partially buffered by direct effects of adipokines on bone.

## P522

### Relationship between benign calcification of breast and lumbar bone mineral density

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#### Introduction

There are many reports that vascular and/or heart valve calcification is good predictor for low bone mineral density. I investigated whether benign calcification of breast can also well predict low bone mineral density.

#### Methods/design

I have reviewed and analyzed the records of 91 women with age more than 50 years old among patients examined bone mineral density and breast mammography from January through December 2010. The lowest value of lumbar bone mineral density was selected and analyzed, but femoral bone mineral density was not analyzed due to no routine examination and frequent omission.

#### Results

On breast mammography, benign calcification was observed in 21 patients (group A) and was not observed in 70 patients (group B). Lumbar bone mineral density was  $0.832 \pm 0.150$  g/cm<sup>2</sup> on the average with benign calcification and  $0.782 \pm 0.141$  g/cm<sup>2</sup> without benign calcification. There was not a significant difference between group A and B ( $P=0.169$ ). Average age of patients of group A and B was  $59.9 \pm 6.1$  years and  $61.1 \pm 5.8$  years respectively, which had no significant difference ( $P=0.4$ ). In addition, there was not a significant difference between group A and B for body weight, body mass index, waist circumference, smoking habitus, alcohol drinking habitus and physical activity index ( $P>0.05$ ).

#### Conclusion

Lumbar bone mineral density with benign calcification on breast mammography was not statistically different compared to that without calcification. In the future there is a need for prospective, greater mass study.

## P523

### Vitamin D and bone metabolism in adult patients with cystic fibrosis

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#### Objective

To evaluate Vitamin D levels and bone metabolism in adult patients with cystic fibrosis (CF).

#### Patients and methods

One hundred patients with CF (53 F, 47 M; age  $29 \pm 8$  years), and 100 healthy controls were enrolled. Patients were assessed through clinical, and laboratory investigations. Evaluation of serum 25 hydroxy vitamin D (25OHD), vertebral fractures (quantitative morphometric analysis) and bone mineral density (BMD) at lumbar spine and total hip (dual energy X-ray absorptiometry) was done.

#### Results

Serum 25 OHD vitamin D was lower in patients than in controls ( $P<0.001$ ). Vitamin D deficiency (<10 ng/ml) was observed in 14%, vitamin D insufficiency (10–30 ng/ml) in 47% and normal value of vitamin D (30–100 ng/ml) in 39% of CF patients. Osteoporosis was found in 15%, while osteopenia in 28% of CF patients. Normal values of BMD was found in 57% of them. Furthermore, in 10 patients we evaluated vertebral fractures: the fractures were mild in two patients (20%), moderate in five (50%), and severe in two patients (20%). 25OHD vitamin D levels was not related to T and Z score at lumbar spine and femoral neck ( $P=0.431$ ;  $P=0.487$ ;  $P=0.629$ ;  $P=0.849$ , respectively).

#### Conclusions

This study reports a high prevalence of vitamin D deficit and osteoporotic vertebral fractures in adult patients with CF, suggesting that complicated osteoporosis is an important comorbidity of CF patients. Adequate supplementation with vitamin D and treatment for osteoporosis could improve the quality of life of these patients.

## P524

### Association of the A1330V and V667M polymorphisms of LRP5 with bone mineral density in Greek peri- and postmenopausal women

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Wnt signaling through low-density lipoprotein receptor-related protein 5 (LRP5) is an important determinant of bone mass regulation. Polymorphisms in the LRP5 gene have been associated with either osteoporotic phenotypes or normal bone mineral density (BMD) variation.

#### Objective

To explore the influence of two LRP5 single nucleotide polymorphisms (SNPs) A1330V and V667M on BMD and serum levels of osteoprotegerin (OPG), receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) and bone metabolic markers in a Greek female population.



**Study design**

Two hundred and twenty-one peri- and postmenopausal women aged 40–63 years were enrolled. All participants underwent spinal BMD evaluation. Genotyping of A1330V and V667M polymorphisms was performed by real-time polymerase chain reaction. Levels of OPG, soluble RANKL (sRANKL) and bone metabolic markers were measured.

**Results**

Both SNPs were significantly associated with low spinal BMD. As regards A1330V SNP, women carrying CT/TT genotypes had lower spinal BMD than women with CC ( $P < 0.0001$ ). Regarding V667M SNP, spinal BMD was lower in women with GA/AA genotypes than in women with GG ( $P < 0.0001$ ). The associations remained significant after adjustment for age, years since menopause (YSM) and body mass index (BMI). The two SNPs were in strong linkage disequilibrium. A significant interaction between A1330V and V667M SNPs on spinal BMD was revealed. No effect was observed on circulating OPG, sRANKL levels and bone metabolic markers even after adjustment for age, YSM and BMI.

**Conclusions**

These findings demonstrate that A1330V and V667M polymorphisms of LRP5 gene are strongly implicated to osteoporosis susceptibility in peri- and postmenopausal Greek women.

**P525****Efficacy of Alendronate treatment in women with postmenopausal osteoporosis: results after 3-year study**

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**Introduction**

In Romania, the prevalence of postmenopausal osteoporosis in urban centers was estimated by a study in 2007 to be 16.5%. Using alendronate we are increasing bone mineral density and reducing bone turn-over in women with postmenopausal osteoporosis.

**Materials and methods**

There were examined 55 postmenopausal women with osteoporosis (average age  $62 \pm 4.5$  years). Bone mineral density (BMD) was determined by dual-energy X-ray absorptiometer (HOLOGIC Delphi W; SN 7044909) on lumbar spine. Examination was performed before the onset of treatment and after a period of 1, 2 and 3 years of treatment. Alendronate was taken in a dose of 70 mg weekly. The patient also received 1000 mg of calcium and 800 IU Vit D daily.

**Results**

Alendronate (FOSAMAX) significantly increase BMD at lumbar spine after one year of therapy (with 1.9%) with further increase after two and three years of treatment (2.44 and 0.8%). In this study we had no serious side-effects or significant gastro-intestinal adverse effects. Also, no fractures were encountered during this period.

	Baseline	1 year	2 years	3 years
BMD (g/cm <sup>2</sup> )	0.723	0.737	0.755	0.761
%		↑ 1.9%	↑ 2.44%	↑ 0.8%

**Conclusion**

Our study suggest that alendronate therapy is effective and well tolerated in the treatment of women with established postmenopausal osteoporosis, but we need full support from the patient and long term-compliance.

**P526****Bisphosphonates therapy in women with post-menopausal osteoporosis: comparative results**

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**Aim**

To evaluate the effect of risedronate vs ibandronate in treatment of systemic osteoporosis in postmenopausal women.

**Materials and methods**

We examined 57 postmenopausal women with systemic osteoporosis (average age  $61 \pm 3.5$  years). Bone mineral density (BMD) was determined by dual-energy

X-ray absorptiometer (HOLOGIC Delphi W; SN 7044909) on lumbar spine. Examination was performed before the onset of treatment and after a period of 1, 2 and 3 years of treatment. Risedronate was taken in a dose of 35 mg weekly by 24 patients, while ibandronate 150 mg once a month by 33 patients. The women also received 1000 mg of calcium and 800 IU Vit D daily.

**Results**

After the first year of treatment, we observed an increase of 3.65% in BMD in risedronate group and a 4.7% in ibandronate group. The main difference came after 2 years of treatment, when, in the ibandronate group, we noticed a decrease in BMD by 4.25%, comparing with the other group (possible explanation – lack of compliance). In the third year there was an increase in both groups. No fractures were reported during this period.

	Baseline	1 year	2 years	3 years
Risedronate				
BMD (g/cm <sup>2</sup> )	0.767	0.795	0.789	0.796
%		↑ 3.65%	↓ 0.76%	↑ 0.9%
Ibandronate				
BMD (g/cm <sup>2</sup> )	0.741	0.776	0.743	0.769
%		↑ 4.7%	↑ 4.25%	↑ 3.49%

**Conclusion**

Our experience shows that both risedronate and ibandronate are efficient against post-menopausal osteoporosis, with minimal side-effects.

**P527****Experience with Cinacalcet in primary hyperparathyroidism: results from the Swiss primary hyperparathyroidism cohort study**

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**Objective**

Evaluate cinacalcet use and effect based on follow-up within a cohort study of recently diagnosed Primary Hyperparathyroidism (PHPT) patients. Characteristics, treatment indications and biochemical data are presented with measures of cinacalcet efficacy and safety.

**Methods**

The Swiss Primary Hyperparathyroidism Cohort Study is a prospective, non-interventional project to collect clinical, biochemical and outcome data in patients with PHPT initiated in June 07. It is multicentric, including 253 patients in 15 centres in Switzerland. Patients started on cinacalcet were identified and the indication for treatment recorded. We compared pre and post treatment calcium and parathyroid hormone levels (PTH).

**Results**

From June 2007 to December 2010, 30 patients were treated with the cinacalcet. Indications for treatment were: 3 patient refusals of parathyroidectomy (PTX), 14 preoperative (4 symptomatic), 4 recurrences /persistences post-PTX, 3 symptomatic hypercalcemia patients, 1 case where surgery was contraindicated, 4 to decrease serum calcium levels and 1 missing. Cinacalcet patients were younger than cohort population (mean age  $\pm$  SD:  $63.2 \pm 16.1$  vs  $69.5 \pm 14.1$  years). Median ( $\pm$  IQR) pre-treatment calcium was significantly higher at  $2.83 \pm 0.36$  mmol/l amongst cinacalcet recipients versus  $2.66 \pm 0.27$  mmol/l ( $P = 0.02$ ). PTH levels tended to be higher at  $16.6 \pm 9.3$  vs  $11.7 \pm 7.8$  pmol/l. The median cinacalcet start dose was  $30 \pm 30$  mg/day, thereafter adapted to 30 to 420 mg. Median post-treatment calcium decreased significantly to  $2.72 \pm 0.5$  mmol/l ( $P = 0.0005$ ). PTH levels decreased insignificantly to  $14.3 \pm 4.7$  mmol/l. Forty-five and ten percent of patients had normalised serum calcium and PTH levels, respectively. Adverse effects were observed in 3 subjects: hypocalcemia, muscle cramps and nausea.

**Conclusion**

Treatment with cinacalcet is an effective and safe way of reducing calcium levels pending surgery or when surgery is contraindicated.

**P528****Diabetes mellitus and the incidence of fracture**

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**Objective**

To study if people with diabetes mellitus (DM) have increased risk of fracture.

**Methods**

The study population consisted of 29 512 people aged 50 years or more. There were 1981 diabetic patients among them. The records of the local trauma clinic for two-year period were analyzed.

**Results**

Women with DM had fewer fractures during two years than women without DM (relative risk 0.43; 95% confidence interval 0.30–0.61). The frequency of fractures in men was similar for those with and without DM (RR 0.97; 95% CI 0.59–1.60). We haven't also found an increased risk of hip fracture (RR 0.7, 95% CI 0.31–1.61) in diabetic people compared with non-diabetic population. However, the incidence of multiply or repeated fractures was significantly higher in diabetic population (1.5% vs 0.3% in non-diabetic one), it resulted in 5-fold higher risk of these fractures for DM (95% CI 1.94–12.89).

**Conclusion**

Our data indicate that patients with diabetes don't have an increased total fracture risk. DM in women is associated with a lower frequency of fracture, which could be explained by low physical activity of these patients. Nevertheless, the increase of multiply/repeated fracture risk indicates poor quality and/or higher risk of traumatic falling in diabetic population.

**P529****The postprandial response of bone turnover in  $\beta$ -thalassemia major**

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**Background**

Osteoporosis is considered to be an important cause of morbidity in patients with  $\beta$ -thalassemia major. Despite recent advances in the identification of the molecular pathways involved in the thalassemia-induced osteoporosis, less is known about the responses of the thalassaemic skeleton to various physiological and pathological conditions. The aim of the present study was to evaluate the postprandial response of bone turnover, which is an important component of the circadian rhythm of bone remodeling, in thalassaemic patients.

**Design/methods**

Thirty-two adult patients with  $\beta$ -thalassemia major (10 male, 22 female, aged  $33.09 \pm 7.81$  years) and thirty-one healthy individuals matched for age were included in the analysis. Exclusion criteria were concomitant diseases and medication that affect bone metabolism. All participants underwent an oral glucose tolerance test (OGTT) with 75 g of glucose after an overnight fast. Serum carboxy-terminal collagen crosslinks (CTX), procollagen I N-terminal propeptide (S-P1NP) and osteocalcin were assayed. All results are presented as mean  $\pm$  S.E.M.

**Results**

Baseline values of CTX and P1NP were significantly higher in the patient group compared to control ( $0.45 \pm 0.046$  vs  $0.34 \pm 0.18$   $\mu\text{g/l}$ ,  $P=0.049$  and  $56.42 \pm 7.08$  vs  $40.12 \pm 2.80$  ng/ml,  $P=0.039$ , respectively). At two hours after the glucose load, CTX levels demonstrated a significant reduction compared to baseline values ( $0.25 \pm 0.02$  vs  $0.45 \pm 0.04$   $\mu\text{g/l}$ ,  $P<0.001$ ), while markers of bone formation did not change significantly. The postprandial reduction of CTX was comparable to the reduction observed in the control group ( $43.6 \pm 0.02\%$  vs  $45.3 \pm 0.02\%$ ,  $P=0.684$ ).

**Conclusion**

Despite the high bone turnover state in thalassaemic patients, the postprandial reduction of bone resorption remains unaltered, suggesting that the physiological responses to nutrients are conserved in the thalassaemic skeleton.

**P530****Recent changes in clinical presentation and therapeutic approach of primary hyperparathyroidism in MEN1 patients**

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**Introduction and aim**

Primary hyperparathyroidism (PHP) is the most frequent endocrine abnormality in MEN1 affecting about 100% of cases and inducing severe bone and kidney complications. Although surgery represents the only curative approach in MEN1 PHP, novel drugs are now available to effectively control PHP. The objective of this study was to show the recent changes in clinical presentation and therapeutic approach in MEN1-related PHP occurred in the last years.

**Patients and methods**

The study population included 27 consecutive patients with MEN1 related-PHP in follow-up between 1990 and 2010. The differences in clinical presentation of PHP and type and outcome of the therapeutic approaches for PHP between patients diagnosed before 2008 (13) and those diagnosed in 2008–2010 (14) were investigated.

**Results**

In the subgroup before 2008, 31% were asymptomatic, while 69% presented nephrolithiasis and/or osteoporosis; in the subgroup 2008–2010 64% were asymptomatic while 36% presented nephrolithiasis and/or osteoporosis.

Total parathyroidectomy was performed in 31% of cases diagnosed before 2008 and was never performed in those diagnosed in 2008–2010, while partial parathyroidectomy was performed in 46% of patients diagnosed before 2008 and in 14% of those diagnosed in 2008–2010. Medical therapy consistent with cinacalcet was administered to 7 patients diagnosed before 2008 (54%), while it was administered to 12 patients diagnosed in 2008–2010 (86%).

In all of patients treated with cinacalcet, serum calcium levels were normalized and PTH levels significantly decreased. No patient treated with total parathyroidectomy relapsed, however, all of them developed hyperparathyroidism. All but one of the patients undergone partial parathyroidectomy relapsed after surgery.

**Conclusions**

Clinical presentation of PHP is recently changed in patients with PHP. Based on the attenuation in the severity of clinical manifestations and on availability of an effective medical therapy, the therapeutic management of these patients is suggested to switch from surgery to medical therapy.

**P531****Restitution of severe bone changes after successful treatment of primary hyperparathyroidism**

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Primary hyperparathyroidism is an autonomous form of hyperparathyroidism, whose characteristics are: increased secretion of parathyroid hormone with clinical signs of hypercalcaemic syndrome and various organ lesions, including bones.

**Case report**

A 51-year-old Caucasian female presented with walking difficulties, polyuria, polydipsia, palpitations and headaches. Examination showed thoracic spine hyperkyphosis with consecutive chest deformity, staggered walk, loose teeth and severe periodontal disease. Using basic diagnostic procedures the diagnosis of primary hyperparathyroidism (total serum calcium: 2.62 mmol/l, phosphorus: 0.51 mmol/l, PTH: 1721 pg/ml) with dominant bone lesions in the form of cystic von Recklinghausen osteodystrophy was made. Kidneys and gastrointestinal tract were intact. Hand X-ray showed marked diffuse osteoporosis with reduction of bone elements; edge defects due to lacunar resorption of corticalis dominantly in the middle phalanges; bone fractures; thinner ungular extensions; 'bubble' structure due to group of cysts with thin septa predominantly on the V metacarpal bone, giving the look of bone inflates; the presence of brown tumors. Primary hyperparathyroidism was treated surgically (adenoma of left inferior parathyroid gland). Calcium and vitamin D supplementation was administered until correction of hypocalcaemia with normalization of PTH levels. X-ray of hands 7 months after the initiation of therapy: reparative changes were dominant with phalangeal deformities and hyperostosis. Hand X-ray 20 months later: filling of bone defects is complete, reduced inflates of V metacarpal bone, the picture of old

fractures with mild bone deformities, gradual reduction of periosteal lacunar resorptions with sclerotic margins, osteoporosis. Hand X-ray after 6 years: marked diffuse osteoporosis; inflates of V metacarpal bone with anarchic distribution of bone elements and thinner corticalls, small pseudo cystic attenuation. Other earlier seen changes are not present.

#### Conclusion

The restitution of severe bone lesions in primary hyperparathyroidism may take several years after successful surgery.

### P532

#### Intrathymic localisation of parathyroid adenoma: case report

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#### Introduction

The most common localization of parathyroid glands is behind the thyroid gland poles. According to different authors ectopic localization is present in 37–45.7%.

#### Case report

Caucasian male, 31 years old, with elevated serum calcium was sent to an endocrinologist after pyelolithotomy. He has the history of ulcerative colitis treated with anti-inflammatory therapy. Laboratory analysis: total serum calcium: 2.85 mmol/l, phosphorus: 0.63 mmol/l, alkaline phosphatase: 137 J/l, parathyroid hormone (PTH): 114 pg/ml, osteocalcin: 90.0 ng/ml, cross laps: 1447.0 pg/ml. Spine DXA *T*-score: -2.8; *Z*-score: -2.9. Laboratory analysis suggested diagnosis of primary hyperparathyroidism. Ultrasonography did not show enlarged parathyroid glands. Two-phase parathyroid gland scintigraphy (99mTc-MIBI) with SPECT of the thorax was done. The result was suggestive for the ectopic parathyroid adenoma localized in the anterior mediastinum. CT examination of the neck and thorax showed the formation of 12 × 12 mm in aortopulmonary window, along the pulmonary artery main stem, which could correspond to ectopic parathyroid glands. CT angiography of the thoracic aorta described oval soft tissue nodule 8 mm in diameter at the same location, which could correspond to ectopic parathyroid or lymph gland. The patient was referred to surgical treatment. Intraoperatively glandular formation ~1 cm in diameter was found in thymic tissue in upper anterior mediastinum. Ex-tempore and further pathohistological analysis confirmed intrathymic parathyroid adenoma. Substitution of calcium and vitamin D was introduced until achieving normal levels of serum calcium and PTH levels (total calcium: 2.50 mmol/l, PTH: 36.3 pg/ml). Other controls in the same period: osteocalcin: 13.13 ng/ml, cross laps: 108.9 pg/ml, DXA of the spine after 13 months *T*-score: -1.3; *Z*-score: -2.0.

#### Conclusion

This case is presented because of relatively rare localization of parathyroid gland adenoma.

### P533

#### Thyroid gland dysfunction and osteoporosis in elderly people

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Osteoporosis is a chronic noninfectious disease, which changes the structure and mineral density of bones, causing fractures after small trauma, deformities and disability. Every minute two breakages happen, due to osteoporosis. Thyroid gland dysfunction could disturb the bone remodelling process.

The aim of this study is to check the bone quality in females suffering from thyroid diseases. This prospective study included 164 women of the average age of 74 ± 0.48 (range: 60–90). None of them have taken hormone replacement therapy during the menopause.

The bone mineral density has been defined by the method of densitometry, with double absorptiometry of X ray (DXA) using Hollogix Explorer instrument (S/N 91038) on lumbar spine (L1–L4), hip and thigh bone neck.

We investigated thyroid hypophyseal hormonal axis, and parameters indicating the quality of calcium metabolism (parathormone (PTH), ionized calcium (Ca<sup>++</sup>), 25OHD<sub>3</sub>).

In 43/164 (26%) patients, thyroid gland dysfunction was proved. In this group of patients 21/164 (13%) have been diagnosed as hyperthyroid, but 22/164 (13%) of them suffered from hypothyroidism.

Densitometry (DXA) has showed spine osteoporosis but worse finding has been confirmed in hip trabecular bone (*T* score L1–L4: -3.03 s.d. ± 0.07 and -3.39 s.d. ± 0.14 respectively).

In 22/164 elderly women hypothyroidism has been proved. Their trabecular bone is osteoporotic both of spine and hip (-2.87 s.d. ± 0.02, and -2.99 s.d. ± 0.24 respectively), while in those suffering from hyperthyroidism deterioration of trabecular bone is higher (-3.2 s.d. ± 0.04, and -3.40 s.d. ± 0.24 respectively). Deficiency of 25OHD<sub>3</sub> has been confirmed both in hyper and hypothyroidism.

We have to recommend all aspects of any bone changes prevention, i.e. osteopenia and osteoporosis.

### P534

#### Dietary fructose inhibits lactation-induced adaptations in rat calcitriol synthesis and calcium transport

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An adequate intestinal uptake of calcium is critical for the maintenance of calcium homeostasis during pregnancy and lactation. We previously found in rat models of end-stage renal disease that dietary fructose reduces intestinal calcium absorption and perturbs kidney morphology. Despite the dramatic rise in fructose consumption and the evidence of its deleterious effects for adult health, there is no information about fructose effects on maternal health and pregnancy outcomes. We investigated the interactions between pregnancy/lactation and dietary fructose, to evaluate if fructose compromises intestinal calcium absorption and homeostasis and whether calcitriol (active form of vitamin D and one of the main regulators of calcium transport) mediates this effect. Three groups of pregnant rats and virgin controls were fed isocaloric 63% glucose, fructose or starch diets during gestation and lactation. Excessive fructose intake prevents lactation-induced increases in intestinal calcium transport, in intestinal expression of calcium transporting and binding proteins (TRPV6 and CaBP9k), and in calcitriol plasma level. The binding of VDR, the transcription factor mediating the activity of calcitriol, to the promoters of *TRPV6* and *CaBP9k* genes was also significantly reduced in dams fed fructose. Changes in calcitriol levels were tightly correlated with alterations in expression of the 1 $\alpha$ -hydroxylase but not 24-hydroxylase, indicating that excessive fructose intake perturbs specifically the final step in calcitriol synthesis. Bone mineral density and content as well as mechanical strength decrease with lactation, and dietary fructose and glucose significantly exacerbate these effects. Thus, we showed that over-consumption of fructose during pregnancy and lactation impairs renal production of calcitriol which leads to a decrease in active intestinal calcium transport due to lowered expression of calcium transporters in duodenum. These results are all the more important since there is evidence that the current daily recommendations for vitamin D intake may be inadequate to ensure calcitriol sufficiency in humans.

### P535

#### Prevalence of metabolic bone disease in Wilson's disease (WD) patients

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#### Background

Skeletal changes including demineralization have been reported in WD. The exact mechanism originating these changes isn't completely understood but some authors have proposed a link to vitamin D (VitD) metabolism.

#### Aim

To investigate the prevalence of demineralization in a cohort of WD patients and correlate it with VitD status and severity of hepatic and neurological involvement.

#### Methods

Thirty-five patients (17 males and 18 females), mean age of 35.1 ± 14.0 years, have done a complete liver profile, calcium, phosphorus, vitD and bone densitometry. Osteopenia and osteoporosis were defined by WHO criteria. VitD deficiency was defined as levels < 50 nmol/l and insufficiency between 50 and 74 nmol/l. Hepatic and neurological disease was evaluated by MELD score, EuroWilson scale (EW) and functional independence measure (FIM). Statistical analysis was performed using SPSS. The significance level was established at 0.05.

#### Results

A total of 40% of the patients presented osteopenia and 13% osteoporosis. 63% had deficient and 27% insufficient VitD levels. Significant correlations were found between *T*-score at the femoral neck, EW and FIM (*r* = -0.55, *r* = 0.47); *T*-score at lumbar spine, MELD, AST and ALP levels (*r* = -0.42, *r* = -0.39, *r* = -0.36).

After multiple regression EW was significantly associated with decreased mineralization at femoral neck and MELD score and ALP levels were significantly correlated with demineralization at lumbar spine. It was found a significant correlation between VitD levels, AST and ALT levels ( $r = -0.36$ ,  $r = -0.60$ ), but there wasn't significant association between VitD and severity of hepatic or neurological disease.

#### Conclusion

Decreased bone mineralization in our WD population doesn't seem to be associated with low VitD levels, but to the degree of hepatic and neurologic impairment. We didn't find any correlation between VitD deficiency and severity of neurologic or hepatic disease. We advocate that the high rates of demineralization and VitD deficiency found are significant and it is therefore advisable to evaluate and treat these two issues in this population.

### P536

#### Total parathyroidectomy without autotransplantation in the surgical treatment of 'refractory' renal hyperparathyroidism

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#### Background

The optimal surgical technique in patients with refractory renal hyperparathyroidism (RHP) on hemodialysis for end stage renal disease is still a point of debates.

The high percentage of recurrences after standard surgical procedures, i.e. subtotal parathyroidectomy (SPtx) and total parathyroidectomy with autotransplantation (TPtx + At) reactualised the practice of total parathyroidectomy (TPtx).

#### Patients and methods

Forty-three patients with RHP underwent surgery in the last 16 years period. There were 24 SPtx, 6 TPtx + At, both procedures determining 6 recurrences (20.7%) so in the last years TPtx was performed in a series of 13 cases (7 males and 6 females, with median age 43.6 – range 22–65 years, and median dialysis time before PTx 8.2 – range 3–13 years. Parameter studies included demographics, preoperative and follow-up laboratory tests, surgical techniques, pathology results and postoperative immediate and medium term results.

#### Results

Main indications for TPtx were severe bone disease, soft tissue calcifications, neuromuscular phenomena, grossly elevated iPTH and sometimes hypercalcemia. TPtx was done in 12 patients, the 13th one suffering a completion PTx 1 year after outward exeresis of only two glands. Postoperatively the majority of symptoms markedly improved and the values of calcemia, phosphatemia, and alkaline phosphatase normalised together with low or no measurable level of iPTH. One patient required a reexploration for cervical hematoma but no one presented permanent hypocalcemia or recurrent hyperparathyroidism. Pathology revealed nodular hyperplasia in all the cases, a parathyroid carcinoma of one gland and also a incidentally thyroid papillary microcarcinoma in a completion thyroidectomy.

#### Conclusions

TPtx alone proves to be an equally safe and successful as another techniques currently used in the management of RHP eliminating the hyperparathyroid status but being superior with regard of recurrences. The procedure is indicated especially in cases with aggressive, refractory forms of RHP without the prospect of renal transplantation.

### P537

#### The efficacy of four monthly 200 000 IU vitamin D<sub>3</sub> supplementation to treat vitamin D deficiency in institutionalized elderly

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#### Introduction

Vitamin D deficiency has been frequently observed in the elderly population. Improving vitamin D status may be an important modifiable risk factor to reduce fractures; however, adherence to daily supplementation is poor. We tested whether oral administration of an alcoholic solution of vitamin D<sub>3</sub>, 200 000 IU every 4 months in elderly could assure an optimal serum 25(OH)D concentration of 80–120 nmol/l.

#### Method

We enrolled 50 institutionalized elderly (70% women, age 72.7 + 6.8 years; 30% men, age 72.1 + 5.8 years) living in a nursing home for elderly people in Iasi, Romania (latitude: 47°N). Using a factorial design, we randomly allocated subjects to cholecalciferol therapy or placebo.

#### Results

At baseline, 25(OH)D levels were 25.5 ± 16 nmol/l and 60% of participants had of <25 nmol/l. Cholecalciferol treatment using an alcoholic solution of vitamin D<sub>3</sub>, 200 000 IU every 4 months did not succeed to rise 25(OH)D levels above 80 nmol/l. The 25(OH)D levels were 34 ± 12 nmol/l at 4 months, 45 ± 21 nmol/l at 8 months and 49 ± 19 nmol/l at 12 months.

#### Conclusion

In elderly, the correction of low status of vitamin D<sub>3</sub> require doses above 2000 IU/day. In our study, the oral administration of vitamin D<sub>3</sub>, 200 000 IU every 4 months (equivalent of oral doses of vitamin D<sub>3</sub> 2000 IU/day) may be a convenient way to maintain sufficient vitamin D status but failed to establish the optimal levels of serum 25(OH)D concentrations.

### P538

#### Recurrent primary hyperparathyroidism

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Recurrent primary hyperparathyroidism (RPHPT) is defined as hypercalcemia occurring after 6 months of normocalcemia following initial operation. The aim of this study was to analyze our experience in RPHPT.

Between 1980 and 2010 33 patients (1.2%) with primary hyperparathyroidism (HPT1) were reoperated in our tertiary referral centre for recurrence of disease: 24 adenomectomies, six subtotal parathyroidectomies, two total parathyroidectomies with autotransplantation and one negative exploration. Twenty-five patients had their primary intervention in our department.

Mean age at primary and secondary intervention was 50 and 63 years respectively. Mean time till recurrence was 12.8 years, not significantly different in uni- or multiglandular disease. In 22 patients contributing factors for recurrence could be identified: eight had a genetic compound (positive familial history, MEN-1 syndrome), eight had previous medical history of cervical radiotherapy, three cases of parathyroid carcinoma and three surgery itself (twice parathyreomatosis, one devascularised but not-identified adenoma). In 11 patients no risk factor was identified of which five had a metachronous adenoma. Mean calcium was preoperatively 2.71 mmol/l and PTH 120 pg/ml and postoperatively 2.25 mmol/l with PTH 27.5 pg/ml. Positive findings on ultrasound and MIBI-scintigraphy allowed for eighteen focussed surgical approaches. Morbidity following secondary intervention included one vocal cord palsy due to oncologic resection. Four patients (13%) were treated for temporary hypocalcemia and three patients (9.7%) had definitive hypoparathyroidism. Six patients were not cured, of which three patients died eventually from parathyroid carcinoma.

We may conclude that RPHPT is a rare disease. In 67% of the cases predisposing factors can be identified that should initiate prolonged follow-up of the patient. In contrast with persisting HPT1, recurrent HPT1 is rarely due to surgical failure.

### P539

#### Vitamin D and dark skin

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Vitamin D is synthesized in the skin. Low vitamin D levels have been observed in African Americans. However, the relationship between vitamin D and skin color has not been completely evaluated.

The aim was to study the relationship between dark skin color and vitamin D levels in healthy individuals.

Vitamin D (25(OH)D<sub>3</sub>) levels were measured in 15 dark skinned individuals. All subjects were immigrants, having migrated to Greece for financial reasons. They had migrated to Greece at least a year before the study, thus residing in Greece for at least a year before vitamin D measurement. Immigrants were from Egypt and the Middle East. However, they were not wearing the traditional clothes covering the whole body, having already adapted to the clothing of the western civilization. Vitamin D, 25(OH)D<sub>3</sub> levels were measured by RIA.

All individuals had low vitamin D<sub>3</sub> levels, mean 25(OH)D<sub>3</sub> levels being 13.6 ng/ml, range 11–17 ng/ml, s.d. 1.4.

Vitamin D was found to be low in all dark skinned individuals studied. Vitamin D deficiency observed in the immigrant population studied may be related either to dark skin color or to nutritional deficiency. Dark skin may protect the skin from the photon energy thus protecting it from carcinogenesis, preventing however vitamin D synthesis.

## P540

### Comparison of oral versus intramuscular treatment of vitamin D deficiency or insufficiency

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#### Background

Different dosing protocols have been used for vitamin D supplementation, but there has been a lack of comparative data among them. This clinical trial study was aimed to compare the efficacy of two different regimens of vitamin D supplements on serum 25(OH)D<sub>3</sub> level, at the end of a 3 months therapy period.

#### Design

Eighty-four cases of vitamin D deficiency or insufficiency who did not meet exclusion criteria were randomized to two different vitamin D supplementation protocols as pearl 50 000 IU weekly for one month then monthly for 2 months or injection of 300 000 IU vitamin D during the 3 months for whom had insufficient level of vitamin D, while the deficient ones were under treatment of pearl 50 000 IU weekly for 2 months and one for another month or injection of 300 000 IU vitamin D during the 3 months. The primary outcome measure was the serum 25-hydroxyvitaminD (25(OH)D) concentration 3 months after onset of treatment.

#### Results

Pretreatment level of vitamin D was not different meaningfully in oral and injection methods but the vitamin D level after treatment in oral method was higher than in injection one. ( $P=0.023$ ). In addition, at the end of the treatment 23.8% (10) of the patients on oral therapy did not receive to sufficient level of vitamin D while in the injection method this feature was 42.9% (18) ( $P=0.064$ ). Furthermore oral therapy had better effect on serum 25(OH)D<sub>3</sub> level in overweight persons ( $P=0.046$ ).

#### Conclusions

Based on the results, we concluded, oral method had better effect on serum 25(OH)D<sub>3</sub> level, 3 months after therapy than injection route.

## P541

### Outcomes of parathyroidectomy with autotransplantation in nephrogenic secondary hyperparathyroidism

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#### Background

One of the major complications developed by patients with end stage renal disease (ESRD) is secondary hyperparathyroidism (SHPT), due to hypocalcaemia, hyperphosphatemia and 1,25(OH)<sub>2</sub>D deficiency with parathyroid hyperplasia and high PTH levels as earliest changes, which may require parathyroidectomy (PTX).

#### Study design and objectives

This study aimed to evaluate the indications, the particularities and outcomes post-PTX of patients suffering from secondary hyperparathyroidism due to chronic kidney disease (CKD).

#### Patients and methods

A total of 196 patients, 112 F (57.14%), aged 51.66 ± 11.27 (ranged 21–81 years) with hyperparathyroidism and ESRD underwent PTX with (194 patients) or without (2 patients) autotransplantation from 1999 to 2009.

#### Results

Duration of the ESRD was 8.76 ± 5.06 years. The ESRD was treated by hemodialysis in 65.82% cases. A total of 16 cases were submitted to kidney transplantation with a rejection in 10 cases. At the time of the PTX, 6 patients were with kidney transplantation. The medical therapy of SHPT was vitamin D and phosphate binders, calcimimetics were not used in this group. The increased parathyroid volume was evaluated by ultrasonography (sensitivity 92.45% versus pathology), in 159 patients. Tertiary HPT was diagnosed in 53 (27.04%) patients despite medical treatment. The evaluation was performed 24 h before and after surgery. Preoperative values were for PTH = 1721.84 ± 839.84 pg/ml, Ca = 9.94 ± 1.28 mg/dl and P = 6.38 ± 1.90 mg/dl; Ca x Ph product above 75 mg<sup>2</sup>/dl<sup>2</sup> was found in 23.97% ( $n=47$ ) cases. Surgery was performed by removing all parathyroid glands but one which was dissected to a equivalent volume of a normal parathyroid. Autotransplantation consisted in placement of this remnant parathyroid in the jugular notch area, by keeping the vascular supply from the cervical vessels. PTX effect was the decrease of PTH values to 48.13 ± 58.18 pg/ml, calcium to 6.93 ± 1.29 mg/dl and phosphate to 4.90 ± 1.90 mg/dl, while the product Ca x Ph decreased in 195 cases under 75 mg<sup>2</sup>/dl<sup>2</sup>. Postoperative evolution was uneventful in all cases, but the global prognosis is decided by the kidney disease.

#### Conclusion

Parathyroidectomy in ESRD is an important part of management in secondary or tertiary HPT, cervical autotransplantation being an useful surgical alternative.

## P542

### Pseudohypoparathyroidism and GNAS epigenetic alterations: quantitative analysis of methylation defects and correlation with clinical characteristics

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The two main subtypes of pseudohypoparathyroidism (PHP), PHP-Ia and -Ib, are caused by mutations in GNAS exons 1–13 and methylation defects in the imprinted GNAS cluster, respectively. PHP-Ia patients show Albright hereditary osteodystrophy (AHO) and resistance toward multiple hormones (PTH/TSH/GHRH/gonadotropins), whereas PHP-Ib patients classically display hormone resistance limited to PTH and TSH. Incoming data suggest that the two diseases share more genetic and clinical similarities than previously thought. In particular, methylation defects have been detected in a subset of patients with PHP and AHO, but the correlation between molecular findings and the severity of the disease has not been investigated yet.

In the present study, we included 30 patients with either classical PHP-Ib ( $N=16$ ) or PHP with different degrees of AHO ( $N=14$ ), all found to have broad GNAS methylation defects in the absence of mutations. Differential methylation of GNAS DMRs A/B, AS, XL, NESP was assessed using highly quantitative analysis based on PCR-pyrosequencing. The data obtained were correlated with the following clinical characteristics: age at diagnosis, endocrine function (PTH, TSH, FT<sub>4</sub>, calcium, phosphorus levels) and the presence or absence of AHO signs (short stature, brachydactyly, round face, ectopic ossifications, mental retardation).

No statistical difference was observed between the group of patients with classical PHP-Ib and the group with PHP plus AHO. In particular, the degree of the imprinting defect (percentage of methylation at each DMR expressed in respect with a pool of 20 normal subjects age- and gender-related) did not correlate with the onset of the disease, the severity of endocrine resistances, nor with the presence/absence of specific AHO signs.

In conclusion, similar molecular alterations may lead to a broad spectrum of diseases, from isolated PTH resistance to complete PHP-Ia. Our study further confirms the existence of an overlap between molecular and clinical features of PHP-Ia/Ib, highlighting the need of an updated classification that takes into account the recent knowledge on the molecular basis underlying these defects.

**P543****Does total parathyroidectomy with autotransplantation simplify reinterventions for persistent or recurrent secondary hyperparathyroidism?**

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Subtotal parathyroidectomy (SPX) and total parathyroidectomy with autotransplantation (TPXAT) are adequate techniques in treatment of secondary hyperparathyroidism (HPT2) in hemodialysed patients with identical results. Reinterventions required for persistence or recurrence of HPT2 may seem easy after TPXAT: no redo-surgery in the cervical area and removal of grafts in the forearm under local anaesthesia. The aim of this study was to analyze our results of reiterative surgery in HPT2.

Between 1981 and 2010, 44 hemodialysed patients were reoperated for HPT2 in our tertiary referral centre. Twelve cases were excluded for missing data or an incomplete primary intervention. Five patients were operated initially elsewhere. Primary interventions consisted of 18 SPX and 14 TPXAT that were performed correctly. Male/female ratio was 3:5. Operative indication for reintervention was PTH-level > 500 pg/ml (mean: 967 pg/ml). Localization studies included cervical ultrasound and MIBI-scintigraphy, plus CT-scan or invasive PTH-gradient measurement if necessary.

Mean time to reintervention was 60 and 111 months after SPX and TPXAT respectively ( $P < 0.05$ ). After SPX two patients had persisting hypercalcemia caused by a mediastinal supernumerary gland and inadequate judgment of the parathyroid remnant size. Twelve patients had recurrence following SPX caused by remnant hyperplasia and four patients had a supernumerary gland ( $n = 4$ ). 81% was cured after 1.1 reoperation without any postoperative morbidity. Following TPXAT, no persistence occurred and recurrence was caused by hyperplasia on parathyroid autografts ( $n = 11$ ) and by supernumerary glands ( $n = 4$ ). Significantly more imaging studies were required for localization of pathological parathyroid tissue after TPXAT. 79% was cured after 2 (mean) reinterventions ( $P < 0.05$ ). In our experience reoperations for persistent or recurrent HPT2 were not easier to perform after TPXAT than after SPX.

**P544****Bone turnover markers and vitamin D status over a training year in female elite alpine skiers**

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**Introduction**

The training year of elite athletes is characterized by different kind and intensities of workload implying different level of metabolic responses of the skeletal tissue, assessable by measuring serum levels biochemical markers.

**Methods**

Fourteen top-level female skiers, from the Italian Women's Alpine Ski Team (slalom and giant slalom), were tested at the end of the relative rest period (T1), the pre-competitive season (T2) and the competitive season (T3). Anthropometric and clinical data (age, height, weight, BMI, body fat%,  $VO_{2max}$  ( $lO_2/min$ ), counter-movement jump (h)) were recorded at each timepoint. Serum levels of bone-specific alkaline phosphatase (BAP) and tartrate-resistant acid phosphatase (TRAP5b) activities and of osteocalcin (OC) and the carboxyterminal crosslinked telopeptide of type I collagen (b-CTX), were assayed together with the determination of 25(OH)D levels.

**Results**

No significant variation were found in anthropometric and clinical data over the training year. Formation markers, BAP activity ( $P < 0.01$ ) and OC ( $P < 0.05$ ) and the resorption marker TRAP5b activity ( $P < 0.05$ ) significantly increased from T2 to T3, while beta-CTX showed no significant decreases all over the study. The trends of all bone markers correlated one to each other at T2 versus T3. 25(OH)D levels increased from T1 to T2 and from T1 to T3 ( $P < 0.01$ ) but its variations do not correlate with any of bone markers.

**Discussion**

The passage through T2 to T3 stimulated the whole bone metabolism, as consequence of the heavy mechanical stimulation during the highly demanding competitive period. These variations were completely independent from the corresponding changes of 25(OH)D. All athletes showed an insufficiency of vitamin D even though they exercised outdoor, mainly at latitudes in which sunlight exposure is adequate.

**P545****Some epidemiological aspects of primary hyperparathyroidism in Russia**

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Primary hyperparathyroidism (PHPT) is the third most frequent endocrine disorder and has a variable clinical presentation. Asymptomatic PHPT became the predominant form of the disease with increase of its incidence after the introduction of automated serum calcium measurement in North America and Europe. Data from Russia is lacking.

**Aim**

To present the clinical profile of PHPT in Russia.

**Materials and methods**

This retrospective study was conducted at endocrinology centers in 8 regions of Russia. We analyzed the clinical presentation, and treatment options in patients with confirmed PHPT (1995–2010).

**Results**

Seven hundred and thirty-eight patients (F:M-8:1) with age ranging from 13 to 83.4 years (mean 54.3) were included: 54% – from Moscow ( $n = 397$ ), 11% – from Moscow region ( $n = 79$ ) and 35% – from 53 regions of Russia ( $n = 262$ ). 33% of patients were in 50–59 years age-group. PHPT was diagnosed in woman more often in 50–59 years (23.8%) and 60–69 years (28.8%) age groups while in men it was irrespective of age. Symptomatic PHPT was the most common form (74%) and was revealed with osteoporosis in 56%, nephrolithiasis – in 45% and ulcer disease – in 18%. Our data showed an increase in the incidence of PHPT after 2005 compared with earlier period (from 4–29 per year to 93 in 2006) with peak in 2009 ( $n = 126$ ) and a tendency to more frequent detection of mild PHPT in the last decade (from 15 to 41%). 64.9% of patients were treated surgically, 17 patients undergone repeated parathyroid surgery. 28.3% received bisphosphonates, calcitonin and/or cinacalcet. 11.5% was observed without treatment.

**Conclusions**

PHPT still remains symptomatic disorder in our country most frequently with skeletal and renal manifestations. Although our data demonstrates marked changes in diagnostics of PHPT in the course of time, apparently many cases (especially the mild forms of PHPT) are unrecognized.

**P546****Conservative therapy by cinacalcet for primary hyperparathyroidism management: first results of two center prospective study**

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**Introduction**

Parathyroid hormone (PTH) secretion is regulated by the concentration of extracellular ionized calcium. The calcium sensing receptors (CaSR) of parathyroid chief cells play a central role in calcium homeostasis. Their activity is reduced by calcimimetics, the compounds that directly increase the sensitivity of CaSR to extracellular ionized calcium. Cinacalcet hydrochloride, an oral calcimimetic, is indicated in treatment of primary hyperparathyroidism (PHP), for patients with failure of previous surgery, patients with serious comorbidity that makes surgery impossible, or patients who do not accept surgery or do not carry out the surgical criteria.

**Aim**

To assess the efficacy, tolerability and safety of cinacalcet in the patients with PHP.

**Patients and methods**

Project is an ongoing prospective two center study in 33 patients (29 women) with PHPT, mean age 64.0 years (95% CI 55.4–74.6). From these, surgery was contraindicated in 16 patients, in 12 patients previous surgery failed, and 5 patients did not accept surgery. Serum concentrations of calcium, iPTH, phosphates were evaluated before and during the treatment.

**Results and conclusion**

All patients responded to cinacalcet. Applied doses of cinacalcet ranges from 15 to 90 mg. Serum calcium was 2.80 mmol/l (95% CI 2.74–2.86) at baseline, iPTH concentration was 19.5 pmol/l (95% CI 15.6–28.4) and phosphatemia was 0.83 mmol/l (95% CI 0.76–0.89). After 6 months, mean calcium concentration was 2.35 mmol/l (95% CI 2.27–2.44) ( $P < 0.001$ ) and mean iPTH concentration was 14.5 (95% CI 11.4–17.7) ( $P < 0.001$ ). The most common adverse event was nausea, which affected 5 patients. Cinacalcet effectively normalizes calcium concentration and significantly reduces (but not normalizes) iPTH concentration. Therapy is safe and well-tolerated.

**P547****The QUS fracture risk groups: unifactorial analyze regarding bone markers and DXA assessment**

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**Introduction**

The quantitative ultrasound (QUS) is an useful tool in the evaluation of the fragility fracture risk.

**Aim and study design**

We performed the QUS fragility fracture risk groups (validated by the golden standard DXA) by a unifactorial analyze including the bone markers.

**Method**

The cut offs used were:  $SI \leq 59U$  (high risk),  $SI \geq 83U$  (low risk), and medium risk with  $SI$  between 59 and 83U. The QUS analyze was performed by a heel GE Achilles Device. The statistical analyze used student *t* test. We studied 130 postmenopausal women who were not treated for osteoporosis.

**Results**

In a prospective study, the anamnesis, serum bone markers, DXA and QUS were performed. Low risk patients ( $n=48$ ) and high risk ( $n=19$ ), without age difference. 31.57% of the patients from the high risk group were diagnosed with osteoporosis, versus 7.6% in the low risk category. The percent of the women with normal DXA was 21.05 vs 56.41%. The percent of the false positive patients (high risk at QUS and normal DXA) was 21.05%. The percent of the false negative patients with osteoporosis was 7.6%. In the high risk group, serum PTH, 25OH-D, osteocalcin, alkaline phosphatase were not significantly different from the group with low risk. In the high risk group, the BMI, total calcium, and Beta Cross Laps were statistically significant different from the low risk group. The percent of the patients with an already known fragility fracture was: 12.5% in the low risk group, versus 26.32% in the high risk group.

**Conclusion**

Bone markers as PTH, 25 OH D have no influence. The DXA assessment proved significant difference: the osteoporosis was almost 4 times more frequent in the high risk groups while the normal DXA was found two times more frequent in low risk patients (QUS) versus high risk patients.

**P548****Bone density in patients with ACTH-dependent and ACTH-independent Cushing syndrome**

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**Background**

The presence of functional ACTH receptors (MC2R) on osteoblasts and fact, that their activation lead to the proliferation of osteoblasts is consistent with the presence of receptors for another anterior pituitary hormones (FSH and TSH) on bone cells. Does ACTH directly regulate bone? Is it possible, that this is another hormone of a novel regulatory axis, pituitary-bone axis, in which hormones bypass traditional endocrine targets to regulate bone mass directly? Supraphysiological doses of glucocorticoids lead to different metabolic complications as well as to osteoporosis and osteonecrosis. However, osteonecrosis is not a cardinal feature of ACTH-producing adenomas.

**Aim**

To compare bone mineral density (BMD) in patients with ACTH-dependent and ACTH-independent Cushing syndrome (CS).

**Patients and methods**

Retrospective analysis of BMD by X-ray absorptiometry (Hologic Discovery) in patients with ACTH-dependent ( $n=15$ ) and ACTH-independent ( $n=7$ ) form of CS.

**Results**

Patients with ACTH-dependent (ACTH 92.7 pg/ml) and ACTH-independent (ACTH 3.0 pg/ml) form of CS did not differ by their body height (165.1 vs 164.4 cm), body weight (80.6 vs 74.4 kg), BMI (29.6 vs 27.6 kg/m<sup>2</sup>),  $UFC_{rel}$  (3.78 vs 3.54 xURR), nor BMD at femoral neck (neck 0.69 vs 0.73 g/cm<sup>2</sup>, trochanter 0.61 vs 0.61 g/cm<sup>2</sup>, inter 1.01 vs 0.99 g/cm<sup>2</sup>, total 0.85 vs 0.85 g/cm<sup>2</sup>), neither at the lumbar spine (L<sub>1</sub> 0.82 vs 0.83 g/cm<sup>2</sup>, L<sub>2</sub> 0.89 vs 0.93 g/cm<sup>2</sup>, L<sub>3</sub> 0.91 vs 0.95 g/cm<sup>2</sup>, L<sub>4</sub> 0.92 vs 0.97 g/cm<sup>2</sup>, total 0.88 vs 0.91 g/cm<sup>2</sup>).

**Conclusions**

We did find differences in BMD in patients with ACTH-dependent and ACTH-independent CS. However, the fact that low ACTH is associated with osteonecrosis and that ACTH administration reduces such necrosis in animal model, even despite methylprednisolone acetate, provides a firm rationale for the use of ACTH in humans to decrease the risk of osteonecrosis.

**P548.1****Effects of atorvastatin and rosuvastatin therapy on serum 25-hydroxyvitamin D levels: a comparative study**

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**Introduction**

There is accumulating evidence on the favourable effects of some statins on bone and mineral metabolism. Although some studies have reported drop of serum 25-hydroxyvitamin D levels in subjects receiving statin therapy, comparative studies evaluating various statins in prospective randomized design is lacking. Our purpose is to compare the effects of variable doses of rosuvastatin and atorvastatin on serum 25-hydroxyvitamin D levels adjusted for seasons.

**Methods**

Hyperlipidemic patients who did not receive lipid lowering therapy before were included. They were randomly assigned to receive rosuvastatin 10 mg, atorvastatin 20 mg, atorvastatin 10 mg and atorvastatin 20 mg. We planned to evaluate serum 25-hydroxyvitamin D, LDL-c, HLL-c, triglyceride levels at baseline, 12, and 24 weeks of therapy. Preliminary results are reported.

**Results**

Seventy-six cases were recruited to the ongoing study till now. 16 hyperlipidemic men and women, 8 of whom received rosuvastatin 10–20 mg/day, and the remaining atorvastatin 10–20 mg/day completed the first twelve weeks. The mean basal lipid and 25-hydroxyvitamin D levels were similar in both groups (155.8 ± 23.4 mg/dl and 54.4 ± 20.9 UG/l for atorvastatin; 172.1 ± 46.8 mg/dl and 48.6 ± 21.2 UG/l for atorvastatin group, respectively) (mean ± s.d.). LDL-c levels decreased significantly in both groups to a mean value of 85.5 and 100.9 mg/dl, respectively ( $P < 0.01$ ). No significant effects of atorvastatin and rosuvastatin were observed on 25-hydroxyvitamin D levels at the end of twelve weeks (65.8 ± 31.7 and 47.3 ± 31.7 UG/l, respectively;  $P > 0.05$ ). There was no correlation between LDL-c and 25-hydroxyvitamin D level changes in both of the groups.

**Conclusion**

In contrast to some previous data, rosuvastatin and atorvastatin, two potent and widely prescribed statins, did not lead to a significant change in 25-hydroxyvitamin D levels. Long-term prospective studies are needed to evaluate the effects of statins on vitamin D levels.

**Cardiovascular endocrinology and lipid metabolism****P549****Hashimoto's disease presenting as cardiac tamponade**

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**Introduction**

Chronic longstanding hypothyroidism is a known cause of pericardial effusion. However, cardiac tamponade as the primary presentation of Hashimoto's hypothyroidism is relatively uncommon.

**Case description**

A 34-year-old African American woman presented to the ER complaining of shortness of breath, fever, cough and precordial chest pain of 1 day duration. Examination revealed an obese female with patchy alopecia and dry skin in mild respiratory distress. Further examination revealed tachycardia, raised JVD and pulsus paradoxus. Chest radiograph showed marked cardiomegaly. ECG revealed sinus tachycardia at 80 bpm, and reduced QRS voltage. Echocardiography demonstrated a large pericardial effusion and diastolic RV collapse consistent with cardiac tamponade physiology. Pericardial window was performed and a total of 1500 ml of serous fluid was drained. Pericardial fluid culture, cytology and pericardial biopsy were performed and analysis returned as exudative fluid. All causes of massive pericardial effusion including infection, neoplasm, trauma, uremia, post-acute myocardial infarction or aortic dissection were ruled out. Patient's TSH level returned as 65.8 μIU/ml and a subsequent thyroid antimicrosomal antibody level of >600 IU/ml lead to a diagnosis of Hashimoto's thyroiditis. Replacement thyroid therapy was initiated with levothyroxine 0.05 mg/day and empiric antibiotics were discontinued. Subsequently, the patient became asymptomatic and was discharged for follow-up as outpatient. On follow-up her TSH levels have been therapeutic and no signs/symptoms of recurrent effusion are evident till date.

**Conclusion**

Cardiac tamponade is an uncommon presentation or complication of hypothyroidism requiring a high index of suspicion and timely intervention. Treatment with pericardial window may be essential and long term levothyroxine therapy with follow up is imperative in such cases.

**P550****The relation between metabolic syndrome and cognitive decline in elderly Turkish people**

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**Objective**

To determine the relationship between metabolic syndrome (MS) and cognitive decline in people over 60 years age.

**Methods**

The age of the participants were between 60 and 89 years old ( $71.71 \pm 6.58$ ). According to NCEP ATP III criteria 93 had MS and 47 had not. Cognitive performance was determined with the use of standardized mini mental test and the mini mental scores (MMS) of the two groups were compared. Apart from the MS we investigated the relationship between MMS and age, gender, educational status, tobacco and alcohol use, the history of acute myocardial infarction (AMI) and stroke, medication use (antihypertensive, insulin, oral antidiabetic, statin) and body mass index (BMI). Depression, which is a cause of pseudodementia, was also assessed with the use of geriatric depression scale (GDS).

**Results**

The MMS was low in 25 patients (26.9%) in the MS group, and in 7 subjects (14.9%) in the control group. We could not find difference between MMS's of two groups statistically. In this study the MMS was low in subjects with high fasting plasma glucose level, tobacco use, insulin use, advanced age and depression. The GDS's of two groups were similar. There was not difference in the MMS's between patients with high blood pressure, high triglyceride level, low HDL cholesterol level, history of AMI and stroke, low educational status, high BMI and in whom without them. We also could not find difference between MMS's of the patients who use antihypertensives, oral antidiabetics and statins and in whom not using these medications.

**Conclusion**

The number of elderly people increases in Turkey, however, cognitive decline related to diseases also increases. The establishment of the risk factors, which affect the cognitive functions and prevention of them in elderly, would prevent the cognitive decline, one of the important causes of mortality and morbidity in today's world.

**P551****Relationship between N-terminal pro-B-type natriuretic peptide levels and metabolic syndrome**

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**Instruction**

Previous studies have been shown that obese individuals have reduced natriuretic peptide levels. But conflicting data exist on the relation of natriuretic peptide levels to other metabolic risk factors. We investigated the relationship between plasma N-terminal pro-B-type natriuretic peptide levels (NT-proBNP) and metabolic syndrome (MetS), metabolic risk factors in 469 patients free of heart failure.

**Material and methods**

Two hundred and thirty diagnosed MetS cases and 239 non-MetS cases were included in this study. Echocardiography examinations were performed and left ventricular mass index was calculated according to Devereux correction formula. NT-proBNP was measured by electrochemiluminescence. The log-transformed NT-proBNP levels were used for abnormal distribution. Multiple linear regression analysis was performed to assess the association between levels of NT-proBNP and metabolic factors. Covariance analysis was used for group comparisons.

**Results**

Log NT-proBNP levels was independently related with age, gender, body mass index, systolic blood pressure, diastolic blood pressure, fasting blood glucose, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol, left ventricular mass index and left ventricular ejection fraction in multiple linear regression analysis ( $P < 0.05$ ). Adjusted log NT-proBNP levels were lower in persons with MetS compared with those without MetS ( $P < 0.05$ ). Individuals with hyperlipidemia, elevated body mass index, diastolic blood pressure and fasting plasma glucose had lower levels of log NT-proBNP than those without MetS ( $P < 0.05$ ).

**Conclusions**

There is a relationship between metabolic components and lower plasma NT-proBNP concentration. These findings raise the possibility that reduced plasma NT-proBNP levels is a manifestation of MetS, which might possess significant clinical and pathophysiological implications.

**P552****Inflammation indices and insulin resistance in metabolic syndrome with and without coronary artery disease**

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**Background**

Obesity, particularly abdominal obesity, is associated with resistance to the effects of insulin on peripheral glucose and fatty acid utilization, often leading to type 2 diabetes mellitus. Insulin resistance, the associated hyperinsulinemia and hyperglycemia, and adipocyte cytokines (adipokines) may also lead to vascular endothelial dysfunction, an abnormal lipid profile, hypertension, and vascular inflammation, all of which promote the development of atherosclerotic cardiovascular disease (CVD). The aim of our study was to assess the association of insulin resistance and inflammatory markers with the occurrence and extent of CAD.

**Methods**

Plasma level of FBS, oral glucose tolerance test (OGTT), insulin, fibrinogen, TG, LDL-C, HDL-C, ESR, C-reactive protein (CRP), magnesium (Mg) and calcium (Ca) were measured. Measurements were made in samples obtained from 44 patients who had metabolic syndrome with coronary arteries diseases (cases), and 45 patients who had metabolic syndrome but without CAD (controls). Cases were patients with 3 of 5 criteria of metabolic syndrome whom had documented CAD (resent or old MI, positive angiography for CAD) and controls were the patients with metabolic syndrome but (same the cases) but with no evidence of ischemic heart disease (IHD) such as normal electrocardiogram, echocardiogram, exercise tolerance test and no sign and symptom of IHD.

**Results**

After adjustment for factors, high levels of hsCRP and fibrinogen were significantly related to an increased risk of coronary heart disease in cases group ( $P < 0.001$ ). Although the insulin level, ESR and smoking were higher in cases group but these difference were not significant level of HDL in cases group was lower than control group but no significantly.

**Conclusion**

Elevated levels of inflammatory markers, particularly hsCRP and fibrinogen level indicate an increased risk of coronary heart disease.

**P553****Regulation of fuel metabolism during exercise in hypopituitarism with growth hormone-deficiency (GHD)**

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**Introduction**

Hypopituitary patients with GHD tend to have a reduced exercise capacity. GH is secreted during exercise, has a strong lipolytic action and has been shown to be positively correlated with exercise performance. We hypothesized that the lack of GH leads to a reduced systemic availability of free fatty acids (FFA) during exercise thereby affecting exercise performance.

**Methods**

Patients with GHD and matched sedentary control subjects (CS) were exposed to an increasing workload on a treadmill for the determination of  $VO_{2max}$ . On a separate day, the patients and CS performed a 2-h exercise session on a treadmill (50–60% of the  $VO_{2max}$ ). Usual hydrocortone replacement therapy was administered prior to exercise as needed. Blood samples were taken at baseline and every 30 min during the exercise. Analysis of the samples included GH, catecholamines (noradrenaline, adrenaline) as well as glucose and FFA. Area under the curve (AUC) as well as peak concentrations of hormones and metabolites were analyzed.

**Results**

Ten patients with GHD (four females, age:  $42.5 \pm 12.4$ , years, mean  $\pm$  s.d.; BMI:  $26.6 \pm 3.8$  kg/m<sup>2</sup>; waist:  $89.3 \pm 12.9$  cm) and 10 CS matched for gender, age, BMI and waist (age:  $42.8 \pm 12.6$  years; BMI:  $25.2 \pm 5.3$  kg/m<sup>2</sup>; waist:  $90.7 \pm 19.1$  cm) volunteered for the study. GHD patients tended to have a reduced  $VO_{2max}$  compared with CS (GHD:  $36.3 \pm 6.7$  ml O<sub>2</sub>/kg per bodyweight; CS:  $41.7 \pm 6.0$ ,  $P = 0.07$ ). GH-AUC and GH peak concentrations were lower in GHD patients compared to CS (by a factor 15). AUC and peak concentrations of catecholamines were similar in patients and CS. FFA-AUC, Glucose-AUC and glucose peak concentrations were not significantly different between the two groups. GHD patients tended to have lower FFA peak-concentrations compared to sedentary controls (patients:  $1.03 \pm 0.39$  mmol/l; CS:  $1.51 \pm 0.53$ ;  $P = 0.054$ ).

**Conclusion**

This study indicates that i) there is a tendency towards a reduced exercise capacity in GHD patients compared to matched control subjects and ii) systemic



availability of FFA may be slightly reduced whereas glucose availability is similar during exercise in GHD.

#### P554

##### **Prevalence of androgen deficiency in males with metabolic syndrome: relation between hormonal and metabolic parameters**

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Androgen deficiency in the aging male has become an actual topic due to the aging population and finding from different studies that shown a progressive decline of serum levels of age-related testosterone. These levels have been inversely related with markers of metabolic syndrome and cardiovascular disease. The aim of this observational study was to investigate the relation of endogenous testosterone and other sex hormones with different components of metabolic syndrome, as defined by the National Cholesterol Education Program (NCEP), in a Spanish middle-aged and elderly male population.

We studied 73 consecutive men with metabolic syndrome (criteria NCEP), aged between 40 and 70 years. The body weight, waist circumference and arterial pressure were measured, and fasting serum levels of glucose, insulin, lipid profile, total testosterone (TT), SHBG, dehydroepiandrosterone sulfate (DHEA-S) and estradiol were determined. The insulin resistance was calculated by use of the HOMA index.

Our male population present an evident androgenic deficit (TT <230 ng/dl) in 9/73 cases (12.3%) and a partial androgenic deficit (TT 230–345 ng/dl) in 34/73 cases (46.6%). We find an inversely significant correlation of values of TT with those HOMA index ( $r = -0.293$ ,  $P < 0.05$ ) and those waist circumference ( $r = -0.312$ ,  $P < 0.05$ ).

In conclusion, our findings show that patients with metabolic syndrome could present a relatively high prevalence of hypogonadism, so this abnormality must be investigated in this population. The decrease in androgen concentrations in men could be related with insulin resistance, and in consequence with the development of diabetes mellitus and cardiovascular disease.

#### P555

##### **Polycystic ovary syndrome and their relationship with metabolic syndrome in female-to-male transsexuals**

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##### **Objective**

To determine the prevalence of polycystic ovary syndrome (PCOS) and hyperandrogenism and their relationships with metabolic syndrome (MS) parameters in female-to-male transsexuals (FMT).

##### **Design and methods**

Seventy-seven 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**

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 secondary to hyperadrenal axis activation and/or gonadal, although many studies assess these phenomena as the cause of these disorders.

#### P556

##### **Is metabolic syndrome a useless category in subjects with high cardiovascular risk? Results from a cohort study in men with erectile dysfunction**

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##### **Introduction**

Although several studies have demonstrated that MetS is associated with a twofold increase in the risk of cardiovascular (CV) diseases, this risk does not appear to be greater than the sum of risks associated with each of its individual components. To determine the association of men with ED and individual components of MetS and their subsequent relationship to cardiovascular (CV) risk, and, more specifically whether the sum of the MetS components is greater than the individual components in predicting CV risk.

##### **Methods**

We longitudinally studied a consecutive series of 1687 (mean age  $52.9 \pm 12.8$ ; range 17–88 years) patients attending our clinic for ED and evaluated different clinical and biochemical parameters. Information on MACE was obtained through the City of Florence Registry Office.

##### **Results**

One hundred and thirty-nine MACE, 15 of which were fatal, occurred during a mean follow-up of  $4.3 \pm 2.6$  years. Subjects with MetS at baseline showed a higher incidence of MACE (HR = 1.77), after adjusting for age, however, the association disappeared in an alternative Cox model, adjusting both for age and for individual MetS components (HR = 1.525 (0.564–4.123);  $P = 0.408$ ). The two most predictive MetS components of CV risk were low HDL cholesterol and high triglycerides. Exploring possible interactions between individual components of MetS and their effect on CV risk using two alternative approaches indicates that the effect of MetS components on CV risk is additive, but not synergistic. Among subjects with hypertension, after adjusting for age, elevated glycaemia and low HDL-cholesterol confer relevant additional risk, while in subjects with high triglycerides, hyperglycaemia increased the risk of incident MACE.

##### **Conclusions**

With regards to CV risk, the MetS construct seems to add little or nothing to the careful assessment of its components. Thus, there is no reason to recommend the use of MetS as a diagnostic category in patients with ED.

#### P557

##### **Prolactin levels independently predict major cardiovascular events in patients with erectile dysfunction**

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##### **Introduction**

We previously reported that in subjects consulting for sexual dysfunction lower PRL plasma levels were associated with worse lipid and glycemic profile, as well as with a higher prevalence of metabolic syndrome and arteriogenic erectile dysfunction (ED). To assess possible associations between PRL levels and incident major cardiovascular events (MACE) in subjects with ED.

##### **Methods**

This is an observational prospective cohort study evaluating a consecutive series of 1687 patients attending our Andrological Unit for ED. Different clinical, biochemical and instrumental parameters were evaluated. Information on MACE was obtained through the City of Florence Registry Office.

**Results**

When only subjects without pathological hyperprolactinemia (PRL <735 mU/l or 35 ng/ml) and pituitary diseases were considered both unadjusted and adjusted analysis showed a significantly lower incidence of MACE in subjects with PRL levels in the highest PRL quintile (246–735 mU/l or 12–35 ng/ml) when compared to the rest of the sample. In particular, the risk of MACE was reduced by 5% (1–9%;  $P=0.03$ ) for each 10 ng/ml increment of PRL. Conversely, comparing patients with hyperprolactinemia to matched controls, no significant difference was detected between cases and controls in MACE.

**Conclusions**

In subjects at high risk for cardiovascular diseases, such as those with ED, a relatively high PRL plasma level is associated with an overall decreased chance of MACE, independently from other known risk factors.

**P558****Body mass index regulates hypogonadism-associated CV risk: results from a cohort of subjects with erectile dysfunction**

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**Introduction**

Obesity is an independent cardiovascular (CV) risk factor. Testosterone (T) is inversely related to body mass index (BMI) in males. There is substantial evidence suggesting that low T could play a role as a moderator of CV mortality in men, and so this study is designed to assess the possible interaction between T and obesity in predicting major cardiovascular events (MACE) in a sample of subjects with erectile dysfunction.

**Methods**

This is an observational prospective cohort study involving a consecutive series of 1687 patients attending our Unit for erectile dysfunction. According to BMI, subjects were divided into normal weight (BMI = 18.5–24.9 kg/m<sup>2</sup>), overweight (BMI = 25.0–29.9 kg/m<sup>2</sup>) and obese (BMI ≥ 30.0 kg/m<sup>2</sup>). Hypogonadism was defined as total T below 10.4 nmol/l. Information on MACE was obtained through the City of Florence Registry Office.

**Results**

Among the patients studied, 39.8% had normal weight, whereas 44.1 and 16.1% were overweight or obese respectively. Unadjusted analysis in the whole sample showed that, while hypogonadism and obesity were significantly associated with an increased risk of MACE, their interaction term was associated with a protective effect. In a Cox regression model, adjusting for confounders, hypogonadism showed a significant increased risk of MACE in normal weight subjects, whereas it was associated with a reduced risk in obese patients.

**Conclusions**

Hypogonadism-associated CV risk depends on the characteristics of subjects. Further studies are advisable to clarify if low T in obese patients is a (positive) consequence of a comorbid condition to save energy or if it represents a pathogenetic issue of the same illness.

**P559****Severe depressive symptoms and cardiovascular risk in subjects with erectile dysfunction**

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**Introduction**

Erectile dysfunction (ED) and mood depression are often associated and both are correlated with an increased risk of cardiovascular morbidity and mortality.

The aim of the present study is to explore biological and clinical correlates of depressive symptomatology in a sample of men consulting for sexual dysfunction and to verify possible associations between depressive symptoms and incidence of major cardiovascular events (MACE).

**Methods**

A consecutive series of 2303 male patients attending the Outpatient Clinic for sexual dysfunction was retrospectively studied. A subset of the previous sample ( $N=1687$ ) was enrolled in a longitudinal study. All patients were investigated using a structured interview on erectile dysfunction (SIEDY), composed of three scales which explore organic, relational and intra-psyche components of ED. MHQ-D scoring from middlesex hospital questionnaire (MHQ) was used as a putative marker of depressive symptoms. Information on MACE was obtained through the City of Florence Registry Office.

**Results**

We found a positive relationship between MHQ-D score and a progressive impairment in obtaining an erection hard enough for penetration, even after adjusting for confounding factors. Moreover, we observed positive relationships between MHQ-D score and the three pathogenetic domains underlying ED. When the longitudinal subset was evaluated, during a mean follow-up of  $4.3 \pm 2.6$  years, 139 MACE, 15 of which were fatal, were observed. Unadjusted incidence of MACE was significantly associated with baseline depressive symptoms. When the presence of severe depressive symptoms were introduced in a Cox regression model, along with the arteriogenic ED and partner's hypoactive sexual desire, after adjusting for age, chronic diseases score, and SMHQ (a broader index of psychopathology), severe depressive symptomatology was independently associated with a higher incidence of MACE.

**Conclusion**

Depressive symptomatology constitutes an independent risk factor for cardiac morbidity and mortality in men with ED.

**P560****Priapus is happier with Venus than with Bacchus**

Giovanni Corona<sup>1,2</sup>, Valentina Boddi<sup>1</sup>, Matteo Monami<sup>3</sup>, Alessandra Fisher<sup>1</sup>, Elisa Bandini<sup>1</sup>, Gianni Forti<sup>4</sup>, Edoardo Mannucci<sup>3</sup> & Mario Maggi<sup>1</sup>

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**Introduction**

The relationship between alcohol consumption and erectile function is still not completely clarified. Aims of the present study are to explore a number of biological and clinical correlates of alcohol consumption in a sample of men consulting for sexual dysfunction, and to verify possible associations with the incidence of major adverse cardiovascular events (MACEs).

**Methods**

A consecutive series of 1956 (mean age  $55 \pm 11.9$  years old) attending our outpatient clinic for sexual dysfunction was retrospectively studied. A subset of the previous sample ( $N=1687$ ) was enrolled in a longitudinal study. Different clinical, biochemical, instrumental (penile Doppler ultrasound (PCDU)), and intrapsychic (Middlesex Hospital Questionnaire (MHQ)) were evaluated. We considered alcohol abuse more than 3 drinks/day.

**Results**

Among the patients studied 81% reported no or mild (<4 drinks/day) alcohol consumption whereas 14.3 and 3.9% declared a moderate (4–6 drinks/day) or severe (>6 drinks/day) alcohol abuse respectively. After adjustment for confounders, both moderate or severe alcohol abuse was associated with low perceived partner's sexual desire, worse couple relationship, and smoking abuse. Furthermore, moderate and severe alcohol abuse was associated with low prolactin and thyroid-stimulating hormone levels, as well as an increase in triglycerides and total cholesterol levels. Penile blood flow was reduced in moderate and severe alcohol drinkers even after adjustment for confounders. In the longitudinal study, after adjusting for confounding factors, any kind of alcohol abuse was independently associated with a higher incidence of MACE (hazard ratio = 2.043 (1.059–3.943);  $P < 0.0001$ ).

**Conclusions**

Our findings demonstrate that, in subjects consulting for erectile dysfunction, severe alcohol consumption is associated with a worse sexual function and a higher incidence of MACE.

**P561****Young women with the polycystic ovary syndrome (PCOS) have evidence of endothelial dysfunction but not of altered arterial structure**  
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The aim of our study was to assess the presence of endothelial dysfunction and of increased carotid intima-media thickness (IMT), two precocious markers of atherosclerosis in PCOS subjects. Simultaneously, their relationship with some metabolic, hormonal and anthropometric parameters was analyzed.

**Methods**

The study group consisted of 45 women with PCOS (age = 23.1 ± 4.1 years, body mass index (BMI) = 28.4 ± 5.9 kg/m<sup>2</sup>). Thirty-two healthy age-matched women (23.0 ± 5.3 years and BMI = 25.8 ± 6.8 kg/m<sup>2</sup>) were included as controls. All women were evaluated clinically and biochemically. Serum insulin, total testosterone, sex hormone binding globulin and estradiol were measured using ELISA commercial kits. Intima-media thickness was assessed by high-resolution ultrasonography at the level of common carotid artery bilaterally, while endothelial dysfunction was investigated by both endothelin (ET)-1 levels determination and measurement of flow-mediated dilatation (FMD) of the brachial artery.

**Results**

ET-1 levels were significantly higher while the FMD was significantly lower in the PCOS women compared with the control women ( $P=0.001$ ). In contrast, no difference was observed between the two groups in carotid artery IMT. In the total study group, both ET-1 (correlation with TT:  $r=0.439$ ,  $P<0.0001$ ) and with the free androgenic index (FAI):  $r=0.448$ ,  $P<0.0001$ ) and brachial artery FMD (correlation with the FAI:  $r=-0.242$ ,  $P=0.046$ ) were significantly associated with hyperandrogenemia markers. In multiple regression analyses for the total population, only PCOS presence represented an independent predictor of FMD levels ( $b=-0.406$ ,  $P=0.001$ ).

**Conclusions**

Our data show that young, non-dyslipidemic, non-hypertensive and eu-glycaemic women with PCOS have altered endothelial function but not evidence of altered arterial structure and that increased androgen levels are linked to impaired endothelial function.

**P562****Metabolic syndrome and the effect of testosterone treatment in young men with congenital hypogonadotropic hypogonadism**

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**Objective**

The relationship between metabolic syndrome (MS) and hypogonadism has always been investigated in study groups confounded with aging, obesity or chronic metabolic disorders. So far there has been no data about the presence of MS in young hypogonadal patients. Also there is controversial data about the metabolic effects of testosterone replacement therapy. We investigated the frequency of MS in treatment naïve, young men with congenital hypogonadal hypogonadism (CHH). We also searched for the effect of testosterone replacement on the metabolic profiles of this specific patient group.

**Design**

Retrospective analysis

**Methods**

A total number of 332 patients (age 21.68 ± 2.09 years) were enrolled. The control group was the 395 age and body mass index (BMI) matched healthy

young men (age 21.39 ± 1.49 years). Standard regimen of testosterone esters (250 mg/3 weeks) were given to 208 patients.

**Results**

MS was more prevalent in CHH ( $P<0.001$ ) according to healthy controls. The patients had higher arterial blood pressure, waist circumference (WC), triglyceride ( $P<0.001$  for all), fasting glucose ( $P=0.02$ ), insulin ( $P=0.004$ ), HOMA-IR ( $P=0.002$ ) and lower HDL cholesterol ( $P<0.001$ ) levels. With a mean follow-up period of 5.63 ± 2.6 months, the BMI, WC ( $P<0.001$  for both), systolic blood pressures ( $P=0.002$ ) and triglyceride levels ( $P=0.04$ ) were increased and the total and HDL cholesterol levels were decreased ( $P=0.02$  and  $P<0.001$  respectively).

**Conclusions**

This study shows increased prevalence of MS and unfavorable effects of testosterone replacement in young patients with CHH. Long term follow up studies are warranted to investigate the cardiovascular safety of testosterone treatment in this specific population.

**P563****Determinants and outcome of amiodarone-associated thyroid dysfunction**

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**Background**

Amiodarone is frequently associated with thyroid dysfunction. Identifying predictors for amiodarone-associated thyroid dysfunction and assessing treatment outcome may aid clinicians in daily practice.

**Patients and methods**

In our retrospective study, we included 303 consecutive patients with amiodarone therapy for cardiac arrhythmias (260 with atrial fibrillation and 43 with ventricular arrhythmias). Thyroid function tests were performed every 6 months.

**Results**

Mean age was 63 ± 12 years and 66% was male. After median follow-up of 3.3 (0.1–24) years, 23 (8%) patients developed amiodarone-associated thyrotoxicosis (incidence rate 1.9 per 100 person years) and 18 (6%) hypothyroidism (incidence rate 1.1 per 100 person years). The only predictor for amiodarone-associated thyrotoxicosis was age < 62 years (HR = 2.4 (95% CI 1.0–5.7),  $P=0.05$ ). Predictors for amiodarone-associated hypothyroidism were thyroid stimulating hormone > 1.4 mU/l at baseline (HR = 5.1 (95% CI 1.1–22.4),  $P=0.03$ ), left ventricular ejection fraction < 45% (HR = 3.8 (95% CI 1.1–13.3),  $P=0.04$ ) and diabetes mellitus at baseline (HR = 3.3 (95% CI 1.1–10.3),  $P=0.04$ ). Gender was not a predictor for amiodarone-associated thyroid dysfunction. Five out of 23 (22%) patients with thyrotoxicosis exhibited spontaneous normalization of thyroid function on continuation of amiodarone therapy. Mean time to normalization in the total group was 6.2 ± 3.3 months, with no difference between continuing or discontinuing amiodarone (6.6 ± 3.8 versus 5.8 ± 2.8 months,  $P=0.5$ ).

**Conclusions**

During median follow-up of 3.3 years, the incidence of amiodarone-associated thyrotoxicosis was higher compared to hypothyroidism. Only general predictors for amiodarone-associated thyroid dysfunction were observed. Discontinuation of amiodarone did not influence treatment outcome.

**P564**

Abstract withdrawn.

**P565****C-reactive protein and fibrinogen are strong predictors of the cardiovascular outcome in subjects with subclinical atherosclerosis and the metabolic syndrome**

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**Background**

The presence of the metabolic syndrome (MS) increases cardiovascular morbidity and mortality but few data is available on the outcome in subjects with the MS and subclinical atherosclerosis.

**Aim**

We aimed to assess cardio- and cerebro-vascular events in subjects with MS and subclinical atherosclerosis.

**Methods**

We followed-up for 5 years 339 subjects with asymptomatic carotid intima-media thickness >0.9 mm, of whom 130 had the MS, evaluating at baseline traditional cardiovascular risk factors (including male gender, older age, obesity, hypertension, diabetes, smoking, family history of cardiovascular diseases, dyslipidemia) and plasma levels of C-reactive protein and fibrinogen.

**Results**

Cardio- and cerebro-vascular events were registered in the 29% of subjects with the MS and in the 20% of those without it and the presence of more criteria for the diagnosis of the MS was significantly associated with vascular morbidity and mortality: with transient ischemic attack ( $P<0.0001$ ), angina ( $P=0.0022$ ), cardio- and cerebro-vascular death ( $P=0.0019$ ) and the presence of any clinical event ( $P=0.0003$ ). Further, we used logistic regression analysis to search for possible independent associations of any parameter evaluated at baseline with the occurrence of clinical events and we found a predictive role for elevated markers of inflammation (OR 3.8, 95% CI 2.6–12.5,  $P=0.0022$ ), elevated fasting glucose (OR 2.1, 95% CI 1.2–4.0,  $P=0.0134$ ) and elevated triglycerides (OR 1.4, 95% CI 1.1–2.9,  $P=0.0351$ ).

**Conclusions**

These findings confirm a worst vascular outcome in subjects with more criteria for the diagnosis of the MS and further suggest the need of future research to understand the combined role of inflammation and the MS in the progression from subclinical to clinical atherosclerosis.

**P566****Apolipoprotein A1 and B100 and hormones in postmenopausal women**

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Menopause is a physiological condition associated with an important change in the hormonal status, an adverse lipid profile and cardiovascular risk. Apolipoproteins are proteins that bind to lipids to transport them. Apolipoprotein intestine synthesis is regulated principally by the fat content of the diet, while in the liver by dietary composition, hormones, alcohol intake and lipid lowering drugs. Are they changed due to aging? Do they interfere in the hormonal changes? The aim of the study was to evaluate apolipoprotein A1 and B100 values correlated to hormones in postmenopausal women compared with fertile women.

**Subjects and methods**

One hundred and forty-four postmenopausal, healthy women with a MMSE score over 28 and 170 fertile women were enrolled. Fasting blood samples were taken in order to determine the biochemical and hormonal status.

SPSS version 15 was used for statistical analyze.

**Results**

Postmenopausal women had higher mean values for BMI, waist circumference, triglycerides, total and LDL-cholesterol, apolipoprotein A1 ( $P<0.001$ ) and B100 ( $P=0.007$ ), HOMA, cortisol, GHBP, IGFBP1, PTH and cross laps; lower mean values for fT3, T4, DHEA, DHEAS, ADION, PRL, GH, IGF1 and IGFBP3 compared with fertile women.

Apolipoprotein B correlated positively with BMI, waist circumference, triglycerides, total and LDL cholesterol, uric acid, GHBP ( $P<0.001$ ) and negatively with DHEA ( $P<0.001$ ), DHEAS ( $P=0.011$ ), ADION ( $P=0.06$ ), T4, fT4 ( $P<0.001$ ) and IGFBP3 ( $P=0.034$ ).

Apolipoprotein A correlated positively with HDL-cholesterol ( $P<0.001$ ), apolipoprotein B ( $P=0.001$ ) and negatively with estradiol ( $P=0.017$ ), estrone ( $P=0.022$ ), alkaline phosphatase, crosslaps ( $P=0.001$ ) and osteocalcin ( $P=0.023$ ).

**Conclusion**

Postmenopausal women have higher apolipoprotein B and surprisingly higher apolipoprotein A levels that correlate positively.

Their correlations with the hormonal status point to changes due to aging and joint action regarding cardiovascular risk.

**P567****Cortisol secretion and HPA axis activity in patients undergoing coronary angiography**

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**Introduction**

Several studies have shown that cortisol and altered HPA axis activity may participate in pathogenesis and severity of coronary artery disease (CAD).

**Aim**

To examine possible interaction between cortisol secretion and HPA axis activity with severity of CAD.

**Patients and methods**

Diurnal cortisol secretion and dexamethasone suppression test (DST) were analyzed in 200 consecutive patients (mean age  $59.3 \pm 8.6$ ) referred for coronary angiography due to suspected CAD. Basal cortisol secretion was measured as well in 60 age, sex, and BMI-matched healthy control subjects (CS). Patients were divided in two groups according to positive (PA) and negative (NA) coronary angiogram. Severity of and the extent of disease was assessed by Gensini and disease extent scores.

**Results**

There was no difference in the basal cortisol concentration between NA and PA patients ( $474.9 \pm 153.8$  vs  $449.2 \pm 155.6$ ,  $P>0.05$ ), however both NA and PA patients had significantly higher basal cortisol in comparison to CS ( $394.9 \pm 127.6$ ,  $P<0.05$ , for both). No difference in diurnal cortisol rhythm and the ability to suppress cortisol after 1 mg of dexamethasone was found between NA and PA patients. A weak and negative correlation between basal cortisol and severity of CAD was obtained only in male patients by both Gensini ( $r=0.16$ , two-tailed  $P=0.01$ ) and extent of disease score ( $r=0.17$ , two-tailed  $P=0.02$ ). After adjustment for potential confounding factors, cortisol during DST proved to be an independent predictor of severity of CAD obtained by the extent score ( $B=2.6$ ,  $P=0.019$ ,  $R^2=0.15$ , adj.  $P=0.13$ ).

**Conclusion**

Patients undergoing coronary angiography have higher basal cortisol level than healthy subjects. There is no difference in diurnal cortisol secretion or HPA sensitivity between patients with positive and negative angiogram. HPA axis suppression ability is an independent predictor of severity of CAD obtained by the extent score of disease.

**P568****Plasma levels of the neurohormones A and B-type natriuretic peptides increases after weight loss in severe obesity**

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**Introduction**

The neurohormones A and B-type natriuretic peptides are markedly increased in patients with heart disease, and are well established as useful diagnostic

biomarkers. Obesity is a known risk factor for cardiovascular disease. Cross sectional data suggest however that plasma levels are reduced among obese. Therefore, the aim of the present study was to investigate the impact of weight loss and corresponding changes in body composition on plasma levels of A and B-type natriuretic peptides.

#### Methods and design

Fifty-two obese individuals 47 women, BMI  $36.5 \pm 5.6$  kg/m<sup>2</sup>; age  $62 \pm 7$  years (mean  $\pm$  s.d.). Randomized to either intervention on a weight reduction program, using a low energy diet between 3.4 and 5 MJ/day, or to a control group which was offered diet counseling. Body composition measuring fat and fat free mass using whole body Dual energy X-ray (DEXA) scan, and plasma levels of NT-proBNP, MR-proANP and were measured at baseline and after 52 weeks.

#### Results

In the intervention group ( $n=28$ ), diet induced a substantial weight loss (BMI  $31.6 \pm 6.2$  vs.  $37.1 \pm 6.1$  kg/m<sup>2</sup>;  $P < 0.001$ ) and loss in body fat ( $23.5 \pm 15.5\%$ ;  $P < 0.001$ ) after 52 weeks. Corresponding, plasma NT-proBNP ( $97 \pm 55$  vs.  $55 \pm 31$  pg/ml;  $P < 0.001$ ) and MR-proANP ( $74 \pm 26$  vs.  $59 \pm 21$  pmol/l;  $P < 0.001$ ) levels increased. A modest weight loss ( $33.6 \pm 5.5$  vs.  $35.8 \pm 5.5$  kg/m<sup>2</sup>;  $P = 0.001$ ), and body fat ( $9.2 \pm 11.0\%$ ;  $P < 0.05$ ) was observed among controls ( $n=24$ ), with no changes in NT-proBNP or MR-proANP concentrations. The loss in total weight and in fat mass were significantly higher in the intervention as compared with the control group ( $P < 0.001$ ), for both.

#### Conclusions

NT-proBNP and MR-proANP concentrations increases in circulating plasma during substantial weight loss, underlining the impact of BMI on plasma levels of A and B-type natriuretic peptides.

## P569

### $\alpha$ -Defensins are novel biomarkers of low grade inflammation with strong prognostic impact in patients with heart failure

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#### Introduction

$\alpha$ -Defensins are recently described peptides, which are part of the innate immune system. Increased circulating levels are observed in conditions associated with chronic low grade inflammation, such as ischemic heart disease and in diabetes mellitus (DM). Recently we reported adverse prognostic implications of high  $\alpha$ -defensins in patients with type 1 DM. However, it is not clear whether this finding may be generalized to include other groups such as patients with chronic heart failure (CHF). Therefore the aims were to compare plasma levels of  $\alpha$ -defensins in CHF patients and healthy controls, and to examine the predictive ability of  $\alpha$ -defensins with respect to all-cause mortality and future cardiovascular outcome.

#### Methods and design

Prospective observational study, median follow-up of 2.6 years, the prognostic value of plasma  $\alpha$ -defensins with respect to overall mortality and new ischemic events in 194 CHF patients and 98 age-matched healthy controls.

#### Results

$\alpha$ -Defensins levels were two times higher among patients with severe heart failure (NYHA class III/IV) than in patients in NYHA class I/II and healthy controls ( $P = 0.001$ ). After adjustment for potential confounders including NT-proBNP, high  $\alpha$ -defensins ( $> 540$  ng/ml) corresponding to the optimal cut-off value, were associated with a twofold increased mortality risk hazard ratio (HR) being 2.1 (95% CI: 1.2–4.1;  $P = 0.029$ ) and of new ischemic events HR: 1.86 (95% CI: 1.15–3.01;  $P = 0.01$ ). The combination of high  $\alpha$ -defensins and high NT-proBNP levels (above the respective median values) were associated with a threefold increase in mortality HR: 3.19 (95% CI: 1.68–6.06;  $P < 0.001$ ), as compared to patients with low levels of both biomarkers.

#### Conclusion

$\alpha$ -Defensins are novel interesting biomarkers of chronic inflammation. In heart failure patients  $\alpha$ -defensins provide incremental prognostic information to established clinical risk markers.

## P570

### B-type natriuretic peptide modulates the response to intravenous glucose in a placebo-controlled cross-over study in healthy volunteers

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B-type natriuretic peptide (BNP) is a hormone secreted from the heart in response to volume load and serves clinically as a reliable biomarker in the diagnosis of cardiac dysfunction and heart failure. As patients with heart failure present an increased risk for developing diabetes, we aimed to investigate the role of BNP on parameters of glucose metabolism in a placebo-controlled crossover study performed in 10 healthy volunteers ( $25 \pm 1$  years; BMI  $23 \pm 1$  kg/m<sup>2</sup>; fasting glucose  $83 \pm 2$  mg/dl). Participants received intravenously either placebo or 3 pmol/kg per minute BNP-32 for 4 h. One hour after beginning the BNP/placebo infusion, a 3 h intravenous glucose tolerance test ( $0.33$  g/kg glucose +  $0.03$  U/kg insulin at 20 min) was started. Plasma glucose, insulin and C-peptide were frequently measured and minimal model analysis was performed.

BNP increased the glucose distribution volume ( $13 \pm 1\%$  BW vs.  $11 \pm 1$ ,  $P < 0.002$ ), leading to an overall reduction of glucose concentrations ( $P < 0.001$ ) especially during the initial 20 min of the test ( $P = 0.001$ ). This was accompanied by a reduction of the initial C-peptide levels ( $4.3 \pm 0.4$  ng/ml vs.  $4.9 \pm 0.3$ ,  $P = 0.015$ ). On the other hand, BNP had no specific impact on beta cell function ( $129 \pm 17$  vs.  $124 \pm 11$  pmol<sub>C<sub>P</sub></sub>/mmol<sub>G</sub>), insulin clearance ( $8.7 \pm 1.1$  vs.  $8.3 \pm 0.7$  ml/min per kilogram) and insulin sensitivity ( $10 \pm 1$  vs.  $9 \pm 2 \times 10^4$  min<sup>-1</sup>/( $\mu$ U/ml)), all  $P > 0.6$ .

Intravenous administration of BNP increases glucose distribution volume lowering plasma glucose concentrations after a glucose load without affecting beta cell function and insulin sensitivity. These results speak for the concept that BNP does not worsen, but improves diabetes in patients with heart failure, and open otherwise new questions regarding BNP-induced differences in glucose availability and signaling in several organs/tissues.

## P571

### D-Lys-GHRP-6, an antagonist of ghrelin receptor type 1a, did not antagonize the effect of acylated ghrelin but antagonized the effect of unacylated ghrelin on the growth of HECa10 murine endothelial cell line

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Ghrelin is expressed in endothelial cells but its precise role remains unknown. Some authors treat ghrelin as an endogenous regulator of angiogenesis.

The aim of this study was to examine the direct effect of human acylated and unacylated ghrelin and rat acylated ghrelin applied alone or together with D-Lys-GHRP-6, an antagonist of ghrelin receptor type 1a, on the growth of murine endothelial cell line HECa10 assessed by Mosmann method *in vitro*.

Human ghrelin is homologous to rat ghrelin apart from two amino acids, but their effects on the growth of the examined line differ significantly. Human acylated and unacylated ghrelin inhibited the growth of HECa10 line at all examined concentrations ( $10^{-5}$ – $10^{-12}$  M) in 24 h and 72 h culture with almost the same potency. Rat acylated ghrelin inhibited the growth of this line only at 2 out of 8 concentrations ( $10^{-7}$ ,  $10^{-11}$  M) in 24 h culture and at 3 out of 8 concentrations ( $10^{-7}$ ,  $10^{-8}$ ,  $10^{-11}$  M) in 72 h culture. D-Lys-GHRP-6 used as an antagonist of ghrelin receptor type 1a applied alone also inhibited the growth of this cell line at all examined concentrations ( $10^{-4}$ – $10^{-6}$  M) in 48 h culture in dose-dependent manner, and in 72 h culture without dose–response effect. D-Lys-GHRP-6 did not modify the inhibitory effect of rat acylated ghrelin in 48 h culture, but in 72 h culture we observed even stronger inhibitory effect of this combination in comparison with the effect of each substances applied alone. Unexpectedly D-Lys-GHRP-6 at the concentration of  $10^{-4}$  M decreased, abolished or even reversed the inhibitory effect of human unacylated ghrelin in 72 h culture dependent on the ghrelin concentrations.

These data indicate that both ghrelins have an antiangiogenic properties. However, D-Lys-GHRP-6 seems not to be an appropriate antagonist of ghrelin receptor type 1a in this experiment.

**P572****Phosphate levels inversely correlate with aortic stiffness in patients with primary hyperparathyroidism**

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**Introduction**

Although data on the association of primary hyperparathyroidism (pHPT) with cardiovascular disease are accumulating, there is still controversy on this issue. Moreover, possible associations of aortic stiffness and central aortic pressures with pHPT and associated effective molecules have not been addressed.

**Methods**

Seventy patients with pHPT were consecutively recruited from the outpatients' clinic of an academic department of endocrinology. In all patients, arterial stiffness was assessed by measurement of pulse wave velocity (PWV) between carotid and femoral arteries while reflected waves and central blood pressures were evaluated by pulse wave analysis. Laboratory evaluation included parathyroid hormone (PTH), 25(OH)vitD, total calcium, phosphate and albumin levels.

**Results**

Phosphate levels but not calcium, PTH or vitamin D3 levels inversely correlated with PWV ( $-0.244$ ,  $P=0.046$ ) but not with reflected waves or central blood pressures. By multivariate analysis, among other risk factors, phosphate levels independently determined PWV ( $P=0.032$ ).

**Conclusions**

In patients with pHPT, phosphate levels independently correlated with aortic stiffness as expressed by PWV. Further research is needed to assess the possible effect of low phosphate levels on atherosclerosis in pHPT.

**P573****Low sex hormone binding globulin – strong association with metabolic syndrome in patients with acute coronary syndrome**

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**Objective**

Despite of conflicting data, reduced levels of endogenous sex hormones and especially sex hormone binding globulin (SHBG) seem to be associated with metabolic syndrome (MS). In this study we determined relationship between endogenous sex hormone levels and SHBG and components of metabolic syndrome in both sexes.

**Methods**

In 39 men (median age 70.2) with total testosterone level in the lowest quartile of normal range (median 12.8 nmol/l) and 29 postmenopausal women (median age 74.5, median estradiol 78.6 pmol/l) without hormone replacement therapy who were admitted with acute coronary syndrome we determined concentrations of endogenous sex hormones: total testosterone (TT), calculated free androgen index (FAI), estradiol (E) and SHBG. Using correlation analyze we determined their relationship to components of MS: waist circumference, serum concentration of triacylglycerides (TAG) and high density lipoproteins (HDL), diabetes mellitus, arterial hypertension and body mass index (BMI).

**Results**

In men low TT was associated with higher BMI ( $P=0.0009$ ) and waist circumference ( $P=0.017$ ), low SHBG was associated with higher BMI ( $P=0.0014$ ), waist circumference ( $P=0.0073$ ) and level of TAG ( $P=0.03$ ). FAI did not have relationship to MS. In women low estradiol was associated with higher level of TAG ( $P=0.02$ ). Lower TT was associated with higher BMI ( $P=0.02$ ) and waist circumference ( $P=0.03$ ) and low SHBG was associated with higher BMI ( $P=0.05$ ), arterial hypertension ( $P=0.006$ ) and TAG ( $P=0.007$ ). FAI in women correlated negatively with BMI ( $P=0.05$ ), waist circumference ( $P=0.04$ ) and diabetes mellitus ( $P=0.02$ ).

**Conclusions**

We confirmed inverse association between metabolic syndrome and endogenous sex hormones but especially SHBG. This supports the theory of possible nongenomic action of sex steroids mediated by SHBG although the exact pathogenesis of this relationship is still not well understood.

**P574****Thyroid and parathyroids' dysfunction in patients suffering from chronic renal failure**

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The aim of this study was to investigate the contribution of thyroid and parathyroid glands dysfunction to the arterial hypertension etiology in elderly patients suffering from chronic renal failure.

In 34 patients, average age of 67 years (range: 65–68), with glomerular filtration rate (GFR, <sup>99m</sup>TcDTPA) <60 ml/min, as well as the creatinine clearance (Cockcroft) values between 60 and 30 ml/min, thyroid gland function was analysed by thyrotropin releasing hormone (TRH, 200 µg/ml iv).

In 18 out of 34 patients (53%) stimulated TSH response in 20th and 60th minutes were elevated (28 mU/l ± 8 and 56 mU/l ± 12 respectively), that characterize the primary hypothyroidism, with peak of free thyroxine serum value in 60th minute of 11.8 ± 2.6 pM/l as typical quality of lower fast thyroid gland reserve.

Elevated inorganic phosphates (1.72 ± 0.8, range: 1.56–1.88 mM/l) were confirmed in 28 out of 34 (82%) patients, lower 25OHD<sub>3</sub> (42 ± 8.4 (X ± s.e.), range: 34–58 nM per litre) and secondary hyperparathyroidism (PTH: 132 pg/ml ± 12, range: 102–186). In 11/28 (39%) suffering from hyperparathyroidism, the primary hypothyroidism was established.

Elevated ionized serum calcium (28/34 patients: 1.36 ± 0.12 mM per litre, range: 1.32–1.52), followed lower serum magnesium (0.82 mM/l ± 0.12, X ± s.e.).

In 76% (26/34) people suffering from secondary hyperparathyroidism as well as in 67% (12/18) those with primary hypothyroidism arterial hypertension have been confirmed (TA 160/100, range 145–180 for systolic and 90–110 for diastolic value respectively).

Chronic, progressive renal failure influence both the thyroid and parathyroid glands function, contributing to hypothyroidism, secondary hyperparathyroidism and arterial hypertension, as well as to the blood vessels atheromatosis and calcifications.

**P575****Possible association between polyunsaturated fatty acids and parameters of metabolic syndrome in type 2 diabetes mellitus-pilot study**

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**Background**

In type 2 diabetes mellitus (T2DM), the level of polyunsaturated fatty acids (PUFA), is usually decreased, and could be accused for metabolic disturbance, chronic inflammation, and accelerated atherosclerosis. The aim of this study was to determine if there is any relationship between composition of plasma phospholipids PUFAs and parameters of metabolic syndrome.

**Methods**

The study was cross-sectional. Fifteen T2DM patients 63.22 ± 7.20, BMI 25.73 ± 3.24 kg/m<sup>2</sup>, waist 87.25 ± 5.84 cm, and 22 healthy subjects, age 59.30 ± 3.84, BMI 25.52 ± 2.90, waist 86.45 ± 3.26 cm, participated. Plasma FAs phospholipids profile was determined by GC chromatograph. Demographic and biochemical data, including serum glucose, HbA1c, insulin, lipids, creatinine, albumin, were collected. Univariate analysis and multiple linear regression analysis were performed.

**Results**

Diabetic patients have shown higher levels of some n-6 PUFA, 18:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, and higher levels of metabolic variables HbA1c, triglycerides,

and insulin resistance calculated by HOMA-IR. 18:2n-6 correlated directly with HbA1c ( $P < 0.05$ ), triglycerides ( $P < 0.05$ ), inversely with HDL-C ( $P < 0.05$ ), and directly with HOMA-IR ( $P < 0.05$ ). By multiple regression analysis 18:2n-6 was still independent predictor of HOMA-IR.

#### Conclusion

It looks like that n-6 PUFAs, particularly 18:2n-6, could contribute to insulin resistance in T2DM. Therefore, the treatment of insulin resistance in T2DM should include diet modification with reduce intake 18:2n-6 polyunsaturated fatty acid.

## P576

### Parathormone 1-84 in type 2 diabetic patients

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#### Objective

Increasing evidence suggests a role for mineral metabolism in cardio-vascular disease risk. We aimed at determining in type 2 diabetes patients the relationship between serum levels of 1-84 parathormone (PTH1-84), 25-hydroxy-vitamin D (25(OH)D) or calcium and the glycated hemoglobin (HbA1c), insulin sensitivity or  $\beta$ -cells function, but also the relationship between these factors and coronary arterial disease confirmed by myocardial scintigraphy.

#### Research design and methods

Serum PTH1-84, 25(OH)D and calcium were determined in 60 adult patients with type 2 diabetes divided into two groups: group A-normal myocardial scintigraphy ( $n=37$ ) and group B-positive scintigraphy ( $n=23$ ). The groups were matched regarding age, HbA1c, body mass index, glomerular filtration rate and pharmacotherapy with either vitamin D, angiotensin-converting enzyme inhibitors, sartans or calcium channel blockers.

#### Results

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 (positive myocardial scintigraphy) versus group A (negative scintigraphy) (Table). We found a negative correlation between PTH1-84 and glycated hemoglobin HbA1c ( $P=0.015$ ). There were no significant correlations between calcium, vitamin D or PTH1-84 and HOMA insulin-sensitivity or  $\beta$ -cells function (HOMA-B).

**Table 1**

	Group A	Group B	P value (B versus A)
Serum 1-84 PTH (16-81 pg/ml)	21 (10-42)	27 (17-47)	0.01
Serum 25(OH)D (30-100 ng/ml)	11 (5-66)	10.1 (5-31)	0.58
Serum total calcium (8.6-10 mg/dl)	9 (8.4-10.2)	8.9 (8.4-9.7)	0.35

#### Conclusion

In type 2 diabetic patients higher levels of PTH1-84 were associated with pathological myocardial scintigraphy, suggesting a role for PTH1-84 in cardiovascular disease risk. Serum PTH and HbA1c were inversely correlated.

## P577

### Rhabdomyolysis associated with fenofibrate: a case report

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Rhabdomyolysis is characterized by muscle necrosis and release of intracellular muscle constituents into the circulation. Most cases are associated with a predisposing factor such as high dose statin therapy, diabetes, old age, female gender, renal failure or hypothyroidism.

An 80-year-old woman presented with severe and generalized muscle pain. Her medical history included hypertension, congestive heart failure, chronic atrial

fibrillation and a *thyroidectomy* while an adolescent (unknown diagnosis). Recently she had been hospitalized due to newly diagnosed type 2 diabetes and treated with insulin. During her hospitalization, fenofibrate (267 mg) was added to her daily regimen, as her triglyceride, total cholesterol and HDL cholesterol levels were 561 mg/dl, 187 mg/dl, and 33 mg/dl respectively. Her other medications included furosemide, aspirin, spironolactone, ramipril, verapamil, warfarin tablets, and short- (aspart) and long-acting (detemir) analog insulins. Her physical examination was normal except for muscle tenderness. Upon hospitalization, creatinine, creatinine clearance, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine kinase and INR were 1.5 mg/dl, 28 ml/min, 377 IU/l (normal range (NR)15-41), 136 IU/l (NR 7-35), 7.081 IU/l (NR 98-192), 10.466 IU/l (NR 38-234) and 9.76 respectively. Serum creatinine values were similar to previous laboratory tests. To date, only a few cases of rhabdomyolysis due to fibrate treatment have been reported; its mechanism is unknown. The combination of statin and fibrate agents may increase the risk of rhabdomyolysis. Fenofibrate is highly (99%) bound to plasma proteins, mainly to albumin. Similarly, it interacts with drugs (such as warfarin) that are highly bound to plasma proteins. Fenofibric acid is inactivated by UDP-glucuronyltransferase into fenofibric acid glucuronide and is excreted mainly in urine. When starting fibrate treatment, factors such as age, drug interactions, and renal function should be taken into account.

## P578

### Utility of brain natriuretic peptide (BNP) as a biomarker of risk assessment in acute pulmonary embolism

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#### Introduction

Acute right ventricular dysfunction (RVD) on echocardiography (ECHO) represents the actual gold standard in risk stratification of patients with pulmonary embolism (PE). We previously demonstrated that plasma BNP above a cut-off level of 50 pg/ml could predict acute RVD in PE.

#### Aim and objective

Analysis of the utility and accuracy of plasma BNP in detecting acute RVD in PE.

#### Methods

Seventy patients with confirmed PE, 42 (60%) men, mean age  $52.51 \pm 8.82$  were prospectively investigated. Plasma BNP levels were measured on admission by a quantitative fluorescence immunoassay (Triage BNP). RV function was evaluated by ECHO in the first hour after admission. Patients were divided in two groups: group 1 – with acute RVD on ECHO,  $n=24$  (34.29%) patients; group 2 – without acute RVD on ECHO,  $n=46$  (65.71%) patients. Study protocol was approved by the local Ethics Committee.

#### Statistics

SPSS 16.0.; MedCalc 11.4.4.

#### Results

Plasma BNP proved good in discriminating between patients with and without acute RVD – area under the receiver operating characteristic curve (AUC)=0.86 (95% CI (0.77-0.94),  $P < 0.0001$ ). Stepwise multiple regression analysis identified some patients' characteristics independently and significantly associated with RVD: BNP  $\geq 50$  pg/ml ( $R=0.61$  (0.44-0.74),  $P < 0.0001$ ), syncope ( $R=0.57$  (0.38-0.71),  $P < 0.0001$ ), a Qr type ventricular complex (a prominent Q wave of  $\geq 0.2$  mV and a ventricular depolarisation  $< 120$  ms) in  $V_1$  lead on electrocardiography ( $R=0.55$  (0.36-0.69),  $P < 0.0001$ ), a heart rate  $> 100$  beats/min ( $R=0.54$  (0.35-0.69),  $P < 0.0001$ ). Stepwise logistic regression (meta-analysis) proved BNP  $\geq 50$  pg/ml to have the best accuracy in detecting RVD: odds ratio (OR) (95% CI)=21.00 (5.55-79.50), compared to: syncope (OR=9.33 (2.87-30.41)), Qr in  $V_1$  (OR=6.67 (2.06-21.55)), a heart rate  $> 100$  beats/min (OR=5.61 (1.86-16.99)).

#### Conclusion

Plasma BNP  $\geq 50$  pg/dl was the best predictor of acute right ventricular dysfunction and a valuable biomarker of risk stratification in the patients with pulmonary embolism from our study group.

## P579

**Correlation of atherogenic dyslipidemia with other plaque instability biomarkers and outcome in non ST-segment elevation acute coronary syndromes (NSTE-ACS)**

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**Introduction**

Atheromatous plaque instability biomarkers are the most important predictors for the evolution with major acute cardiovascular events (MACE) in patients with NSTE-ACS.

**Objective**

To evaluate the impact of dyslipidemia, endothelial dysfunction, oxidative stress, platelet hyperactivity and hypercoagulable state and their inter-correlation on the outcome of NSTE-ACS.

**Methods**

Two hundred and forty patients with NSTE-ACS, 154 (64.17%) men, mean age 58.31 ± 8.92, were investigated for dyslipidemia (LDL-Cholesterol > 100 mg/dl, HDL-Cholesterol < 50 mg/dl (men) and < 40 mg/dl (women)), oxidative stress [total antioxidant status < 1.3 mmol/l, Myeloperoxidase (MPO) (IgG ELISA) > 20 U], hypercoagulable state (Von-Willebrand factor activity > 169.7%, Protein C < 70%, Protein S < 72.2%, Antithrombin < 71%, Factor V Leiden – APC Resistance V ratio < 2.18), and platelet hyperactivity (multiple electrode aggregometry aspirin test (ASPtest) > 30 U, and adenosine diphosphate test (ADPtest) > 50 U), and were followed up for MACE (cardiovascular death, non-fatal myocardial infarction, recurrent angina with readmission, stroke) during 1 year. Local Ethics Committee approved the study protocol.

**Statistics**

SPSS 16.0.

**Results**

Except for the stroke, the incidence of MACE in patients with NSTE-ACS was significantly correlated with high LDL-Chol ( $r=0.74$ ,  $P<0.0001$ ), and low HDL-Chol levels ( $r=0.65$ ,  $P<0.001$ ), low serum total antioxidant status ( $r=0.69$ ,  $P<0.0001$ ), hypercoagulable state ( $r=0.63$ ,  $P<0.001$ ), and platelet hyperactivity ( $r=0.53$ ,  $P<0.01$ ). In patients with NSTE-ACS and MACE from our study group, a high LDL-Chol level (> 100 mg/dl) and a low HDL-Chol level (< 50 mg/dl in men and < 40 mg/dl in women) were both best correlated with a low total antioxidant status ( $r=0.63$ ,  $P<0.001$ ;  $r=0.54$ ,  $P=0.02$ ).

**Conclusion**

In patients with NSTE-ACS from our study group atherogenic dyslipidemia was significantly correlated with endothelial dysfunction, oxidative stress and platelet hyperactivity biomarkers, and with MACE at 1-year follow-up.

This work was supported by CNCIS-UEFISCU, Project number NII-ID\_727/2008

## P580

**Relationship between plasma aldosterone concentration and soluble cellular adhesion molecules in patients referred to coronary angiography**

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**Introduction**

An increasing notion suggests that aldosterone contributes to the development and progression of atherosclerosis and cardiovascular disease. Experimental studies documented an upregulation of cellular adhesion molecules, which are

involved in the pathogenesis of atherosclerosis, after administration of aldosterone. However, evidence for an association between circulating aldosterone levels and soluble cellular adhesion molecules in humans is sparse.

**Methods**

We investigated the relationship between plasma aldosterone concentration (PAC; median: 82.0 (51.0–129.0) pg/ml) and soluble cellular adhesion molecules in a large cohort of patients referred to coronary angiography. After exclusion of patients with ongoing oral contraceptive or hormone replacement therapy 1752 patients (mean age: 62.5 ± 10.8 years; 26.4% women) remained eligible for analyses.

**Results**

Age and gender adjusted partial correlation analyses revealed a positive association between PAC and soluble (s) selectin levels but not with sICAM1 and sVCAM1 respectively. In multivariate adjusted analyses of covariance (ANCOVA) sE- ( $P=0.003$ ), sL- ( $P=0.027$ ) and sP-selectin ( $P<0.001$ ) levels increased steadily from the first (reference) to the third gender-specific tertile of PAC. No significant variation across PAC tertiles was found for sICAM1 and sVCAM1 levels respectively. Finally, multivariate regression analyses revealed circulating aldosterone as an important predictor for soluble selectin levels.

**Conclusion**

Our findings in a large cohort of patients indicate that the upregulation of selectins might represent a novel mechanism of aldosterone mediated development and progression of atherosclerosis. Our findings warrants further interventional studies which should evaluate anti-atherosclerotic effects of aldosterone blocking treatment strategies in humans.

## P581

**Long term recreational physical exercise decreases angina susceptibility and induces cardiovascular protective shifts in nitric oxide synthase, heme oxygenase and matrix metalloproteinase enzyme regulation in rats**

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<sup>2</sup>Institute of Physical Education and Sport, Gyula Juhász Faculty of Education, University of Szeged, Szeged, Csongrad, Hungary.

**Introduction**

Physical inactivity, i.e. sedentary life style increases circulatory disease risk. Carbon monoxide and nitric oxide produced by the heme oxygenase (HO) and by the constitutive nitric oxide synthase (cNOS) protect circulation. In contrast, the enhanced level of the matrix metalloproteinase (MMP) augments cardiovascular risks. We examined the actions of recreational physical exercise on the expression of these enzyme systems in conjunction with cardiovascular protection.

**Design**

Male Wistar rats were placed into cages installed with running wheels allowing them the self administration of physical exercise over 6 weeks. We studied i) the activity and expression of HO in the aorta and heart left ventricle (LV); ii) the activity and expression cNOS in the aorta and LV; iii) the plasma level of MMP-2 and; iv) the angina susceptibility of the heart (assessed by lead II. surface ECG following adrenaline plus phentolamine challenge).

**Results**

We found that physical exercise: i) increased LV and aortic HO activity (from 0.83 ± 0.21 to 5.35 ± 0.36 and from 0.73 ± 0.15 to 1.60 ± 1.13 nmol bilirubin/h/mg protein respectively;  $n=12-15$ ;  $P<0.001$ ) and LV HO-1 isoenzyme expression (from 106.3 ± 3.132 to 164.0 ± 16.479%;  $n=6-7$ ;  $P<0.05$ ) and ii) increased LV and aortic cNOS activity (from 16.32 ± 3.71 to 59.11 ± 7.94 and from 105.38 ± 55.72 to 181.78 ± 30.29 pmol/min per milligram protein respectively;  $n=9-15$ ;  $P<0.001$ ) and LV endothelial NOS isoenzyme expression (from 109.15 ± 5.247 to 163.1 ± 10.67%;  $n=3$ ;  $P<0.05$ ); and iii) decreased MMP-2 plasma level (64 kDa; from 1002.71 ± 37.50 to 679.73 ± 34.35 intensity × mm<sup>2</sup>;  $n=12-13$ ;  $P<0.001$ ); and iv) decreased heart ischaemia susceptibility (ST segment depression: from -0.14 ± 0.018 to -0.019 ± 0.019 mV;  $n=11$ ;  $P<0.001$ ).

**Discussion**

Long term recreational physical exercise protects the heart against angina, which might be associated with the up-regulation of the HO and NOS enzyme system, and the down-regulation of MMP-2 activation.

**Grant supports**

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**P582****Heme-oxygenase mediates cardiovascular protection by oestrogen and raloxifene in menopause**A Pósa<sup>1</sup>, C S Varga<sup>1</sup>, A M Berkó<sup>1</sup>, M Veszelka<sup>1</sup>, R Szabó<sup>1</sup>, K Orbán<sup>2</sup>, I Pávó<sup>3</sup> & F László<sup>1,2</sup><sup>1</sup>Department of Physiology, Anatomy, Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Csongrad, Hungary;<sup>2</sup>Institute of Physical Education and Sport, Gyula Juhász Faculty of Education, University of Szeged, Szeged, Csongrad, Hungary;<sup>3</sup>Lilly Area Medical Center, Vienna, Austria.**Introduction**

Selective oestrogen receptor modulators function as oestrogen receptor antagonist in the breast and gonads, while they have agonistic effects on the bone and the cardiovascular system. It is also known that endogenously produced carbon monoxide by the heme-oxygenase (HO) enzyme exerts beneficial actions in cardiovascular protection.

**Design**

Intact females, ovariectomized (OVX), raloxifene or oestrogen treated OVX Wistar rats were used. We studied i) the activity and expression of HO enzymes in the left ventricle (LV) and aorta ii) the ST segment depression (standard lead II surface ECG) provoked by the administration of adrenaline (1 µg/kg, i.v.) and 30 second later phentolamine (1 mg/kg, i.v.), iii) the increase of blood pressure *in vivo* and iv) heart perfusion *ex vivo* induced by arginine vasopressin (AVP).

**Results**

We found that OVX i) decreased in the LV HO activity (from  $2.65 \pm 0.29$  to  $0.78 \pm 0.12$  nmol bilirubin/h per milligram protein;  $n=9-10$ ;  $P<0.05$ ) and HO-2/HO-1 expression (HO-2: from  $93.14 \pm 1.79$  to  $48.0 \pm 2.76\%$ ; HO-1: from  $87.43 \pm 3.02$  to  $39.86 \pm 4.79\%$ ;  $n=10$ ;  $P<0.05$ ) and in the aorta (HO activity from  $6.72 \pm 0.44$  to  $3.56X \pm 0.31$  nmol bilirubin/h/mg protein;  $n=8-10$ ;  $P<0.05$ ; HO-2: from  $92.25 \pm 2.03$  to  $56.51 \pm 3.24\%$ ; HO-1: from  $83.80 \pm 3.30$  to  $57.47 \pm 2.32\%$ ;  $n=12$ ;  $P<0.05$ ), ii) increased the susceptibility of the heart towards ischemia (ST segment depression: from  $-0.011 \pm 0.025$  to  $-0.13 \pm 0.037$  mV;  $n=10$ ;  $P<0.05$ ), iii) increased the response of blood pressure and iv) decreased the heart perfusion to AVP. The oestrogen and raloxifene replacement restored the differences to the level observed in the ovary-intact females. Finally, HO inhibition by tin-protoporphyrine pre-treatment augmented the response of blood pressure, the ST depression and heart perfusion in all groups investigated.

**Conclusion**

Oestrogen improves cardiovascular defence in menopause, at least in part, by a HO-mediated pathway. In our system, raloxifene exerts oestrogen agonistic effect.

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**Clinical case reports****P583****MGMT immunoreactivity in adamantinomatous craniopharyngiomas**Sayid Shafi Zuhur<sup>1</sup>, Ahmet Murat Musluman<sup>2</sup>, Canan Tanik<sup>2</sup>, Ozcan Karaman<sup>1</sup>, Feyza Yener Ozturk<sup>1</sup>, Hanife Ozkayalar<sup>3</sup> & Yuksel Altuntas<sup>1</sup><sup>1</sup>Sisli Etfal Training and Research Hospital, Endocrinology and Metabolism Department, Istanbul, Turkey; <sup>2</sup>Sisli Etfal Training and Research Hospital, Neurosurgery Department, Istanbul, Turkey; <sup>3</sup>Sisli Etfal Training and Research Hospital, Pathology Department, Istanbul, Turkey.**Background**

Currently, no effective medical treatment exists for recurrent and aggressive craniopharyngiomas that are resistant to conventional therapies, including repeat surgeries and adjuvant radiotherapy (RT). Temozolomide is an alkylating chemotherapeutic agent and is used routinely in the management of high grade gliomas. The response to temozolomide is suggested to be dependent on the tumoral expression of O-6 methylguanine DNA methyltransferase (MGMT). Evidence supports that low MGMT immunoreactivity correlates with positive response to temozolomide

**Purpose**

We aimed to evaluate MGMT immunoreactivity in adamantinomatous craniopharyngiomas, in an effort to predict the likelihood of response to temozolomide.

**Methods**

Our material consisted of 23 adamantinomatous craniopharyngiomas operated at the Sisli Etfal Training and Research Hospital during the interval 1993–2009 and identified by histological analysis. Immunostaining for MGMT was performed

using the avidin–biotin–peroxidase complex method. MGMT immunoreactivity was evaluated microscopically and recorded as percentage of nuclear MGMT immunostaining.

**Results**

Of the 23 specimens evaluated, 22 (96%) demonstrated negative (<10%) and 1 (4%) demonstrated low (10%) MGMT immunoreactivity.

**Conclusion**

The data provided by the current study suggests all adamantinomatous craniopharyngiomas exhibit low MGMT immunoreactivity and could be treated with temozolomide. Our findings provide a ground for the assessment of TMZ's efficacy in clinical trials as an alternative agent in this rare tumor subtype, if conventional therapy, including repeat surgeries and RT, fails.

**P584****Tolvaptan versus conventional treatment in hyponatraemia secondary to syndrome of inappropriate anti-diuretic hormone (SIADH): first case experience in West-Midlands region, UK**

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**Introduction**

Tolvaptan is an oral vasopressin V<sub>2</sub> receptor antagonist which offers a targeted approach in regulating body water and serum [Na<sup>+</sup>] and is a novel treatment for hyponatraemia secondary to SIADH. Here we report our first case experience with tolvaptan comparing its efficacy with conventional treatments in the same patient.

**Case report**

A 52-year-old lady was admitted with confusion, nausea and vomiting. She gave a 3-month history of increasing breathlessness, anorexia and weight loss. Her comorbidities included hypothyroidism, hypertension and hyperlipidaemia. She had 45 pack years of smoking history. On clinical examination, left lateral rectus palsy resulting in diplopia was noted. From subsequent investigations she was diagnosed with hyponatraemia secondary to SIADH (serum [Na<sup>+</sup>] 115 mmol/l) due to metastatic small cell lung cancer T2N2M1b (pituitary fossa). Palliative chemotherapy was commenced. Symptomatic hyponatraemia was initially treated with fluid restriction (0.8–1 l/24 h). Serum [Na<sup>+</sup>] improved to 135 mmol/l in 2 weeks with a gradual decline thereafter reaching 110 mmol/l at 2 months. During this time she had two recurrent admissions with symptomatic hyponatraemia which delayed her chemotherapy. She then received demeclocycline 300 mg TDS during her inpatient stay and serum [Na<sup>+</sup>] increased from 110 to 115 mmol/l over 5 days. As any improvement in serum [Na<sup>+</sup>] with conventional treatments had been slow and marginal, tolvaptan 15 mg PO OD was initiated. Within 36 h, serum [Na<sup>+</sup>] showed significant improvement (from 115 to 127 mmol/l). Her clinical condition improved and she was discharged on tolvaptan. Serum [Na<sup>+</sup>] remained normal and she experienced no adverse effects while taking tolvaptan for 35 days. Unfortunately, she then died from the combination of progressive disease and chemotherapy complications.

**Conclusion**

In a patient with hyponatraemia secondary to SIADH, tolvaptan raised serum [Na<sup>+</sup>] levels more promptly than fluid restriction or demeclocycline, improving clinical condition and enabling discharge from hospital, and was well tolerated.

**P585****Coexistence of double small insulinomas with a small glucagonoma in a patient with multiple endocrine neoplasia-type 1 (MEN-1)**Sayid Shafi Zuhur<sup>1</sup>, Ozcan Karaman<sup>1</sup>, Sumeyra Yildirim<sup>1</sup>, Sibel Erdamar<sup>3</sup>, Hakan Senturk<sup>2</sup> & Yuksel Altuntas<sup>1</sup><sup>1</sup>Sisli Etfal Training and Research Hospital, Endocrinology and Metabolism Clinic, Istanbul, Turkey; <sup>2</sup>Gastroenterohepatology Department, University of Istanbul, Cerrahpasa Faculty of Medicine, Istanbul, Turkey; <sup>3</sup>Pathology Department, University of Istanbul, Cerrahpasa Faculty of Medicine, Istanbul, Turkey.**Background**

MEN-1 is a rare disease characterized by parathyroid adenoma or hyperplasia, pituitary adenoma and pancreatic neuroendocrine tumors (PNET). Gastrinomas are the most common functional PNET in MEN-1 disease. Insulinomas and glucagonomas constitute approximately 20 and 3% of PNET's in MEN-1 disease. Most of insulinomas are benign in nature but about 70% of cases of glucagonomas present with metastasis at diagnosis. Coexistence of various PNET's are very rare entity in MEN-1 disease.

## Case

A 27-years-old female presented with loss of consciousness and headache during fasting. Neurological and physical examination was normal. Her laboratory findings were consistent with insulinoma and primary hyperparathyroidism. Prolonged fasting test was consistent with insulinoma. A 0.4 cm pituitary mass was found on MRI which was non-functional. Tc 99 m sestamibi SPECT/CT demonstrated a left lower parathyroid adenoma. There was not pancreatic mass on USG, CT and MRI. EUS demonstrated a 0.9 cm mass in distal pancreas. EUS guided FNAB was consistent with PNET. The patient underwent parathyroid adenectomy and thymectomy followed by distal pancreatectomy. Histopathological analysis of distal pancreas demonstrated three well-differentiated PNET. Immunohistochemical analysis was positive for glucagon and negative for insulin in the largest tumor (0.8 cm), and positive for insulin but negative for glucagon in remaining two tumors (0.6 and 0.5 cm). All of three tumors were strongly positive for chromogranin and synaptophysin and negative for somatostatin. After surgery, glycemia became immediately normal.

## Conclusion

Very small number of the coexistent double small insulinoma along with a small glucagonoma in MEN-1 disease have been reported in English literature. However, insulinoma could be multifocal and could also be associated with glucagonoma, as in the present case. Small tumors could be missed easily on pathologic analysis, therefore, careful histopathological examination of pancreas should be made in cases of MEN-1 disease

## P586

**A case report of macronodular adrenal hyperplasia with combined cortisol and aldosterone oversecretion in response to  $\beta$ -adrenergic and vasopressin stimuli**

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## Introduction

Adrenocorticotropin (ACTH)-independent macronodular adrenal hyperplasia (AIMAH) is a rare cause of Cushing's syndrome, commonly involving the aberrant expression of several membrane-bound adrenal receptors. Recent studies have investigated whether the latter is also implicated in the renin-independent aldosterone secretion of hyperaldosteronism cases.

## Case report

A 35-year-old man presented to our department for evaluation of new-onset arterial hypertension without other symptoms or clinical signs. An adrenal computerised tomography revealed bilateral macronodular adrenal hyperplasia. Adrenal hormonal investigation showed cortisol and marginal aldosterone oversecretion (urinary free cortisol (UFC) 192  $\mu$ g/24 h, basal ACTH <5 pg/ml, serum cortisol following low dose dexamethasone suppression test 216 nmol/l and post-saline infusion serum aldosterone 136 pmol/l). Following an extensive diagnostic protocol for the detection of aberrant hormone receptors, a positive response of cortisol and aldosterone was evident in the isoproterenol infusion and the insulin tolerance test pointing to  $\beta$ -adrenergic stimulation. A positive response of cortisol was also shown in the vasopressin administration mediated via V1-receptor activation. The nor-iodo-cholesterol adrenal scintigraphy demonstrated increased uptake on the right adrenal gland. Following a 3-month trial of propranolol therapy, a right adrenal-sparing adenectomy was performed. The post-operative follow-up revealed persistent cortisol oversecretion and resolution of prior aldosterone autonomy.

## Conclusions

We report a clinical case of AIMAH with combined cortisol and aldosterone oversecretion in response to  $\beta$ -adrenergic and V1-receptor mediated vasopressin stimuli. The *in vivo* identification of aberrant adrenal receptors would confirm our clinical findings and aid to clarify the mechanisms underlying this rare form of adrenocortical disorder.

## P587

**Virilizing ovarian tumor – case report**

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Virilizing ovarian tumors are a rare cause of hyperandrogenism; they account for 1–2% of all ovarian tumors being the Leydig cell tumor the most common one.

We report the case of a 76-year old woman with type 2 diabetes that was referred for androgenic alopecia with 3 years evolution and a recent facial, peri-areolar and dorsal hirsutism. A diagnosis of hypertension was made 18 months previously, and she was on a four anti-hypertensive drug regimen. On examination she had central obesity (BMI 34.5 kg/m<sup>2</sup>), hypertension (180/110 mmHg) and hirsutism (score 18); there was no clitoromegaly, cutaneous bruising or striae. Laboratory tests showed normal ACTH, cortisol, S-DHEA and androstenedione but elevated total testosterone- 1.7 ng/ml (0.4–0.8) and free testosterone- 3.9 pg/ml (<1.55); 1 mg dexamethasone suppression test and ACTH stimulation test were normal. Endovaginal ultrasound and abdominal CT scan showed no evidence of adrenal or ovarian masses. She was submitted to bilateral salpingo-oophorectomy. Histopathological studies revealed a left 1 cm ovarian Leydig cell tumor. Post-operatively testosterone declined to normal levels and an improvement in alopecia and hirsutism was obtained in the following 6 months.

In post-menopausal women the appearance of signs of masculinization and high testosterone levels are suggestive of an ovarian virilizing tumor. Although endovaginal ultrasound is useful for the diagnosis, it was negative in this patient, and clinical and biochemical criteria were determinant for surgery.

## P588

**Congenital adrenal hyperplasia and adrenal myelolipoma – incidental or causal relationship?**

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## Introduction

There are fewer than 20 reported cases involving congenital adrenal hyperplasia (CAH) and myelolipoma. This is a rare benign tumor, often located on the adrenal, consisting of mature adipose and hematopoietic tissues. Its origin is unclear; the most accepted theory is the occurrence of blood capillaries reticuloendothelial cells metaplasia, in response to infection, stress or necrosis. In a small number of cases ACTH hypersecretion coexists.

## Case report

Woman 56 years, is referenced to endocrinology due to external genitalia virilization. She presented melanoderma, hirsutism, androgenic alopecia and ambiguous genitalia. Height = 141.2 cm; weight = 60 kg; BMI = 30.2 kg/m<sup>2</sup>. Previous diseases: meningitis at 8 years, primary amenorrhea, hypertension, dyslipidemia, osteoarthritis and left adrenalectomy for myelolipoma (12.5 cm) in 2007. Laboratory results: FSH 66 mIU/ml (<34), LH 21 mIU/ml (<25), estradiol 52 pg/ml (<14), DHEAS 1.3  $\mu$ g/ml (0.35–4.3), androstenedione 44 ng/ml (0.5–3.4), 11-desoxycortisol 20 ng/ml (<8), 17-OHP 196 ng/ml (0.2–1.6), total testosterone 4.0 ng/ml (<0.9), ACTH 490 pg/ml (9–52), cortisol 9.7  $\mu$ g/ml (5–25), IGF1 127 ng/ml (81–225), prolactin 10 ng/ml (<20), TSH 1.3  $\mu$ IU/ml (0.4–4), FT4 1.3 ng/dl (0.8–1.9), renin 6770  $\mu$ IU/ml (7–76), aldosterone 302 pg/ml (40–310). Tetracosactide test (60 min): 17-OHP 200 ng/ml; 11-desoxycortisol 23 ng/ml. Pelvic US: uterus and ovaries not shown; pituitary MR: '... intra-pituitary 5 mm nodular area... microadenoma can't be excluded...'; abdominal CT: 'left adrenal absence; right adrenal: 15 mm nodule... consistent with adenoma... hepatic steatosis... 2.1 cm cyst in left kidney'. Karyotype: normal. Genetic analysis: '...homozygous for g.999 T>A severe mutation, associated with enzyme 21-hydroxylase activity reduced to 1–2% of normal...' Dexamethasone 0.5 mg/day was started.

## Conclusions

This is a very challenging case in terms of diagnosis: a simple virilizing CAH in a patient with a large myelolipoma. This case reinforces the theory that the prolonged prolonged hypersecretion of ACTH can lead to myelolipomatous changes in adrenal gland due to chronic stress. The finding of ACTH receptors in myelolipoma will be necessary to prove it.

## P589

**Agnesis of the dorsal pancreas, a rare cause of diabetes: a case report and review of 25 Japanese patients**

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## Introduction

Agnesis of the dorsal pancreas, with the absence of the pancreatic body and tail, is a very rare developmental anomaly. To date, only 51 cases have been reported in the English literature. This anomaly can be a cause of diabetes mellitus.

Case report

A 19-year-old Japanese male visited our department because of glucosuria that had been detected during a routine school health check. The patient had undergone cardiac surgery for atrial and ventricular septal defects at the age of 4 years. He denied any history of abdominal trauma, alcohol intake or gastrointestinal problems. Laboratory investigations showed that his fasting hyperglycaemia was 19.7 mmol/l (354 mg/dl), HbA1c level was 12.1% and daily urinary excretion of C-peptide was 43.9 µg/day (normal range: 50–100 µg/day). Anti-islet cell antibody and anti-glutamic acid decarboxylase antibody were negative. Computed tomography confirmed that the body and tail of the pancreas were absent, and a magnetic resonance cholangiopancreatography showed that the dorsal pancreatic duct was absent. Based on these findings, a diagnosis of agenesis of the dorsal pancreas was made. The patient's blood glucose levels improved with insulin treatment.

Discussion

We found 25 Japanese patients (22–83 years, 12 males and 13 females) with clinical descriptions of agenesis of the dorsal pancreas by systematically searching the international and Japanese medical database. In approximately 70% of the reported patients, glucose intolerance or diabetes was demonstrated, and dysfunction of the pancreatic exocrine was found in 60%. Moreover, 28% of the patients had other organ anomalies in cardiopulmonary, gastrointestinal or genitourinary systems.

Conclusion

Agenesis of the dorsal pancreas is rare. However, it is important not to overlook this anomaly, particularly in young patients with diabetes and a history of other congenital organ anomalies.

P591

**X-linked adrenomyeloneuropathy presenting as Addison's disease**

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Introduction

It has been estimated that up to 30% of idiopathic Addison disease in young boys is due to X-linked Adrenoleukodystrophy. We report the case of a 31-year-old previously asymptomatic man, who presented with hyperpigmentation and fatigue. Investigations revealed hyponatraemia (116 mEq/l), low serum cortisol (2.6 µg/ml) and high ACTH levels (>2000 pc/ml), plasma potassium was 4.37 mEq/l. Adrenal antibodies were absent and abdominal computerized tomography scan showed only atrophied adrenal glands. He started hormone replacement therapy but 2 months later he developed, mild cognitive impairment, behavioural disturbances and abnormal sphincter control. Cerebral MRI imaging showed altered signal intensities on the pontine area. The diagnosis of adrenomyeloneuropathy was confirmed by raised circulating concentrations of very long chain fatty acids (VLCFA). (C22: 2.13 mcg/ml (0.18–0.48) C24:O/C22:O: 1.38 (<1.00) C26:O/C22:O: 0.09 (<0.02)). Genetic testing showed a missense mutation causing asparagine to tyrosine exchange at position 289 (A289T) of ALD protein, that to our knowledge has not yet been described. .30 ml/day of Lorenzo's oil was added, 2 months later VLCFA levels were lower (C26: 1.21 mcg/ml C24:O/C22:O: 1.1 C26:O/C22:O: 0.07) and no neurological progression was observed.

Conclusions

Adrenoleukodystrophy should be considered in young adults with a primary adrenal insufficiency of unknown etiology. Lorenzo's oil may be effective in reducing very long chain fatty acids in plasma. Although no clinically significant neurological improvement was observed in our patient, more studies are clearly needed to establish a potential role of this therapy

P590

**A case of two novel mutations in the LHCGR gene in a patient with 46,XY DSD**

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Introduction

Leydig cell hypoplasia (LCH) is a rare autosomal recessive condition that interferes with normal development of male external genitalia in 46,XY individuals. It is mediated by mutations in the luteinizing hormone receptor gene (LHCGR), most frequently located in the coding sequence, resulting in impairment of either LH/CG binding or signal transduction.

Case report

We report a 32-year-old female which presented with primary amenorrhea, female external genitalia and 46,XY karyotype. Breast and pubic hair development were B2 and PH2 respectively. Magnetic resonance imaging revealed inguinal testicles in the absence of uterus and ovaries. At first presentation serum luteinizing hormone (LH) was about threefold upper the normal limit while testosterone was in the female adult normal range. Estradiol was significantly low (10.6 pg/ml) and serum follicle-stimulating hormone was unremarkable. Dehydroepiandrosterone sulphate, 17-OH-progesterone and SHBG were normal. Anti-Müller-hormone and inhibin B were markedly elevated. Mutation or deletion of the SRY-, androgen receptor- and steroid 5-alpha-reductase-gene could be excluded. Finally, sequencing of the LHCGR gene revealed homozygote frameshift mutation in exon 10 (c.907C>T, p.Gln303Trm). Furthermore, a second homozygote mutation was found (c.935A>G, p.Asn312Ser), although this mutation seemed to be of no clinical relevance, since it was located downstream the disruption of the gene sequence. We therefore concluded to the diagnosis of severe Leydig cell hypoplasia. A hormone replacement therapy with estradiol was inducted and the further operative removal of the inguinal testicles as well as the genetic analysis of the parents is planned.

Conclusions

This case-report is the first demonstrating two novel different homozygote mutations in the same hormone binding domain and therefore expands the genotypic spectrum of LHCGR mutations.

P592

**Central precocious puberty related to a tuber cinereum hamartoma in neurofibromatosis type 1 (NF1): case report**

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Background

The prevalence of precocious puberty (PP) and its relationship to optic pathway tumours in patients suffering from neurofibromatosis type 1 (NF1) is significantly greater than in normal population.

Objectives

We present a rare case of precocious puberty in neurofibromatosis type 1 which is not associated with optic pathway tumour but with a tuber cinereum hamartoma. Case report

We present the case of a 5-year and 5-month old male subject examined in pediatric endocrinology for growth spurt, testicular enlargement, pubic hair appearance in association with diffuse disseminated 'café au lait spots'. Family history of confirmed neurofibromatosis type 1 reveals the associated pathology of our case. The patient has the Tanner stage P2G4; triptorelin test reveals the diagnosis of central precocious puberty (LH has increased from 2.85 to 21.89 mIU/ml, FSH increased from 1.98 to 3.52 mIU/ml and testosterone from 3.36 to 8.16 ng/ml). Cerebral MRI revealed a tuber cinereum hamartoma in the floor of the third ventricle in intimate contact with the optic chiasm. Triptorelin treatment has been successfully initiated, with limitation of growth velocity and puberty signs at 3-month interval.

Conclusions

Precocious puberty is a frequent endocrinologic feature in patients with genetically confirmed NF1, and is not related only to optic nerve gliomas. Children with NF1 need early hormonal screening, in order to unravel precocious puberty even at a preclinical level, which is critical for rapid diagnosis and introduction of a proficient treatment

**P593****Hypoparathyroidism and Morbus Fahr case report**

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Hypoparathyroidism is rare endocrine-metabolic disease, characterized by hypocalcemia, hyperphosphatemia and low level of the parathyroid hormone (PTH). Hypocalcemia sometimes is associated with calcifications at the basal ganglia (Morbus Fahr), leading to epileptic seizures, hypocalcemic cataracts and depression. The patient was admitted at the Clinic of endocrinology in Skopje, due to history of long term epileptic seizures, treated with antiepileptic therapy, operated from cataracts on both eyes by unknown origin and episodes of depression and complete clinical confirmation of this disease. His laboratory results showed low level of ionized  $\text{Ca}^{++}$  0.47 mmol/l (1.3–1.6 mmol/l), total Ca 0.83 mmol/l (2.1–2.6 mmol/l), hyperphosphatemia P- 3.1 mmol/l (0.8–1.4 mmol/l), hypophosphaturia 10 mmol/l (20–60 mmol/l), hypocalciuria 1 mmol/l (1.3–10 mmol/l), low level of PTH 1.76 pg/ml (15–65 pg/ml), high level of LDH 1332 U/l (213–423 U/L) and CPK 1204 U/l (24–173 U/l), creatinine level 79 mmol/l (45–109 mmol/l), total proteins 76 g/l (63–83 g/l). Chvostek and Trousseau were negative. Calcifications at the basal ganglia were proven by computed tomography and nuclear magnetic resonance. Dual-energy X-ray absorptiometry (DXA) showed osteosclerosis. During the hospitalization, he was substituted with calcium gluconate parenterally, later orally with calcium carbonate a 1.5 g, Tabl.Rocaltrol a 0.5 µg and Sol.Vigantol 1200 IU. As a result of this treatment, the seizures disappeared and the antiepileptic therapy was discontinued.

**Conclusion**

Patients with epileptic seizures and cataracts should always be clinically and laboratory evaluated for calcium, phosphates and PTH, in order to diagnose properly and to avoid inadequate treatment.

**P594****Acute respiratory distress syndrome in a nonsurgical transsexual**

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**Introduction**

Liquid silicone is an inert material that as minimal local tissue reaction, but it is the systemic complications, such as silicone embolism and acute respiratory distress syndrome (ARDS) that carry the highest associated morbidity. Liquid silicone is frequently used for illegal cosmetic procedures in transsexual patients, and the most common injection sites are the hips, buttocks, face and breasts.

We report a case of silicone embolism with ARDS in a nonsurgical transsexual. Case report

A 35-year-old African non-surgical male-to-female transsexual with type 1 diabetes mellitus was admitted to the hospital complaining of dyspnea, cough and hemoptysis 2 days after illegal silicone injection in the gluteal region by nonmedical personnel. Laboratory screening showed leukocytosis. Chemistry was normal. EKG was unremarkable. Bilateral patchy alveolar infiltrates were present on the chest radiograph. Arterial blood gas analysis revealed severe hypoxemic respiratory failure.

Oxygenation worsened, the patient developed ARDS and was admitted in an intensive care unit for intubation and mechanical ventilation. Steroid therapy was started. CT scan of the chest demonstrated peripheral airspace consolidations in both lungs. Echocardiogram was normal.

Fiberoptic bronchoscopy showed hemorrhagic alveolar fluid and cytology showed polymorphonuclear leucocytes, macrophages and vacuolated deposits of silicone.

After extubation on the 10th day, the patient gradually improved with supportive care, including supplemental oxygen, antibiotics and steroid therapy.

The patient was discharged in stable condition after 21 days.

**Conclusion**

Silicone embolism should be suspected in patients with a history of illicit liquid silicone injections who present pulmonary symptoms.

**P595****A unique case of multifocal intrathyroid parathyroid carcinoma**Ivan Kruljac, Petra Sulentic, Maja Berkovic Cigrovski, Davorka Herman Mahecic, Gorana Mirosevic, Mirko Ivkic & Milan Vrkljan  
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Intrathyroidally located parathyroid carcinoma is extremely rare cause of primary hyperparathyroidism. Multifocal papillary and follicular thyroid carcinomas are rather common.

We report a case of 40-year old man presenting with substantial weight loss, anemia and multiple osteolytic lesions of both tibiae. Peak serum calcium level was 5.0 mmol/l, parathyroid hormone (PTH) level was 989 pg/ml. Left thyroid lobe was firm and enlarged. Routine percutaneous fine needle aspiration (FNA) of intrathyroidal mass disclosed poorly differentiated follicular thyroid carcinoma. FNA of the nodule beneath the left lobe indicated parathyroid origin, possibly parathyroid neoplasm. We performed en block resection of the left thyroid lobe and the nodule beneath it. Frozen section identified likely medullary carcinoma, as well proven in mediastinal lymph node which was previously misinterpreted as enlarged parathyroid gland. Total thyroidectomy and bilateral modified radical neck dissection were then performed. Negative thyroglobulin and calcitonin, but positive PTH expression confirmed parathyroid carcinoma with a single upper mediastinal lymph node metastasis and multiple diffuse microscopic metastases in both thyroid lobes.

This is the first reported case of parathyroid carcinoma with multiple foci within thyroid tissue. Presurgical and intraoperative diagnosis of parathyroid carcinoma can be difficult, especially if located within thyroid gland. In patients with primary hyperparathyroidism and intrathyroid suspicion of malignancy finding, parathyroid carcinoma should be considered. The exact mechanism of intraglandular multifocal thyroid carcinomas has not been determined yet. The first reported presence of multiple foci of intrathyroid parathyroid carcinoma, suggests the intraglandular dissemination of the primary tumor is the main mechanism of multifocality.

**P596****Conservative or radical approach in patients with 46 XY disorders of sex development?**C S Martin<sup>1,2</sup>, S Fica<sup>1,2</sup>, A Miron<sup>1,2</sup>, C Gulea<sup>1</sup>, D Terzea<sup>3</sup>, S Enache<sup>3</sup>, F Andrei<sup>1</sup> & C Nitipir<sup>1</sup><sup>1</sup>Elias Hospital, Bucharest, Romania; <sup>2</sup>Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; <sup>3</sup>Victor Babes National Institute, Bucharest, Romania.**Introduction**

The risk for the development of germ cell tumors is hard to predict in patients with disorders of sex development (DSD) and their optimal management has been a continuous matter of debate. The risk varies between unknown and 40–60% in Denys-Drash/Frasier syndrome. This malignant potential opposes itself firmly to the recent interest in a more conservative approach regarding gonadectomy.

**Case presentation**

We present the case of a 20-years old phenotypic female, diagnosed at birth with 46 XY DSD, and clitoromegaly (clitoroplasty at 12-months old). She presented to our clinic in July 2010; no personal/ family history, BMI 29 kg/m<sup>2</sup>, height= 172 cm, android habitus, feminine secondary sexual development, acne, hirsutism, euthyroid (TSH= 1.22 µUI/ml, FT4= 1.29 ng/dl, ATPO= 14.7 UI/ml), DHEAS= 449.4 µg/dl, total testosterone= 8.13 nmol/l, low AMH= 0.26 µg/l (N for males 2-5), 17OHprogesterone= 1.8 ng/dl, estradiol= 127.1 pmol/l, FSH= 17 mUI/l, LH= 10 mUI/l, slightly elevated LDH= 217 U/l, normal tumor markers: CA125= 14.9 U/ml, CEA= 1.4 ng/ml, αFP= 1.13 ng/ml, total hCG= 1.1 mUI/ml. Abdominal IRM gadolinium enhanced: normal adrenal glands, 5/1.7/4.6 cm uterus, 9 mm endometrium, and two parauterin 2/1 cm and 2.3/1.5 cm gonadal structures. We decided total anexectomy; the histopathology exam diagnosed mixt ovarian and testicular structures, dysgerminoma associated to gonadoblastoma, mixt tumor of germinal cells and sex cordons; IHC: CD117 positive in dysgerminoma cells, CD30 negative, CK7 negative, INHIBIN positive in sex cordon tumor cells, EMA negative, Ki67 positive in 15% of tumor cells. The patient was referred to the oncology department.

**Conclusion**

In view of the limited patient numbers, an intense and multicenter collaboration is needed. We need safe, evidence- based protocols for a definitively revised management of patients with DSD.

### P597

#### Use of our last pills of iopanoic acid in a 33-year-old patient in acute refractory pulmonary edema and thyroid storm: requiem for a useful therapeutic agent

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#### Introduction

The contrast agents iopanoic acid and ipodate are potent inhibitors of thyroid function, reducing type I deiodinase T<sub>3</sub> generation, inhibiting thyroid liberation of T<sub>4</sub> and T<sub>3</sub>, and T<sub>3</sub> receptor binding, with off-label use in hyperthyroid emergencies. However, since the interruption of iopanoic acid production this year, neither agent is currently available in Spain.

#### Case report

A 34-year-old male was admitted to our hospital's ICU in acute pulmonary edema. He had noted weight loss, palpitations, nervousness, fatigue and hyperhidrosis during the previous months. Dyspnea at rest. Physical examination: Temperature(T) was 39°C, heart rate (HR) 133/min, irregular; bilateral exophthalmos; pronounced eyelid retraction, positive Graefe and Moebius signs; firm grade III goiter; intense thyroid bruit; precordial thrill; systolic IV/VI mitral murmur; bilateral wet rales; peripheral edema reaching knees; intense fine tremor of tongue and hands. TSH: 0.00 (0.34–5.6 mIU/l), fT<sub>3</sub>: 19.99 (2.5–3.99 pg/ml), fT<sub>4</sub> 54.56 (5.8–16.4 pg/ml). EKG: atrial fibrillation. Chest X-ray: cardiomegaly, pulmonary edema. Echocardiography: biventricular dilation; moderate mitral and tricuspid regurgitation; moderate pulmonary hypertension. Patient received 80 mg metimazol, i.v. hydrocortisone, furosemide, 1 mg i.v. propranolol without improvement. Endocrinology was consulted and advised use of the last pills of iopanoic acid: 1 g q12 h, 2 mg i.v. Dexamethasone q6 h. Eight hours later: HR was 97; T 37.3°C; fT<sub>3</sub> 8.95. At 14 h: HR 93; T 36; fT<sub>3</sub> 6.34, no dyspnea. 24 h: HR 89, reduced bruit and thrill. Total thyroidectomy was performed on day 10. Six weeks later the patient was asymptomatic, in sinus rhythm, on levothyroxine and propranolol alone.

We conclude that iopanoic acid should be reintroduced into the thyroid pharmaceutical arsenal.

### P598

#### Hypocalcemic laryngospasm in the emergency department

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#### Introduction

Severe hypocalcemia is a life threatening condition, usually symptomatic with cardiovascular and neuromuscular manifestations. Varying clinical presentations and concomitant infections, however, might obscure the right diagnosis, delaying early treatment.

#### Case

A 51-year-old African male presents himself at the emergency department (ED) with complaints of a soar throat, a productive cough and a striking pinched voice. Investigation revealed a severe corrected hypocalcemia (0.97 mmol/l) with a prolonged QTc interval on ECG of 515 ms. We administered intravenous calcium at the ED, which instantly improved the quality of his voice. He was eventually diagnosed with primary hypoparathyroidism for which he has been treated with alfacalcidol and calcium suppletion and an upper airway infection as well.

#### Discussion

The hallmark of hypocalcemia is tetany. In spite of severe hypocalcemia, tetany was not the presenting symptom in our patient. Laryngospasm is rare to be the presenting symptom of hypocalcemia, especially without signs of overt tetany or seizures.

Failure to recognize hypocalcemia by interpreting the complaints to be caused by an upper airway infection can lead to discharging a patient at risk for serious complications like seizures, dysrhythmias such as torsade de pointes and even cardiac arrest.

#### Conclusion

- Laryngospasm is a rare presenting symptom of hypocalcemia.
- In patients with nonspecific complaints of the throat and voice, laboratorium serum calcium and an ECG should be part of routine investigation in the ED.
- Concomitant infections might obscure the diagnosis of hypocalcemia.
- In case of severe hypocalcemia, calcium should instantly be administered intravenously in the ED.

### P599

#### Thyroid storm in the emergency department

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#### Introduction

Thyroid storm is a potentially fatal disorder if treatment is not initiated promptly upon assessment at the emergency department (ED).

#### Case

A 20-year-old young woman is referred to the ED with rapid acceleration of complaints of palpitations, fever, diarrhoea and agitation, which had been present since several weeks.

On physical examination we saw an uncomfortable, restless woman with a tachycardia of 170/min, and a fever of 38.5°C. Palpation of the neck revealed a small ventral, painless, solid elastic mass, more prominent on the right side, clinically suspicious for goiter. ECG showed an Atrial Flutter of 150/min. Initial laboratory results showed an ESR of 35 mm/h (0–20 mm/h) and urine analysis tested positive for ketones.

We presumed the patient was suffering from a thyroid storm for which we promptly started treatment at the ED with propranolol and thiamazole.

The next day she was diagnosed with graves disease. Lab results showed a TSH of <0.01 mIU/l (0.4–4.0 mIU/l) and a free T<sub>4</sub> of >75 pmol/l (10–22 pmol/l).

#### Discussion

Thyroid storm is an acute, life-threatening, hypermetabolic state induced by excessive release of thyroid hormones. The adult mortality rate is extremely high (90%) if early diagnosis is not made and the patient is left untreated.

Therefore it is critical in case of clinical suspicion for Thyroid storm to start prompt treatment with beta blockade and thiamazole before the diagnosis can be confirmed biochemically.

#### Conclusion

- Recognition of thyroid storm can be challenging, especially if there are no clues on physical examination.
- Prompt treatment of thyroid storm is vital for outcome.
- Definitive diagnosis of thyroid storm can only be made after treatment has been started.

### P600

#### Lady with backache and inadequate work-up for hypercalcemia for 5 years

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#### Introduction

Primary hyperparathyroidism affects 1% of the world's population, with majority of cases due to benign parathyroid adenomas. Parathyroid cancer is a rare cause.

#### Case report

This case report describes a 53-year-old lady presenting with nonspecific bone pains, whose initial workup revealed mild hypercalcemia and MRI lumbosacral spine suggestive of multiple myeloma. Subsequent work up, including two bone marrow examinations and serum protein electrophoresis were negative for myeloma. Sestamibi scan was also negative. A phenomenally elevated PTH level (2105 pg/ml, normal range 16–87) and rising serum calcium ordered years later prompted a repeat sestamibi scan and ultrasonography of neck. DEXA scan was consistent with severe osteoporosis. Based on the PTH levels, and radiologic findings, a diagnosis of primary hyperparathyroidism, with a high suspicion of parathyroid cancer, was made. Renal function tests and neurologic status remained essentially normal. Following this, the patient underwent surgical tumor resection with subsequent histopathological confirmation of the diagnosis.

#### Conclusions

In the setting of hypercalcemia, parathyroid hormone assessment is essential, leading to a comprehensive work up of its cause. Carcinoma needs to be considered in the differentials of primary hyperparathyroidism, particularly in the setting of high parathyroid levels, and mandates urgent surgical removal.

**P601****Primary hyperparathyroidism due to an ectopic intrathymic parathyroid adenoma: case report**M Vlad<sup>1</sup>, M Balas<sup>1</sup>, Al Nicodin<sup>2</sup>, M Cornianu<sup>3</sup>, D Onet<sup>4</sup>, A Popa<sup>1</sup> & I Zosin<sup>1</sup><sup>1</sup>Clinic of Endocrinology, 'Victor Babes' University of Medicine and Pharmacy, Timisoara, Romania; <sup>2</sup>Clinic of Thoracic Surgery, 'Victor Babes' University of Medicine and Pharmacy, Timisoara, Romania;<sup>3</sup>Department of Pathology, 'Victor Babes' University of Medicine and Pharmacy, Timisoara, Romania; <sup>4</sup>Department of Radiology, County Hospital, Timisoara, Romania.**Introduction**

The most common cause of primary hyperparathyroidism is parathyroid adenoma. Unfortunately, between 11 and 22% of these adenomas are located in ectopic positions. In this report, we describe a case diagnosed in our department with symptomatic primary hyperparathyroidism due to an ectopic parathyroid adenoma localized in thymus.

**Case presentation**

A 21-year-old woman was referred to our clinic for investigations, due to multiples osteoclastomas in the maxillary bones. Physical examination performed on admission revealed, in addition to the facial lesions, multiple lumps on ribs and lower limbs. Biochemical investigations confirmed primary hyperparathyroidism: hypercalcemia, hypophosphatemia and significant elevated levels of parathormone (PTH) and alkaline phosphatase. By using ultrasound examination of the neck no extrathyroidal or thyroidal masses, suggestive of a parathyroid adenoma, were detected. The patient was submitted to magnetic resonance imaging of the cervical region and mediastinum. The results revealed an enlarged thymus, which was suspected to be the site of the ectopic parathyroid adenoma. The investigations performed didn't reveal any other clinical manifestations of hyperparathyroidism besides advanced bone lesions.

A thymectomy by using video-assisted thoracic surgery was performed in the Clinic of thoracic surgery. The pathological exam revealed a parathyroid adenoma surrounded by normal thymic tissue. Postoperatively, the plasma level of calcium and PTH decreased significantly. The patient developed hungry bone syndrome that required high amounts of oral calcium (3500 mg daily) and vitamin D for maintaining a low-normal serum calcium level.

**Conclusion**

This case, with an ectopic parathyroid adenoma in thymus confirmed by postoperative histology, presented advanced bone lesions, only, as clinical manifestation of hyperparathyroidism. The presentation illustrates the importance of preoperative imaging localization of parathyroid adenoma, which allows an optimal surgical approach. Mediastinal ectopic parathyroid adenoma as a cause of primary hyperparathyroidism must be always considered if cervical pathological masses are not detected.

This analysis was performed, results are processing. The following therapy has been recommended: hydrocortisone 15 mg/day, L-thyroxine 125 µg/day, Desmopressin 0.5 mg/day, Nebido 4 ml i.m. once in 3 months, Somatropin 7–15 Units s.c. daily.

**P603****Unusual case of congenital adrenal hyperplasia: polymenorrhagia and markedly high 17-OH progesterone levels in a lady with non classic congenital adrenal hyperplasia**A Rizwan<sup>1,2</sup>, M Hayat<sup>1</sup> & Z Sheikh<sup>1</sup><sup>1</sup>National Institute Diabetes and Endocrinology, Dow University Health Sciences, Sind, Pakistan; <sup>2</sup>Aga Khan University Hospital, Sind, Pakistan.**Introduction**

Congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency is one of the most common autosomal recessive hereditary diseases. There are two main forms of CAH: early onset, the classic variety, and late onset or non classic type. Here, we aim to describe the case of a young lady with features of both varieties of CAH.

**Case report**

Twenty-three-year-old lady of Asian descent presented with polymenorrhagia since menarche (age 13 years), hirsutism and clitoromegaly since 7 years. There was no history of ambiguous genitalia or salt wasting crisis at birth. She had previously been labelled as a case of 'polycystic ovaries' and been placed on Diane 35, with some regularization of her cycles. On examination, she had moderate hirsutism (Ferriman Gallway score (FG) score 20/36, normal <8/36), marked clitoromegaly (clitoral index 50 mm<sup>2</sup>, normal: <35 mm<sup>2</sup>). Serum testosterone was 145.8 ng/dl (6–82), while 17-hydroxy progesterone 170.0 ng/ml (0.19–1.82). ACTH stimulation test uncovered borderline cortisol deficiency, with 60 min post synacthen cortisol: 17.0 µg/dl (> 18 µg/dl). Oral dexamethasone was commenced in reverse rhythm, with advice to double the dose at times of stress.

**Conclusions**

Prior to labelling a subject with hirsutism and menstrual irregularity as a case of polycystic ovarian syndrome, other hormonal dysfunction requires exclusion. Congenital adrenal hyperplasia is an important consideration in the differentials, which is all too often overlooked by physicians. Clinicians need to be aware of the possibility of non-classic CAH presenting with the unusual history of polymenorrhagia, versus the usual presentation of oligo/amenorrhea. Phenomenally high 17-OH progesterone levels can also be associated with the non classic variety.

**P602****Clinical case of 48 years old man with panhypopituitarism of unclear etiology**

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When patient was an 14-year-old, he presented with a short stature and underdeveloped external genitalia. The patient had not undergone normal pubertal development. Hormonal studies and clinical examination revealed the findings of anterior and posterior pituitary hormone deficiency: ADH, LH (0.2 mIU/l), FSH (1.22 mIU/l). Since then patient took different therapy for diabetes insipidus. Secondary sexual characters developed during patient received gonadotropin for a very short time for hypogonadism. Some involution in development showed after patient stopped receiving gonadotropin. Nowadays, patient came to medical attention with complaints about fatigue, mood lowering and complete sex appetite absence. Clinical examination revealed a sick-looking, pale and dehydrated middle-aged man with decreased muscle mass. His face looked like kewpie doll (fine, children's facial features). And the same time he looks 'elder': his skin is thinned, with fine wrinkles (geroderma). Patient had absolutely no hair in the groin area, armpit and on the chin. Testicular dimensions was 1.7×0.8 cm (left one) and 1.7×0.7 cm (right one) by the results of ultrasound. Owing to low cortisol, GH and IGF1 pituitary provocative test with insulin was performed, secondary adrenal insufficiency and GH deficiency was shown: ACTH – 48.6 pmol/l (> 150), cortisol – 116 nmol/l (> 550), GH – not exceeding 0.11 ng/ml. MRI with contrast showed: pituitary volume is 0.01 cm<sup>3</sup> (0.15–0.51 cm<sup>3</sup>) structure is homogeneous, no signs of structural alterations and space-occupying lesions was shown. Patient denies any head trauma/surgery in past. Owing to clinical signs we considered deficiency PROP1 or PIT1 factors.

**P604****Schmidt's syndrome: unveiling step-by-step**A Rizwan<sup>1,2</sup>, M Hayat<sup>1</sup> & Z Sheikh<sup>1</sup><sup>1</sup>National Institute of Diabetes and Endocrinology, Dow University Health Sciences, Karachi, Sind, Pakistan; <sup>2</sup>Aga Khan University Hospital, Karachi, Sind, Pakistan.**Introduction**

Autoimmune polyendocrine syndrome type II (APSII) is more common than APSI. It occurs more frequently in female than in male patients, often has its onset in adulthood, and has familial aggregation. Schmidt's syndrome is a subset of APSII, usually associated with primary hypothyroidism, primary adrenal insufficiency, and often, type 1 diabetes. This case describes the interesting evolution of autoimmune polyendocrine disease (APEID) in a young lady with long standing type 1 diabetes.

**Case report**

Twenty-three-year-old Asian lady with type 1 diabetes since 11 years presented with irregular menses, reduced appetite, nausea, constipation, weight loss and cold intolerance for the past 3 months. Her mother had noticed a progressive darkening of her complexion. She was experiencing frequent hypoglycaemic episodes on minimal doses of insulin. Examination revealed a postural drop, generalized hyperpigmentation and grade 2 goitre. Laboratory investigations confirmed autoimmune hypothyroidism and primary adrenal insufficiency. She was treated with hydrocortisone and mineralocorticoid, followed by thyroxine replacement. Insulin was adjusted. Having initially improved, she began experiencing generalized weakness and exertional shortness of breath. Work up revealed hypochromic microcytic anaemia. Celiac disease screen was positive. The patient declined to undergo an esophagogastroduodenoscopy (EGD), therefore, a trial of gluten-free diet was commenced.

#### Conclusions

Clinicians should avoid tunnel vision when treating patients with type 1 diabetes as risk of concomitant/subsequent autoimmune phenomena is high, particularly thyroid and celiac disease. Frequent hypoglycaemic episodes in a previously well type 1 diabetic should raise suspicion of adrenal insufficiency and alert the clinician to the possible development of other components of APED.

#### P605

##### Primary hyperparathyroidism in pregnancy: a case report

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#### Introduction

It is supposed that primary hyperparathyroidism (pHPT) during pregnancy is associated with significant maternal and fetal risks as high as 67 and 80%, respectively. The very few case reports published referred mainly to unrecognized pHPT or diagnosed during pregnancy. We report the case of a woman with known asymptomatic pHPT who became pregnant.

#### Case-report

A woman, 29 years, was diagnosed with pHPT a year before becoming pregnant; her biochemistries were: serum total Ca=11.1 mg/dl (8.6–10.3); P=2.52 mg/dl; PTH=120.8 pg/ml (15–65); 25OHD=4 ng/dl (end of spring) and Ca=11.5 mg/dl; PTH=126.3 pg/ml; 25OHD=24.9 ng /dl (end of summer). She had normal lumbar spine (LS) BMD despite a very high remodeling rate, with serum crosslaps=1.273 ng/ml and osteocalcin =33.42 ng/ml; localization studies were negative and surgery was postponed. She became pregnant and her biochemistries were: Ca=11.1 mg/dl; PTH=144.8 pg/ml; 25OHD=28.6 ng/ml (summer). During the last trimester of pregnancy, she was supplemented with oral vitamin D 1000 IU/day; corrected total Ca was 11 mg/dl, PTH 143 pg/ml. The pregnancy was uneventful until the 36th week of gestation, when premature rupture of the membranes occurred and a caesarian section was performed. The male newborn weighed 3200 g and no abnormalities were noted on physical examination. I.v. calcium and oral vitamin D (500 U/day) were started immediately; ionized calcium had a nadir at 1.28 mmol/l (total calcium 8.12 mg/dl) in the 5th day, when oral calcium was started. After 1 month his biochemistries were: total Ca=10.59 mg/dl (9–11); P=6.68 mg/dl (3.1–6); PTH=12.1 pg/ml (15–68); 25OHD=19.5 ng/ml. One month after delivery, the mother had a total Ca=10.9 mg/dl; P=2.7 mg/dl; PTH=213 pg/ml; 25OHD=34.5 ng/ml and lost 11.6% of LS BMD.

#### Conclusion

Vitamin D repletion of the mother with mild pHPT is safe and could prevent neonatal hypocalcemia.

#### P606

##### Primary hypothyroidism due to sublingual thyroid associated with growth failure, hyperprolactinemia and pituitary enlargement

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#### Introduction

Thyroid tissue may be found anywhere along the course of thyroglossal duct. Sublingual thyroid is a rare type of ectopic thyroid tissue resulting from failure of the embryonic development and migration of the thyroid gland to its normal pre-laryngeal site, reaching between genio-hioid and mylohyoid muscles. In most cases, hypothyroidism develops due to inadequate hormone production. Hypothyroidism may produce pituitary enlargement secondary to thyrotroph hyperplasia. Hyperprolactinemia may occur in these cases.

#### Case report

We present a case of a 13-year-old girl with growth failure and severe headache. At clinical examination: height=121 cm (–4 s.d.), weight=22 kg (–3 s.d.); there was no palpable thyroid gland in the pre-tracheal region. Biochemical findings were high serum cholesterol and triglycerides. Hormonal profile diagnosed primary hypothyroidism based on high levels of TSH (37.9 µIU/ml with normal values of 0.4–7 µIU/ml) and low FT<sub>4</sub>, associated with hyperprolactinemia; GH levels were low but responded to stimulating tests. The cervical US examination showed the absence of the thyroid tissue in its normal location

and a well defined, heterogeneous and hypoechogeneous mass in sublingual region. The thyroid scan with Tc-99m O<sub>4</sub> and the MRI confirmed the presence of sublingual thyroid tissue. Pituitary imaging by X-ray revealed an enlarged sella turcica, confirmed by MRI that described a homogeneous enhancement of the pituitary gland. L-T<sub>4</sub> treatment was initiated and after 5 months TSH, FT<sub>4</sub> and PRL returned to normal levels. At 2 years follow-up the height increased by 24 cm and menarche installed.

#### Conclusions

In this case the diagnosis of growth disorder was challenging, having to distinguish between pituitary tumor and primary hypothyroidism. The efficacy of the L-T<sub>4</sub> treatment proved that pseudoprolactinoma was caused by hyperplasia of the TSH and PRL-producing cells.

#### P607

##### Hypoglycemia in patient with large intraabdominal tumor

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#### Introduction

Various non pancreatic tumors can cause hypoglycemia. Usually they are mesenchymal, retroperitoneal tumors or fibrosarcomas. Hemangiopericytoma is a rare type of mesenchymal tumor, which mostly develops in soft tissue and originates from the Zimmermann's pericyte (modified smooth muscle cells).

#### Case report

Fifty-nine-years old female patient with hypoglycemia was admitted to our department. Physical finding showed mild obesity and hypertension. Endocrine testing confirmed fasting hypoglycemia (about 2.0 mmol/l). Seventy-two hours fasting test was interrupted after 12 h with glycemia 0.8 mmol/l, and low levels of insulin, C peptide and IGF1. Ultrasound and multi slice computed tomography (MSCT) showed massive vasculated intraperitoneal tumor, size 120×175×196 mm. She underwent surgery and large lobulated vasculated tumor was found in right lower hemiabdomen connected to the omentum. Histological and immunohistological finding confirmed hemangiopericytoma (grade II-Trojan-Coinde score: 5/9 with Ki67:10%). Postoperatively, no hypoglycemia was detected. Insulin, C peptide and IGF1 were in the normal range.

#### Conclusion

Hypoglycemia, detected in patients with large non pancreatic tumors occurs due to expression and release of immature form of IGF2 molecules, pro-IGF2, which binds to IGF binding protein 3 (IGFBP3) and remains active. This complex binds to insulin receptors in muscles to promote glucose transport and to insulin receptor in liver and kidney to reduce glucose output. Also, it binds to receptors for IGF1 in the pancreatic beta cells and in pituitary which leads to inhibition of insulin secretion and suppression of GH release, respectively. GH and IGF1 values are decreased, IGF2 values may be increased but often are normal. Immature higher-molecular-weight form of IGF2 can only be detected by special laboratory techniques.

#### P608

##### Pheochromocytoma and paraganglioma in patient with extirpated ganglioneuroma

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Ganglioneuromas are benign sympathetic tissue tumors originating from neural crest cells. A 40-year-old female patient, was admitted to our department for endocrinological evaluation of incidentally discovered tumor of right adrenal gland. For about a year before admission she had a pain under the right costal arch. An ultrasound and computed tomography (CT) showed 2 ovoid cystical tumors of right adrenal (34 and 32 mm, respectively). Three months prior admission she had several hypertensive crises with vertigo, with average blood pressure values of 120/80 mmHg. At 27 years of age she had a surgery because of a mediastinal tumor. Pathohistological (PH) finding confirmed ganglioneuroma. In a family history her father, brother and a cousin suffered from heart disease and hypertension. Physical finding showed Horner Sy as a consequence of previous

surgery. Careful endocrine testing showed very high levels of noradrenalin in urine, and metaiodobenzylguanidine (MIBG) scintigraphy of whole body showed accumulation in both adrenal glands. After preparation with phenoxybenzamine she underwent surgery and right adrenal gland was extirpated along with another tumor. PH confirmed pheochromocytoma of right adrenal and paraganglioma of the same side. Genetic testing showed presence of Von Hippel–Lindau mutation Val84Met, and one of her two tested children was also positive. Postoperative testing showed high levels of catecholamines and CT scan revealed new tumor on the left side in front of abdominal aorta, left renal artery and left renal vein and behind splenic vein. After preparation with phenoxybenzamine she underwent another surgery, tumor was extirpated and PH confirmed paraganglioma. Levels of postoperative catecholamines showed to be normal.

In our case ganglioneuroma occurred in patient with VHL mutation whereas in literature they are usually found in patients with MEN2B.

## P609

### Cushing syndrome due to macronodular adrenal hyperplasia or bilateral adrenocortical adenomas? Case report

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#### Introduction

Adrenal incidentaloma are becoming very frequent. Bilateral adrenocortical adenomas are a rare cause of ACTH-independent Cushing syndrome.

#### Clinical case

A 64-year-old hypertensive woman presented with bilateral adrenal incidentaloma detected on the evaluation as a living kidney donor candidate. She was normotensive (129/87 mmHg) on monotherapy, BMI – 24.8 kg/m<sup>2</sup>, with no recent weight gain, hirsutism, striae or mood disturbances. Angio-TC scan revealed a 16×11 mm right adrenal nodular enlargement and a left 53×51 mm one, with an enhancement washout >50% at 15 min. Hormonal tests were as follow: ACTH <5 pg/ml; serum cortisol 17 µg/dl, urinary cortisol 95 µg/24 h (r.v. 20–90); DHEA-S, 17OHP, renin, urinary aldosterone and metanefrines within normal ranges. After overnight and low dose DXM suppression tests serum cortisol were 7.8 and 7.5 µg/dl respectively and diagnostic of Cushing syndrome. Nor-iodo-cholesterol scintigraphy showed an intense uptake by the left adrenal and a nodular fixation on the right. Laparoscopic left adrenalectomy was done and histopathology revealed a single 6 cm adenoma. Four months later, she is eucortisolemic.

#### Conclusion

A serendipitous diagnosis of Cushing syndrome due to macronodular bilateral adrenal hyperplasia was first made in this patient. Decision of unilateral adrenalectomy was based on dimension criteria and the assumption of resolution of hypercortisolism and no need for replacement therapy.

The (unexpected) histopathologic diagnosis predicts a probable relapse and the need for right adrenalectomy in near future.

## P610

### Cholestatic jaundice methimazole-induced or secondary to heart failure?

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Methimazole-induced cholestatic jaundice is a rare adverse effect, dose-dependent, occurring within the first 3 months of use, usually reversible within 3 months after discontinuing drug therapy.

#### Case report

Woman, 64 years, history of hyperthyroidism since August 2009, under therapy with methimazole 5 mg/day. The patient was referred to endocrinology department in March 2010, with history of heart failure, atrial fibrillation, type 2 diabetes mellitus, penicillin allergy, and allopurinol and fenofibrate toxicodermia. In April 2010, she visited the emergency department complaining of worsening of dyspnea and peripheral edema. Her chest X-ray showed a left pleural effusion and thyroid function revealed (TSH – 44.19 µU/ml (0.35–4.94), FT<sub>4</sub> – 0.48 ng/dl (0.70–1.48), FT<sub>3</sub> – 2.60 pg/ml (1.71–3.71)). She was hospitalized due to acute decompensate heart failure probably due to hyperthyroidism. Methimazole was suspended. Fourth days after she developed sclerotic jaundice, with elevation of alkaline phosphatase (148 U/l (38–145)) and bilirubins (total – 17.8 mg/l (<12), direct – 7.9 mg/l (<4)). Abdominal

ultrasonography and magnetic resonance cholangiography excluded biliary obstruction. Concomitant liver diseases, e.g. viral hepatitis, autoimmune hepatitis, and primary biliary cirrhosis, were also excluded by proper serology. The possibilities of cholestatic etiology were hepatic congestion in heart failure or methimazole toxicity. The hyperthyroidism study revealed a Graves disease, and the patient was submitted to radioactive iodine therapy. Twelve weeks after methimazole withdrawal maintained cholestasis.

#### Discussion

Methimazole-induced cholestasis is unlikely due the absence of a clear chronological relationship between drug initiation and the development of jaundice. Cholestasis due to hyperthyroidism usually occurs in association with marked elevation of thyroid hormones. In this case report, liver biopsy could be relevant to exclude others causes of cholestasis.

## P611

### Remind familial hypocalciuric hypercalcemia as a cause of elevated parathyroid hormone and serum calcium: 5 patients

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#### Introduction

Familial hypocalciuric hypercalcemia (FHH) is a benign disorder, with inappropriately elevated parathyroid hormone, without the complications of hypercalcemia (1). It is important to discriminate FHH from primary hyperparathyroidism (PHPT), because of the consequences on symptomatic disease and therapeutic approach. Ca/Cr clearance ratio will be <0.01 in FHH and most often >0.02 in PHPT (2). In patients with PHPT and vitamin D deficiency, low urinary calcium will increase after vitamin D repletion, thereby differentiating it from FHH.

#### Case reports

Five female patients, mean age 54.4 years, without specific clinical symptoms had elevated PTH (mean value 89.2 pg/ml), elevated or at the upper limit of normal serum calcium (10.3 mg/dl) and low urine calcium (mean value 80.5 mg/24 h). Their Ca/Cr clearance ratio was <0.01 (mean value 0.006). Vitamin D 25 OH deficiency coexisted in 2 patients was supplied without substantial increase in calcium urine values. The lack of symptoms and Ca/Cr clearance ratio <0.01 confirmed the diagnosis of FHH. No further evaluations and treatment was necessary.

#### Conclusion

In clinical practice, frequently we see elevated levels of PTH and serum calcium, which can lead to unnecessary evaluation and unsuccessful treatment if not FHH is considered.

## P612

### Iatrogenic hypoadrenalism: a case report

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#### Introduction

Psoriasis is a chronic skin disease with a prevalence of 0.6 to 4.8%. Among the possible treatments, we highlight topic corticosteroids that can cause not only different local side effects (skin fragility and atrophy, striae, purpura, acne, telangiectasia, hypertrichosis) but also systemic side effects, including diabetes mellitus (DM), hypertension (HT), Cushing's syndrome and hypothalamic-pituitary-adrenal (HPA) axis suppression.

#### Case report

A male patient 47 years old, smoker, with psoriasis for nearly 20 years, followed in the Dermatology consultation and medicated for over 10 years with Dermovate ointment 0.5 mg/g (clobetasol) – once or twice a week and Psodermil ointment 0.5 mg/g + 30 mg/g (betamethasone + salicylic acid) – once a week. He was sent to the morbid obesity consultation by his family doctor. He had obesity (BMI = 37.4 kg/m<sup>2</sup>), DM probably secondary to corticosteroid and HT. The patient reported fatigue, muscle weakness and myalgia when he stopped applying the topical corticosteroids. In the physical exam he had a cushingoid phenotype and exuberant psoriatic lesions that occupied most of the trunk and upper limbs. The analytic baseline study revealed early morning plasma cortisol = 0.63 µg/dl



(*n*: 6.2–19.4), early morning plasma ACTH=9.9 ng/l (*n*<63) and urinary free cortisol=10.9 µg/24 h (*n*: 36–137). Hydrocortisone was started (10+5 mg). The ACTH stimulation test with 250 µg of tetracosactide infused i.v. (without taking the morning dose in the day of the analytic study and the evening dose in the day before): cortisol 0'=3.8 µg/dl and cortisol 60'=9.2 µg/dl. The diagnosis of adrenal insufficiency secondary to corticosteroid therapy was confirmed and hydrocortisone therapy was continued at the dose mentioned above.

#### Conclusion

This case highlights the possibility of HHA axis suppression in patients with prolonged application of topic corticosteroids that if not diagnosed may have serious consequences.

## P613

### Diabetes insipidus in the context of sarcoidosis: a case report

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#### Introduction

Sarcoidosis is a systemic granulomatous disease of unknown etiology with a prevalence of 10–20/100 000 individuals and affects mainly young adults. Neurological involvement occurs in about 5% of the cases and it can affect the hypothalamic–pituitary axis. In this case, the most common endocrine manifestations are hyperprolactinemia and diabetes insipidus.

#### Case report

A 44-year-old male patient complaining of not feeling well and dyspnea was diagnosed with sarcoidosis about 6 years ago and was medicated with deflazacort 6 mg. He developed diabetes mellitus secondary to corticosteroid therapy about 4 years later and started insulin therapy. He was referred to an Endocrinology consultation due to polyuria and polydipsia for about 1.5 years. He ingested up to 5 l of water in a single day. In physical examination he had a cushingoid phenotype. Analytic baseline study revealed plasma osmolarity=294 mOsmol/kg (*n*: 282–300), normal renal function, normocalcemia, urine osmolarity=179 mOsmol/kg (*n*: 50–200), urinary volume=5700 ml, mild hyperprolactinemia=17.9/17.7/17.4 ng/ml (*n*: 4.0–15.2), hypopituitarism: hypogonadism – FSH=0.70 mIU/ml (*n*: 1.5–12.4), LH<0.10 mIU/ml (*n*: 1.7–8.6), total testosterone<0.03 ng/ml (*n*: 2.8–8.0); hypothyroidism – TSH=2.05 µIU/ml (*n*: 0.35–4.94), FT<sub>4</sub>=0.66 ng/dl (*n*: 0.7–1.48) and hypoadrenalism – 0900 h. ACTH<1.0 ng/l (*n*: <63.3), 0900 h. cortisol=1.0 µg/dl (*n*: 6.2–19.4). It was performed a water restriction test that confirmed the diagnosis of central diabetes insipidus (urinary osmolarity during the test was always less than 200 mOsmol/kg and urinary osmolarity increased about 97.5% at 120' after administration of s.c. desmopressin) and the patient was discharged with intranasal DDAVP solution 5 µg twice a day. Pituitary MRI showed hypothalamic and pituitary stalk thickening. Contrast administration revealed multiple nodules of contrast uptake consistent with the diagnosis of granulomatous meningitis.

#### Conclusion

Patients with multisystemic diseases, such as sarcoidosis, should have an appropriate follow-up for early diagnosis of complications.

## P614

### A case of small cell lung cancer of which first finding is hyponatremia

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Small cell lung cancer (SCLC) is accepted as a neuroendocrine tumor and usually accompanied with paraneoplastic syndromes. Our case has been diagnosed as SCLC and the only finding of disease was hyponatremia.

#### Case

Forty-four-year of female patient has admitted because of nausea, vomiting and fatigue. Serum Na level was 116 mEq/l. The patient hospitalized for further investigation. In physical examination BP was 140/80 mmHg, HR was 72 beats/min. Patient didn't have complaints such as coughing, sputum, weight loss, dispnea or chest pain. Serum glucose level 101 mg/dl, urea 9 mg/dl, Cr:

0.6 mg/dl and K: 4.1 mEq/l were. Plasma osmolality was measured as 226 mosm/l, urine osmolality was 306 mosm/l, urine density was 1013 and Na in 24 h collection of urine was 147 mmol/l. In imaging of brain with MRI, there were hyperintense lesions located in white matter which were interpreted as finding of encephalomyelitis or demyelinating disease. It could be related to hyponatremia. In abdominal MRI images adrenal glands were normal. Plasma cortisol and 17-OH progesterone responses to short ACTH stimulating test, pituitary hormones, cortisol and GH response to insulin tolerance test were normal. We thought all these results could be due to SIADH. In thorax CT, we have detected multiple nodules biggest 6 mm in dimension and multiple enlarged hilar and mediastinal lymph nodes biggest in 23 mm in size. Endobronchial biopsy was taken from the enlarged lymph nodes. Histopathology results were compatible with SCLC metastasis and patient was referred to an oncology clinics. SIADH syndrome occurs in oncology patients due to ectopic production of hormone by cancer itself, complications or treatments. Early recognition of it is important in order to reduce morbidity and mortality. It should be kept in mind that hyponatremia might be first sign of cancer as it is in our case.

## P615

### Evaluation of autoimmune endocrine disturbances in celiac patients

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#### Aim

Celiac disease is closely related with other autoimmune diseases. Our purpose is to examine the existence of endocrine diseases in Celiac patients.

#### Materials and methods

Celiac patients admitted to gastroenterology clinic were referred to our endocrinology clinic between the dates of September of 2009 and June of 2010. This patient group was evaluated to see as if they had autoimmune thyroiditis, type 1 DM, primary hypoparathyroidism and primary adrenal insufficiency.

#### Results

Twenty-seven patients were registered in our study and 6 of them (22%) were male where as 21 (77.8%) of them were female. Mean age was 35±11 years (20–61). Mean body mass index was 22.61±4.45 kg/m<sup>2</sup> (17.9–34.9). We have detected chronic thyroiditis in 6 and type 1 diabetes mellitus in 2 of patients. All patients with endocrinopathy were female. Plasma cortisol levels were normal. We have made low dose ACTH test to all patients and the results were normal. Moreover we have detected vitamin D deficiency in 23 of the patients (85.2%) and there was secondary hyperparathyroidism in 17 of these patients. We haven't detected primary hypoparathyroidism in any patient. In addition to endocrinopathies we have detected vitamin B12 deficiency in 13 (48.1%) patients and in 3 (11.1%) of them antiparietal antibody was positive.

#### Discussion

Endocrinological pathologies especially type 1 DM, autoimmune thyroiditis, rarely Addison's disease, chronic autoimmune hepatitis, primary biliary cirrhosis, cystic fibrosis and lastly SLE have been known to accompany celiac disease. We have detected in our study group that the occurrence rate of type 1 DM in celiac patients was 7.4% and chronic thyroiditis was 22%. We assume that periodic screening for endocrinopathies should be done in Celiac patients.

## P616

### Autoimmune polyglandular endocrinopathy associated with collagenous sprue: a case report

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#### Introduction

Polyglandular autoimmune syndrome (PAS) is a syndrome that is characterized by the association of two or more organ specific autoimmune disorders. Collagenous mucosal inflammatory diseases involve the columnar-lined gastric and intestinal mucosa. The coexistence of the two disease has not been reported.

We have encountered a patient with a rare combination of autoimmune thyroiditis, hypoparathyroidism, primary ovarian failure and collagenous sprue. To our knowledge this is the first case of collagenous sprue in association with polyglandular syndromes in the literature.

#### Case report

A 32-year-old woman was admitted to our out patient clinic with weakness, myalgia, muscle cramps and carpedal spasm. Her past medical history included diarrhea since 1 year and she had the diagnosis of primary amenorrhea since 15 years. Laboratory investigations revealed hypocalcemia, hypopotasemia, hypophosphatemia. Hematologic tests revealed anemia. Parathyroid hormone was low. The patient had hypothyroidism. Anti TG antibody and anti TPO antibodies were high. Morning cortisol level was 18 µg. ACTH stimulation test could not be done. Upper gastrointestinal endoscopy and histological examination revealed celiac disease and the patient was diagnosed as gluten enteropathy. She had no response to the medications, gluten free diet, her clinical course deteriorated with worsening malabsorption, diarrhea, and weight loss. Second endoscopic examination pathology of the duodenum revealed collagenous sprue. The patient died 4 days after the initialization of steroid therapy.

#### Conclusion

Collagenous sprue must be considered in patients who have polyglandular endocrinopathy with resistant diarrhea to avoid life threatening complications. The presence of other immune-related diseases in this case also suggests that an immunological mechanism may play a causative role in collagenous sprue. This is important and must be kept in mind.

## P617

### Iatrogenic lymphocytic hypophysitis

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A 61-year-old was diagnosed with CLL in 1986 but received no active treatment until 2006, when the WCC became elevated and bone marrow biopsy showed a dense B cell infiltrate. Despite initial treatment with chemotherapy there was disease progression, hence started on Alemtuzumab (Campath) on 24/5/2010. This was stopped on 18/6/2010 because of recurrent neutropenic sepsis. He was readmitted on 18/7/2010 because of left eye cellulitis and hyponatremic with serum sodium of 122 mmol/l, which was investigated and treated appropriately as SIADH with fluid restriction and discharged with normal sodium. He was readmitted on 2/9/10 with lethargy, hypotension and sodium 119 mmol/l. He had not received any glucocorticoids for 5 months and biochemistry revealed pan hypopituitarism. He was treated with hydrocortisone and then thyroxine and testosterone, and his serum sodium rose to 134 mmol/l after 5 days whilst he was taking a normal fluid intake. In view of the biochemical changes he underwent an urgent pituitary MRI that showed gland larger than normal with heterogeneous enhancement and thickened stalk with dural thickening posterior to the gland and extending down the clivus, features consistent with lymphocytic hypophysitis. Campath is a recombinant humanized monoclonal antibody (Campath-1H) directed against the cell surface glycoprotein, CD52. There is increasing use of similar monoclonal antibodies in the treatment of cancer and inflammatory disorders. These immunomodulatory therapies can also induce an unwanted immune response. As a result, there are now iatrogenic autoimmune disorders arising from the treatment of malignancies. This is the first case report demonstrating lymphocytic hypophysitis in the context of use of Campath, which is even more startling considering the short duration of treatment. Endocrinologists should be aware of this new class of agents and the extended range of autoimmune conditions that that can be affected as illustrated by this case.

## P618

### A rare cause of nontraumatic rhabdomyolysis: central diabetes insipidus

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Nontraumatic rhabdomyolysis is mostly caused by drugs, alcohol consumption or compression of muscles. Severe hyperosmolality rarely can cause rhabdomyolysis. Our case has santral diabetes insipidus that has admitted with the clinical picture of rhabdomyolysis.

#### Case

Twenty-six-years old male has admitted to our clinic with the complaint of weakness, nausea, vomiting, polydipsia, polyuria, difficulty in walking and tendency to sleep. Neurologic examination was normal. In laboratory glucose: 166 mg/dl, Na: 168 mEq/l, Urea: 19 mg/dl, Cr: 1.6 mg/dl, K: 2.8 mEq/l, CK: 2916 IU/l, plasma osmolality 348 mosmol/l, and urine density 1005 were. Serum CK level have increased progressively up to 15 000 IU/l during the follow up. We have thought that rhabdomyolysis might have been due to hyperosmolality caused by high Na and low K levels. We have administered hypotonic fluids and followed the urine output and fluid intake of the patient. His general condition was not well enough to tolerate fluid restriction test and he had severe polyuria that is why we have directly made the desmopressin administration test. Plasma osmolality was 348 mosmol/l before desmopressin administration and it regressed to 330 mosmol/l thereafter. Urine osmolality has increased to 330 mosmol/l from 77 mosmol/l after desmopressin administration. Amount of urine per hour has decreased significantly after desmopressin. Consequently the patient has been diagnosed to have central DI. In pituitary MRI a lesion was detected at the level of hypothalamus in right anterolateral neighborhood of 3rd ventricle and 14×18×11 in size. It was interpreted as low grade glial lesion. Desmopressin was started po 0.1 mg three times daily and clinical picture has improved thereafter. Serum Na, K and CK levels have returned to normal values after the treatment.

#### Conclusion

Rhabdomyolysis is a rare but very important complication of hyperosmolality due to DI. CK levels should be monitor strictly in hyperosmolar states.

## P619

### Our clinical experience in percutaneous ethanol injection into cystic thyroid nodules

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#### Aim

Percutaneous ethanol injection (PEI) is a minimally invasive method which can be preferred in treatment of cystic thyroid nodules. We have presented our cases of PEI below.

#### Material and methods

We have performed PEI to 8 patients in our clinic. We have made ultrasound guided fluid aspiration from the cysts which were confirmed to be benign. We have injected ethanol (98%) into the pouche of the cyst in an amount that is 20–40% of the cystic fluid volume with ultrasound guidance.

#### Results

The mean volume of cystic nodules was 13.6 ml (3.8–36.7 ml) and mean ethanol volume injected was 6.5 ml (1.5–15 ml). After the first performance of PEI, we have recorded 50% reduction in nodule sizes at the first control visit of 2 patients. We had to perform PEI for the second time because we couldn't record any significant reduction in nodule size in 6 patients. During the second PEI performance, mean nodule volume was 10.7 ml (6.5–32.4 ml) where as mean ethanol volume used was 4.5 ml (3–15 ml). After the second PEI, we observed almost 50% reduction in thyroid nodule sizes of extra 4 patients. In two patients, we could not record any effective size reduction even after the 2nd PEI. They were referred to a surgeon for thyroidectomy. At the control visit, made 6 months after PEI, in 4 of 6 patients the cystic component of the nodules completely disappeared. Mean nodule size of these 6 patients was calculated as 0.36 ml (0.25–0.54 ml). One year after PEI, we made fine needle aspiration to the nodules previously received ethanol injection and the cytology results were all benign.

#### Discussion

PEI may be an alternative to surgery in complex nodules which are dominantly cystic in nature after eliminating the malignancy probability with fine needle aspiration.

## P620

### Two cases of allergic reactions which has developed due to usage of insulin analogues

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We have presented two cases of allergic reactions developed due to usage of insulin aspart.

Case 1

Forty-years-old female patient has admitted to emergency with diabetic ketoacidosis. Parenteral hydration with saline solution and insulin infusion has been given to her in ER. After ketoacidosis picture has improved, mixture of insulin aspartate and insulin aspartate protamine was started as maintenance therapy. After the application of second insulin dosage, itchy and erythematous papulopustular lesions have developed all over the patient's body. Therapy with insulin analogue was terminated immediately and human insulin was started instead. Punch biopsy was performed from the lesions. According to the pathological examination the diagnosis of IgE mediated allergic reaction has been made. Right after corticosteroid and antihistaminic therapy was started, the lesions regressed and disappeared.

Case 2

The second patient is 75 years old female patient who has admitted to emergency department of our hospital with hematuria, erythematous papulopustular lesions and disruption of general well being. Ten days before this clinical picture developed, intensive therapy with insulin aspartate and insulin detemir had been prescribed to patient for blood sugar regulation. We have stopped analogue insulins and started human insulin. We have performed punch biopsy from the skin lesions. Biopsy result was compatible with leucocytoclastic vasculitis. We have started glucocorticoid and antihistaminic therapy immediately however the patient has died because of the concomitant acute renal failure picture.

Conclusion

Allergic reactions occurring due to insulin injection may present with localized or systemic reactions. Systemic allergic reactions might be IgE mediated, anaphylactic or due to IgG mediated insulin resistance or rarely it might show itself with the clinical picture of serum sickness. Although analogue insulins have reduced the incidence of immune mediated allergic reactions, clinicians still can experience few cases one in a while.

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**P621**

**Sheehan's syndrome primary presenting as central diabetes insipidus: case report**

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Introduction

Sheehan's syndrome is a rare cause of hypopituitarism secondary to an intra or postpartum severe bleeding. Manifestations are most often caused by deficiencies of hormones of anterior pituitary; involvement of posterior pituitary is rarely described.

Case report

We report a case of a 45 years old hypertensive, multipara, premenopausal woman, who underwent fertilization by oocyte donation, that resulted in twin pregnancy. Preterm vaginal delivery was complicated with severe *post partum* hemorrhage and shock due to surgical placental fragments removal. Agalactia and a persistent polyuric-polydipsic syndrome, with daily diuresis > 5 l, developed on the immediate *post partum*. MRI showed ectopic neurohypophysis; serum osmolality 288 mosm/kg; urinary osmolality 89 mosm/kg. After intranasal desmopressin urinary osmolality raised to 372 mosm/kg and diuresis normalised. Four months after delivery, she was still amenorrheic and presented asthenia and swelling. Multiple hypothalamic-pituitary axis evaluation resulted ACTH, TSH, FSH, LH, PRL and GH deficiencies. Started treatment on prednisone and levothyroxine with clinical status improvement. At 6 months of follow-up she is asymptomatic and the MRI showed normal pituitary.

Conclusion

Central diabetes insipidus is rare in Sheehan's syndrome; in this patient it was the primary manifestation. The rapid onset of the anterior pituitary hormones deficiency is also not frequent.

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**P622**

**Hepatic metastasis of parathyroid carcinoma: case report**

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Introduction

Parathyroid carcinoma is a rare cause of primary hyperparathyroidism. Initial complete surgical resection is crucial. Recurrence is common and primary cause of mortality is severe hypercalcemia.

Clinical case

In 2006, a 51 years old male presented with multiple bone pain, anorexia and weight loss, calcium 11.7 mg/dl, PTH 189 pg/ml. Eight years before a diagnosis of atypical parathyroid adenoma was made after left inferior parathyroidectomy and genetic analysis revealed HRPT2 gene mutation. He was lost for follow-up soon after surgery. On reevaluation, it was not possible to localize any eutopic or ectopic parathyroid mass or metastasis by ecography, CT scan, MRI, and Sestamibi scintigraphy. Surgical cervical exploration was negative, nevertheless a right superior and inferior parathyroidectomy were done and histology revealed no tumor. Calcium levels increased steadily (14.7 mg/dl), PTH 444 pg/ml. Cinacalcet was badly tolerated and unable to reduce hypercalcemia. A good response to Zoledronic acid was obtained, but tachyphylaxis developed in the next four months. Selective venous catheterization could not demonstrate any PTH gradient. A trial of anti PTH immunization (Liège University Hospital) was then made (June 2007), but soon after he was hospitalized in our center due to symptomatic severe hypercalcemia (15.2 mg/dl). A 12 mm solid hepatic nodule was then seen on CT and MRI but not on PET-scan. Surgical enucleation confirmed a hepatic parathyroid metastasis. PTH became immediately undetectable and on the third post operative day calcium normalized and hypocalcemia developed. After 42 months, he is asymptomatic on calcium and vitamin D therapy.

Conclusion

Parathyroid carcinoma as a variable course and tends to recur locally. In this case a unique small hepatic metastasis was responsible for severe hypercalcemia. After its resection a new remission was obtained.

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**P623**

**IGF2 producing prostate tumour causing severe hypoglycaemia: case report**

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Introduction

The IGF1 and 2 are polypeptides that share structural similarities to insulin and affect carbohydrate metabolism mainly by activating the IGF1 receptor. We report a patient presenting with prostate tumour and severe hypoglycaemia.

Case presentation

An 82-year-old male presented with frequent seizures and severe hypoglycaemia. Four year prior to the current presentation the patient had been diagnosed with prostate carcinoma, however, he refused surgical treatment. Our laboratory tests confirmed severe hypoglycaemia with low insulin levels at admission. Similarly, during fasting low glucose and low serum insulin levels were documented. Standard oral glucose load showed a diabetoid response, but the insulin response was delayed. An insulinoma could be excluded by various imaging techniques. Moreover, both glucagon and somatostatin tests showed negative results. Furthermore, physiological hypophysis and peripheral hormone concentrations, as well as normal IGF1 serum levels and chromogranin A were measured. Therefore, we hypothesised that the severe hypoglycaemia might have resulted from IGF2 (or its prohormone) synthesis by the previously diagnosed prostate tumour and thus a prostatectomy was recommended. The patient required continuous glucose substitution and diazoxide therapy under which the hypoglycaemia could be controlled, however, due to the poor general condition a surgical procedure was not feasible and the patient deceased. The subsequent immunohistochemistry of the prostate sections showed a carcinoma with strong IGF2 staining, further suggesting that the IGF2-secreting prostate tumour caused the severe hypoglycaemia.

Conclusions

To our knowledge, this is only the second reported case of an IGF2 secreting prostate tumour. It has been previously reported that some mesenchymal tumours secrete IGF2 at high levels causing severe, therapy refractory hypoglycaemia. Increased awareness of IGF2 may improve diagnosis and treatment of hypoglycaemia.

**P624****17 $\beta$ -hydroxysteroid dehydrogenase type 3 deficiency: a new case of a rare disease with an uncommon gene mutation**

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Deficiency of 17 $\beta$ -hydroxysteroid dehydrogenase type 3 (17 $\beta$ HSD3), an enzyme converting androstenedione (A) to testosterone (T) in the Leydig cells of the testis, is a rare cause of autosomal recessive 46,XY disorders of sexual development (DSD). A 18-year-old phenotypically female patient presented with primary amenorrhea. She had deep voice, macrocephaly, broad forehead, enlarged nasal tip, macrostomia, facial acne, gynecomastia, left-convex dorsal scoliosis, hypoplasia of the first finger of right hand, proximal implant of the fifth metatarsus bilaterally and an increased muscle mass and increased distribution of hair on face, neck, abdomen, pubic region and on upper and lower limbs. Genital exam showed thickened labra majora with absence of labra minora. Gynaecological exam shows a blind-ending pseudo-vagina (15 cm) with micropenis. Karyotype analysis showed a male genotype (46,XY). Hormonal evaluation showed decreased T (188 ng/dl) and increased A (10 ng/ml), considering male reference ranges, resulting in a decreased T/A ratio (0.188). MRI identified testicles in inguinal regions. Human Chorionic Gonadotropin test showed the lack of response of A although T/A ratio remained permanently under 0.8, as classically expected. These evidences were suggestive of a 46,XY DSD due to 17 $\beta$ HSD3 deficiency. A mutation (IVS3 -1 G>C or c.326-1G>C) which alter the splicing of exon 4 of the 17 $\beta$ HSD3 gene was discovered at the genetic evaluation. This mutation was previously reported in Dutch and Brazilian population and is one of 27 actually known mutations of 17 $\beta$ HSD3 gene. Psychologist identified a well determined female gender identity so was decided to proceed with surgical removal of the gonads and of micropenis with vaginal reconstruction. In conclusion, the case described in this study represents a new case of a rare DSD associated to a rare gene mutation of 17 $\beta$ HSD3 gene.

**P625****Testicular microlithiasis: how worried should we be?**

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**Introduction**

Gynecomastia is a relatively common reason for consultation, with a high prevalence in the neonatal period, puberty and with aging. The diagnostic approach should be more thorough in normal-weight individuals with recent onset of gynecomastia, rapid growth of breast tissue, or in the setting of a large painful breast. Besides the evaluation of liver function, testosterone, estradiol, LH and  $\beta$ -hCG, testicular ultrasound evaluation is important to exclude testicular tumors. The prevalence of testicular microlithiasis (TM) is about 2.4% in asymptomatic boys, and increases with age. It is usually found in isolation, but in 30 to 40% of cases, it is associated with testicular germ cell tumor or its precursor carcinoma *in situ*.

**Case report**

A 21-year-old male was referred for endocrinology consultation for unilateral left gynecomastia, which had begun 8 months earlier. His PRL levels were also increased in isolated assay (401 mIU/ml (86–324)). He had a history of bilateral gynecomastia at puberty, which had fully recovered. A painless enlargement of the left breast, without any palpable nodules, was noticed. Physical examination was otherwise unremarkable. Mammary ultrasound showed left mammary gland hyperplasia, without any nodule. PRL increase was not confirmed in multiple catheter samples (20.0–18.6–16.6 ng/ml at 0, 20 and 40 min, respectively (4.0–18.4)). FSH (2.64 (1.1–13.6) mIU/ml), LH (3.78 (1.1–8.8) mIU/ml), total testosterone (4.16 (1.7–8.7) ng/ml), estradiol (35.0 (11–44) pg/ml), liver tests and  $\beta$ -hCG (<1.2 (0–5) mIU/ml) were normal. Testicular ultrasound scan showed bilateral testicular microlithiasis with no suspicious nodules. The patient remains in regular clinical and imaging follow-up.

**Conclusions**

Despite the association between TM and testicular germ cell tumor, the malignant potential of the former is not yet completely understood. There is also no consensus on the best follow-up strategy for patients with TM, but it is nevertheless important to identify those with at least one risk factor for testicular germ cell tumor.

**P626****Virilizing ovarian steroid cell tumor: clinical case**

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**Introduction**

Androgen-producing tumors are a rare cause of hyperandrogenism ( $\pm 0.2\%$ ). Ovarian steroid cell tumors represent <0.1% of all ovarian tumors. They may present at any age and in 12–50% of cases are associated with virilization. The majority of these tumors are benign or low-grade malignancies.

**Clinical case**

A 74-year-old female patient, with history of vaginal hysterectomy for genital prolapse at the age of 50, is referred to Endocrinology consultation for androgenic alopecia, which lasted for 10 years. She also complained of irritability. Mild hirsutism, deepening of voice and clitoromegaly were noticed on physical examination. Biochemical hyperandrogenism was revealed: total testosterone 3.35 ng/ml (*n*: 0.1–0.8), free testosterone 0.79 ng/dl (*n* < 0.20), dihydrotestosterone 0.36 ng/ml (*n*: 0.02–0.37), delta 4-androstenedione 2.2 ng/ml (*n*: 0.3–3.5), 17-hydroxyprogesterone 1.8 ng/ml (*n*: 0.11–1.2), DHEA-s 94  $\mu$ g/dl (*n*: 35–410). There were no significant alterations at both ovaries on pelvic transvaginal ultrasound. Pelvic MRI revealed a 27 $\times$ 15 mm right adnexal mass, isointense on T1- and mildly hyperintense on T2-weighted imaging, which had not been visualized by abdominopelvic CT scans. Tumor markers were negative (CEA, CA19.9, CA125, Ca15.3,  $\alpha$ -fetoprotein). She underwent exploratory laparotomy, which revealed adnexa with normal macroscopic appearance, and no other significant alterations in the abdominopelvic cavity. Peritoneal washing, scraping of the diaphragm and bilateral salpingo-oophorectomy were performed. Frozen section of the surgical specimen revealed a sex cord-stroma tumor in the right ovary of and areas of stromal hyperplasia on the left ovary. Diagnosis of ovarian steroid cell tumor was made by definitive histological and immunohistochemical examination. After surgery, there was improvement of the alopecia and hirsutism and androgen level normalization.

**Discussion**

Symptoms and signs of virilization and total testosterone levels three times the upper limit of normal or greater than 2 ng/ml are suggestive of the presence of an androgen-producing tumor. Despite the recognized value of transvaginal pelvic ultrasonography in the diagnosis of ovarian tumors, its sensitivity and specificity in the diagnosis of ovarian tumors in women with hyperandrogenism are not certain.

**P627****Clinical, hormonal and cytogenetic evaluation of 46,XX male**

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The XX male syndrome, also called 'de la Chapelle syndrome', has been renamed as '46, XX testicular disorder of sex development' (DSD) in 2006. It occurs in about 1/20 000 male newborns. We describe a 57-years-old male who referred to our andrological outpatient clinic because of erectile dysfunction. He was a smoker and suffered from 15 years of type 2 diabetes mellitus; he was also carrying a euthyroid multinodular goiter. The physical examination included the measurement of height (162 cm), body weight (69 kg), body mass index (BMI 26); moderate gynecomastia and regular external sex organs were present. No maldescended testis history at anamnesis. Prostate size was normal at ultrasound. Testicular volume was very low: right 1–2 ml, left 4–5 ml, with reduced consistency. Standard laboratory procedures to assess liver and kidney functions and blood count were normal. Serum levels of LH and FSH were high; testosterone (T) and prostate-specific antigen (PSA) levels were very low. Semen analysis, performed according to the WHO Laboratory Manual, revealed azoospermia in 2 different determinations, even after ejaculate centrifugation. Bone mineral density (BMD) revealed osteoporosis. A testis biopsy showed a histological profile of Sertoli Only Cell Syndrome. Karyotyping on peripheral blood lymphocytes was performed and SRY gene was localized using fluorescent *in situ* hybridisation (FISH) with a SRY probe. FISH study ruled out the presence of a Y-bearing cell line, and confirmed translocation of SRY to Xp terminal part. The patient then underwent hormonal substitutive therapy with mucoadhesive buccal testosterone (30 mg BID) with testosterone level normalization and recovery from erectile dysfunction. Our report indicates that DSD diagnosis is difficult and frequently late. Early diagnosis allows early parental counseling and alerts the physicians for further management.

## P628

### Five year follow-up of recurrent parathyroid carcinoma in a young man: a case report

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#### Introduction

Parathyroid carcinoma is a rare cause of pHPT – <1%.

#### Case report

We report the case of a 25-year-old man who presented with acute severe hypercalcemia (19 mg/dl) and extremely high serum PTH (2737 pg/ml). He was initially treated with i.v. fluids, calcitonin and pamidronate and total calcium decreased to 12.7 mg/dl. A left lower parathyroidectomy (tumour with microvascular invasion and intact capsule) was performed with a prompt decrease in PTH (89.8 pg/ml) and calcium (6.8 mg/dl), followed by severe and prolonged hungry bone syndrome (6-months). During the 5 years follow-up, he developed 4 recurrences, the first after 2 years, and then at shorter intervals of 10 months, 6 and 5 months. Preoperative calcium and PTH levels were higher than initially (18.6–20 mg/dl and 1601–3402 pg/ml, respectively), with incomplete response to iv bisphosphonates (pamidronate, zoledronate) and surgery (serum calcium 12.3–15.9 mg/dl and PTH 107–166 pg/ml). Each time, extensive local invasion required en bloc resection of all involved tissues, including the thyroid. Initial renal failure didn't progress in time (serum creatinine 1.8 mg/dl). The clinical course was aggravated by the occurrence of ischemic heart disease and by severe skeletal involvement with long-bone fractures (humerus, femur) from minimal trauma. The very high bone remodelling (cross-laps up to 19 ng/ml) was only attenuated by BP and surgery. A differential diagnosis with bone metastasis was unsuccessful with MIBI scan and a FDG-PET scan was scheduled.

#### Conclusion

This case confirms that the diagnosis of parathyroid carcinoma is difficult even after histopathologic evaluation and is often established by the presence of recurrences. The clinical course was marked by severe and difficult to control hypercalcemia, severe bone disease (possible bone metastasis), and ischemic cardiac involvement.

## P629

### Unexpected association: Turner syndrome and hypopituitarism: a case report

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#### Introduction

Turner syndrome is a common cause of dwarfism and hypogonadism as is pituitary failure. However the association of the two is rarely thought and reported in the literature. We present two cases of women with hypogonadism diagnosed with Turner syndrome with various degrees of pituitary insufficiency.

#### Case report

The first case presented at 16 years with secondary amenorrhea and showed slightly disharmonic dwarfism. A hypogonadotropic hypogonadism was unraveled, so additional investigations targeting pituitary function were undertaken; low GH levels (<2 ng/ml) and lack of increment of cortisol levels during and after insulin induced hypoglycemia provided additional proves of pituitary failure. The MRI scan denied pituitary mass and showed pituitary hypoplasia, and ectopic posterior pituitary. Abnormal karyotype was also revealed: mosaicism 45X0 (30%)/46XX (70%).

The second case presented at 48 years, with a diagnosis of Turner syndrome since the age of 5. She had had estrogen-induced puberty then discontinued hormone replacement. At presentation the patient was 147 cm tall and had bilateral Madelung's deformity, associated hypothyroidism on thyroxine replacement, partial hearing loss, renal duplication, and hypertension, impaired glucose tolerance test and osteoporosis. FSH level was inappropriately normal for her condition at 7.5 mIU/l and a CT scan of the pituitary revealed empty sella with pituitary hypoplasia. Karyotype: 45X0.

#### Conclusion

We report these two interesting cases of an unusual association of Turner syndrome and hypopituitarism of different etiologies to challenge the use of gonadotroph classification of hypogonadism and widen the perspective of seeking multiple syndrome association.

## P630

### A rare variant of Wolfram syndrome with diabetic microvascular disease, hypergonadotropic hypogonadism and palmar fibromatosis: case report

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#### Introduction

Wolfram syndrome, a very rare condition, is a neurodegenerative disorder characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) which appear in childhood, hampering diagnosis and treatment. Others less frequent features as hypergonadotropic hypogonadism, microvascular disease and local fibromatosis are reported in a male patient diagnosed at the age of 18 years.

#### Case report

An 18-year-old male patient diagnosed with Wolfram syndrome at the age of 5 due to association of diabetes mellitus, deafness and diabetes insipidus is admitted to endocrinological clinic due to short stature and lack of secondary sexual characteristics. The clinical examination revealed a height at – 2.5 s.d., Tanner II puberty stage and palmar fibromatosis (Dupuytren's contracture). Hormonal profile was normal for TSH, FT<sub>4</sub>, cortisol and prolactin but suggestive for hypergonadotropic hypogonadism due to high levels of FSH and LH in association with low levels of testosterone. On fundoscopic examination, he has bilateral optic atrophy and diabetic retinopathy. The urological exam. (ultrasound and urography) diagnosed bilateral hydronephrosis and atonic bladder. Audiometry showed bilateral high frequency hearing loss. The cranial CT scan confirms the optic atrophy without other abnormalities.

#### Conclusion

The presence of diabetes insipidus and sensorineural deafness in the first decade not in the second, dilated renal outflow tracts in the second decade not in the third, the hypergonadotropic hypogonadism, diabetic retinopathy and palmar fibromatosis makes this case particular and emphasize the requirement of complete and complex evaluation of the patient with Wolfram syndrome regardless the age.

## P631

### 'Uterine neuroendocrine tumour: an unusual cause of hyponatraemia' and the role of tolvaptan, a vasopressin V2 receptor antagonist

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#### Background

Hyponatraemia is the commonest electrolyte abnormality in clinical practice, and may be a biochemical manifestation of different diseases including malignancy. About 14% of hyponatremia in medical inpatients is due to underlying tumour related conditions.

We present a case of 68 years widow who was referred by her General Practitioner with 3 weeks history of nausea, occasional vomiting, confusion, increased urinary frequency, and urinary incontinence but no dysuria. She also complained of chronic constipation, and weight loss. Her past medical history included left knee arthroscopy, cochlea implant and sterilisation in 1976. She was non smoker but her husband was a heavy smoker. On admission she was haemodynamically stable with normal observations. Blood test revealed profound hyponatraemia (Na<sup>+</sup> 118 mmol/l), and serum osmolality of 247 mOsm/kg, urine osmolality of 701 mOsm/kg, urinary sodium of 110 mmol/l, random cortisol of 542 nmol/l, in keeping with SIADH. Tumour markers were negative. Chest X-ray was unremarkable but abdominal ultrasound revealed a uterine heterogeneous enhancing mass which was also confirmed on staging CT of chest, abdomen and pelvis. Flexible sigmoidoscopy was normal. Her medications included Paracetamol and Codeine Phosphate. She was started on fluid restriction of 750 ml/24 h. She remained hyponatraemic (116–117 mmol/l) despite this. Oral Demeclocycline 300 mg TDS was added, but she was unable to tolerate it and she was changed to tolvaptan (30 mg od). She was discharged home on tolvaptan (30 mg) and was reviewed by gynaecology team in outpatient clinic; cervical biopsy histology showed a high grade neuroendocrine malignant tumour confirmed by positive immuno labelling for chromogranin, AE1/3 and CAM5.2. She is currently undergoing chemotherapy and still on tolvaptan. Her sodium remains low but at safer levels of 127–130 mmol/l.

#### Discussion

Hyponatraemia secondary to uterine neuroendocrine tumours is rare and generally associated with chemotherapy treatment rather than being related to the uterine neuroendocrine tumour. Conventional treatment for hyponatraemia due to SIADH was ineffective or not tolerated. Tolvaptan treatment was more successful even though it is mainly used in patient with mild to moderate euvoalaemic hyponatraemia. This possibilise hyponatraemia.

**P632****Diagnosis of Bruns–Garland syndrome in a patient with McArdle disease and type 2 diabetes mellitus**

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**Introduction**

McArdle disease is a rare recessive disorder of glycogen metabolism, related to muscle phosphorylase deficiency. It usually presents in adolescence or early adulthood with muscle cramps, exercise intolerance, easy fatigability, and progressive weakness as glycogen is the primary source of energy for intense muscle activity. Few reports described the association of McArdle disease and type 2 diabetes and some authors suggested that hyperglycemia and hyperinsulinemia may improve muscle symptoms. In fact, sucrose ingestion before exercise is one of the proposed treatments for this disease.

**Clinical case**

A 69-year-old caucasian male with previous medical history of insulin-treated type 2 diabetes mellitus (diagnosed 30 years before), McArdle disease (diagnosed 15 years before, by muscle biopsy) and colon carcinoma (6 years before, treated with surgery and chemotherapy, without evidence of recurrence) presented with severe pain and weakness in left lower extremity (during last 4 months) and weight loss. Previously, he did routine moderate exercise, without symptoms. On physical examination, he had proximal muscular atrophy (more in anterior compartment of left thigh), very reduced reflexes (more in left limb), and a small antero-lateral area of hypoesthesia in left thigh. HbA1c was 9.4%. Electromyography revealed a sensitive and motor polyradiculoneuropathy (with predominantly lumbal expression) compatible with the diagnosis of Bruns–Garland syndrome. Patient was submitted to symptomatic treatment (gabapentin and duloxetine), physiotherapy and glycemic control optimization.

**Discussion**

In this patient with simultaneous miopathy and neuropathy predisposing disease, this clinical presentation originated a challenging differential diagnosis. Bruns–Garland syndrome is a rare complication of diabetes and more frequent in patients under good metabolic control. Another relevant point is related to the eventual contribution of hyperglycemia to McArdle disease absence of symptoms. This fact may be taken into account in deciding glycemic treatment goals in this patient with long duration diabetes.

**P633****Lower limb Merkel cell carcinoma with a single metastasis in a thyroid nodule**

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**Introduction**

Merkel cell carcinoma (MCC) is a rare, aggressive cutaneous malignancy, affecting predominantly caucasian and older individuals (slight male predominance). This neoplasm is associated with a high risk of local recurrence and regional lymph node involvement. Distant metastases are less frequent; they were described in liver, bone, CNS, skin, lung and spleen. The authors describe a clinical case of Merkel cell carcinoma with a single metastasis in a thyroid nodule.

**Clinical case**

A 53-year-old caucasian female was submitted to excision of a rapid growing nodule on the internal face of left lower limb. Hystologic examination showed a Merkel cell carcinoma, with 2.5×2.5 cm and positive margins. Additionally, she underwent a thyroid sonography that revealed a left lobe nodule (4×3.2×2 cm) and a small one in right lobe (1.2 cm). Admitted to our hospital, MR scan showed extensive persistent disease in lower limb. 18F-FDG PET showed fixation only in the left lower limb. FNA biopsy of the largest thyroid nodule revealed benign result – colloid. Patient underwent regional hyperthermic perfusion with melfalan and TNF $\alpha$ , and then tumoral lesion excision and plasty. Three years later, she complained of cervical discomfort. Cervical sonography showed thyroid nodule growing. FNA biopsy didn't result in a conclusive diagnosis. Patient was soon after submitted to total thyroidectomy, revealing a Merkel cell carcinoma metastasis in the thyroid nodule (with 6.5 cm) with gland capsule and soft tissues invasion; a right papillary microcarcinoma (0.1 cm) was also found. Repeated 18F-FDG PET suggested left lower limb recurrence, without other metastases. She was submitted to cervical radiotherapy.

**Conclusions**

This clinical case describes a Merkel cell carcinoma with a single thyroid metastasis in a previously known nodule with a benign result in FNA biopsy. In oncologic patients, thyroid secondary involvement is an important differential diagnosis in initial evaluation and follow-up of thyroid nodules.

**P634****An old story again with a new patient in modern world: acute adrenal insufficiency in a 51-year old man with vertebral tuberculosis**

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**Introduction**

Tuberculosis is still an important cause of adrenocortical insufficiency in the developing countries. Adrenal insufficiency is usually the result of hematogenous spread of the pulmonary tuberculosis. Adrenal tuberculosis, especially with enlargement of adrenal glands can cause diagnostic problems and requires differentiation from neoplastic disease and fungal infections. Tuberculosis osteomyelitis is a rare form and surgery may be needed. We report a patient with adrenal insufficiency and tuberculosis osteomyelitis.

**Case report**

A 51-year-old man was admitted to hospital because of anorexia, dizziness, nausea, vomiting and weight loss. On admission, his blood pressure was 90/50 mmHg, had severe orthostatic hypotension; the laboratory examination revealed high erythrocyte sedimentation rate (50 mm/h), hyponatremia (121 mEq/l), hyperkalemia (7.5 mEq/l) and low cortisol levels (5  $\mu$ g/dl). Fluid replacement, methylprednisolone and fludrocortisone -to correct resistant hyperkalemia- were administered with a diagnosis of adrenal insufficiency. ACTH level was 5.41 pg/ml under corticosteroid and mineralocorticoid treatment. Upper abdomen magnetic resonance imaging (MR) revealed asymmetrical enlargement of adrenal glands compatible with bilateral adrenal hyperplasia. In name of excluding tuberculosis, X-ray of the chest was completely normal. We looked up any pathological lymphadenopathy; the pathological examination of a hypoechoic, 2 cm, non-reactive, inguinal lymph node was compatible with granulomatous lymphadenitis which showed caseating granulomas. The patient had been suffering lomber pain for several months. Lomber MR revealed height loss and pathologic signalisation in L1–L2 which could be compatible with compression fracture or metastasis. A biopsy of lomber vertebra was performed. The pathological result showed chronic necrotic caseating granulomatous inflammation revealing tuberculosis osteomyelitis. Antituberculous medicines isoniazid, rifampicin, pyrazinamide were prescribed with steroids.

**Conclusion**

Adrenal insufficiency due to tuberculosis has become a rare condition for developed countries, however, in a patient from a developing country tuberculosis should always come to mind in the differential diagnosis when primary adrenal insufficiency is detected.

**P635****A case of a parathyroid adenoma with Celiac disease: primary or quaternary hyperparathyroidism?**

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**Introduction**

Celiac disease (CD) is gluten sensitive enteropathy with a wide spectrum of severity. Iron deficiency, growth retardation, shortness, delayed puberty can be seen. Osteomalacia and hypocalcemia are results of malabsorption of vitamin D and calcium which may cause secondary hyperparathyroidism. In untreated cases for a long period, tertiary hyperparathyroidism can also be seen due to autonomy of parathyroid glands with chronic stimulation. There are some cases of primary hyperparathyroidism accompanying CD. We report a case of CD presenting with severe hypercalcemia of a parathyroid adenoma.

**Case report**

A 14-year-old girl admitted to our department for delayed puberty and growth retardation. She was 125 cm tall and 28.5 kg; under the 3rd percentile. Her wrist graphy was consistent with 7–8 years. Laboratory examination revealed iron deficiency anemia, low 25OH vitamin D level (7 ng/ml), high parathyroid hormone level (PTH) (955 pg/ml), hypercalcemia (13.4 mg/dl),

hypogonadotropic hypogonadism (FSH: 0.29 mIU/ml, LH: 0.1 mIU/ml, estradiol: 5.32 pg/ml), low levels of GH (6.67 ng/ml) and IGF1 (26.2 ng/ml; 111–551). Endoscopic biopsy was compatible with total villous atrophy, crypt hyperplasia, intraepithelial lymphocyte infiltration, edema, lymphocyte and plasma cell infiltration of lamina propria. Endomysium antibody was positive. Gluten free diet was started. Her neck ultrasound and parathyroid scintigraphy demonstrated a parathyroid adenoma. She underwent parathyroidectomy. Her calcium became 8.8 mg/dl and PTH 12.3 pg/ml. After 4 months of gluten free diet she is now 132 cm tall and weighed 34 kg. She is in stage Tanner 2, her axillary and pubic hair had began to grow up.

#### Conclusion

Tertiary hyperparathyroidism may arise in patients with longstanding undiagnosed CD. It has been suggested that autonomous four gland hyperplasia or tertiary hyperparathyroidism may progress to adenoma formation and that this should be termed 'quaternary hyperparathyroidism'. There should be more studies to explain the relationship between CD and hyperparathyroidism.

### P636

#### A case of congenital adrenal hyperplasia of 11 $\beta$ -hydroxylase deficiency presented with Addisonian crisis

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#### Introduction

Steroid 11 $\beta$ -hydroxylase deficiency is a relatively rare form of congenital adrenal hyperplasia characterized by the overproduction of adrenal androgens and deoxycorticosterone. It usually presents with virilization of the female fetus, precocious puberty in male infants and hypertension with or without hypokalemia in both genders. Because of high levels of mineralocorticoids, patients rarely present with Addisonian crisis. We describe a male patient with 11 $\beta$ -hydroxylase deficiency undiagnosed until 41 years old and presented with adrenal crisis.

#### Case report

A 41-year-old male was admitted to the emergency unit with dizziness, hypotension and unconsciousness which have started after acute gastroenteritis. He had acute renal failure, hospitalized in the intensive care unit. A cortisol level of 12.2  $\mu$ g/dl in the intensive care conditions was compatible with adrenal insufficiency. He was born of a consanguineous marriage, was the shortest in his family (148 cm) and had a history of precocious puberty. He had a history of left orchietomy 6 years ago with a diagnosis of testicular mass and had undergone left adrenalectomy 3 years ago with a pathologic diagnosis of myelolipoma. He also had a history of infertility due to azoospermia; hypertension and hypokalemia. After recovering of gastroenteritis and renal failure, laboratory examination revealed undetectable LH, and FSH, and very high levels of testosterone, progesterone, 17-OH-progesterone, 11-deoxycortisol, 11-deoxycorticosterone and DHEA-S, and a slightly high ACTH. He was diagnosed as congenital adrenal hyperplasia due to 11 $\beta$ -hydroxylase deficiency. He was treated with dexamethasone 0.75 mg daily and after 4 weeks, most of the abnormal values returned to normal, LH and FSH became detectable, testosterone decreased to suboptimal levels. He became normotensive and normokalemic without any treatment. Myelolipoma and testicular mass (rest tissues) in this patient are probably due to metaplastic origin due to chronic stimulation by ACTH.

#### Conclusion

This is an interesting case presented adrenal insufficiency with congenital adrenal hyperplasia of 11 $\beta$ -hydroxylase.

### P637

#### Diabetes and A1C. How a hemoglobin variant affect A1C test: a case report

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#### Introduction

Glycated hemoglobin A1c (A1C) is used for diagnosis and monitoring diabetic patients. The precision of A1C assay methods is affected by the presence of

hemoglobin variants. About 7% of world population is asymptomatic carrier of these variants.

#### Case report

PCIMR, woman, 42 years old, caucasian, followed in consultation for obesity, primary hypothyroidism and bipolar disorder. Had family history of obesity and sudden death of her father at 50 years old. Was treated with levothyroxine and psychotropic drugs. She reported recent hypertension and weight gain. Presented with BMI 32.5 kg/m<sup>2</sup> and BP 140/90 mmHg. Laboratory study showed: TSH 5.6  $\mu$ U/ml (NR: 0.4–4.0), FT<sub>4</sub> 1.2 ng/dl (NR: 0.8–1.9), fasting plasma glucose 108 mg/dl, total cholesterol 182 mg/dl (NR:  $\leq$ 190), LDL 124 mg/dl (NR: <115), triglycerides 119 mg/dl (NR: <150). Reassessment analysis showed: plasma fasting glucose 106 mg/dl and A1C 14% (NR: 4.0–6.0). After confirmation (A1C 18.5%), we initiated investigation for a hematologic disease.

#### Results

Hb 14.6 g/dl (NR: 11.5–15.5), hematocrit 42.8% (NR: 37–47), MCV 85.4 fl (NR: 76–96), MCH 29.2 pg (NR: 27–32), MCHC 34.2 g/dl (NR: 31.0–35.6), RDW 13.1%, reticulocyte 1.52%; normal iron metabolism. Suspecting of interference with A1C test, we studied hemoglobins and identified an X variant, with AXA2 hemoglobins profile: Hb A2 2.1% (NR <2), HbF 0.1% (NR: 2–3.5), Hb variant 13.8 g/dl. The HBA1 gene sequencing revealed heterozygosity for mutation of  $\alpha$ 1 CD40 AAG-AAT (Lys-Asn) – Hb Saratoga Springs. Given the familial transmission risk, we performed family screening in order to genetic counseling.

Conclusions  
In this patient, we found abnormal fasting plasma glucose associated with discrepancy of A1C value. We identified a rare variant of hemoglobin A (Hb Saratoga Springs). Despite the advantages of A1C in the diagnosis and monitoring of diabetes, limitations of the method must be taken into account.

### P638

#### Hyponatremic coma with seizures as onset of isolated ACTH deficiency

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#### Introduction

Isolated acquired ACTH deficiency is rare; it may be due to hypophysitis and association with other autoimmune diseases was reported.

#### Case report

E.L, female, aged 34, was diagnosed with primary autoimmune hypothyroidism and premature ovarian failure; levothyroxine and estroprogestins were administered. Suddenly, the patient developed vomiting, shivers without fever and soon coma installed, with tonic-clonic seizures. There was no history of recent pregnancy or traumatic injury. Biochemical data revealed severe hyponatremia (<100 mmol/l) with normokalemia (4.2 mmol/l); thyroid function was normal on L-T<sub>4</sub> therapy (TSH=1.91 mIU/l, FT<sub>4</sub>=1.2 ng/dl). Adrenal deficiency was suspected. I.v. dexametasone 24 mg/day and saline were administered, with progressive, slow normalisation of natremia, followed by remission of coma and seizures. Further tests supported isolated ACTH deficiency: decreased plasma 0800 h cortisol (2.6  $\mu$ g/dl), with low normal ACTH levels (8.2 pg/ml). Short Synacthen test (250  $\mu$ g) showed inadequate cortisol stimulation (9.9  $\mu$ g/dl at 30 min, 13.6  $\mu$ g/dl at 60 min) and prolonged ACTH stimulation test (3 days) revealed adequate cortisol stimulation at 24 h (39.8  $\mu$ g/dl), 48 h (31.1  $\mu$ g/dl) and 72 h (43  $\mu$ g/dl). Insulin tolerance test could not be performed due recent history of seizures, but insufficient response to glucagon test (maximum cortisol levels=10.9  $\mu$ g/dl) supported the diagnosis. All other pituitary hormones were in normal range. Cerebral and pituitary MRI, adrenal CT scans were normal. Hydrocortisone 20 mg/day was administered, with maintenance of normal natremia and kalemia. Low sodium levels were reported in 28% of cases with isolated ACTH deficiency, but hyponatremic coma was very seldom reported.

#### Conclusion

This case emphasis the importance of considering isolated central adrenal insufficiency when encountering hyponatremic coma.

**P639****GH in Prader-Willi syndrome: to treat or not to treat**

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**Introduction**

Prader-Willi (PWS) is a complex genetic syndrome characterized by dysmorphic features, hypotonia, mental retardation, behavioral abnormalities, hyperphagia with progressive obesity, and endocrine dysfunctions as hypogonadism and GH deficiency. GH treatment is recommended, the major concern being aggravation of sleep apnea.

**Cases report**

We present 2 cases with specific clinical features and genetically confirmed PWS (del 15 q11.2-q13), who had normal stature in spite of confirmed GH deficiency. Both cases are girls of 14 (PD), respectively 6-year-old (MF), born in non-sanguine couples, with low birth weight and important hypotonia. After the age of 1, they both presented hyperphagia with rapid and important weight gain. At 10 years of life, PD had important obesity (+10 s.d.) and a surprising height at +2 s.d. despite of low GH, insufficiently stimulated, and normal low IGF1 (138 ng/ml). Sleep apnea could not be assessed because of agitation. At the age of 14, with rigorous alimentation, her weight did not excessively increased, being at +8 s.d. She had no puberty signs, low hormones of gonadal axis, and a low height velocity, however her actual height remains higher than expected (+1 s.d.). Bazal GH remained low and IGF1 (37.6 ng/ml). She had moderate obstructive sleep apnea. With possible sleep apnea in the first case and confirmed in the second, in front of their normal height, the decision of monitoring of height velocity without treatment was taken.

**Conclusion**

Short stature is common in PWS. Our patients presented unexplained high stature, despite partial GH deficiency. GH treatment remains controversial and armed expectative is probably preferable in these two cases.

**P640****Subacute onset in severe Riedel thyroiditis associated with hypoparathyroidism and left recurrent palsy**

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A 42-years woman presented with hypothyroidism, a large, firm, irregular goiter, severe cervical and preauricular pain, 2 weeks after an episode of respiratory infection. Laboratory findings certified subacute thyroiditis and autoimmune hypothyroidism: TSH > 50 µU/ml, FT<sub>4</sub> = 6.5 pmol/l, ATPO > 1000 U/ml, ATGL > 1000 U/ml, ESR = 115 mm/h. Thyroid ultrasound showed hypoechoic pattern with a nodule on the right lobe and low Doppler signal. Fine needle aspiration biopsy showed features of chronic thyroiditis and fibrosis. Prednisone 30 mg/day and levothyroxine replacement treatment were started temporary relief of symptoms and improvement of biological profile. However, she relapsed after 3 months when the steroid was tapered, while the goiter size increased and it became harder. Four months after the first admission, she was submitted to surgery, and total thyroidectomy was performed with difficult dissection due to severe fibrosis and local invasion towards the left recurrent nerve, hyoid muscles, trachea and esophagus. The pathology report confirmed thyroid fibrosis with Riedel features.

Postsurgery ATPO level decreased but she presented dysphonia and documented hypoparathyroidism. ENT evaluation certified left recurrent nerve palsy. She was placed on calcium (1000 mg) and vitamin D<sub>3</sub> (0.5 µg/day). Despite surgery, she continued to present cervical constriction, oedema and pain in the anterior cervical area, therefore she required methylprednisolone pulse therapy, then switched to oral treatment. Computed tomography certified residual left cervical fibrosis of 3 cm diameter and 10 cm length, from pharyngolarinx up to the supraclavicular fossa, in addition to posterior left juxtaleural pulmonary fibrosis. Euthyroidism was maintained with L-thyroxine 100 µg/day. Addition of tamoxifen at 20 mg/day improved the clinical and biological status with decrease of ESR, allowing progressive reduction of steroid treatment, with clinical relief of symptoms.

In conclusion, tamoxifen treatment added to corticosteroids, is a useful association in Riedel's thyroiditis.

**P641****Multiple pituitary deficiencies in a young patient with primary empty sella**

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**Introduction**

Usually, the hormonal profile is normal in patients with empty sella. However, when present, hormonal deficiency most commonly consist in growth disturbance in children and mild hyperprolactinaemia in adults. Hypopituitarism with signs of symptomatic dysfunction occurs in <10% of patient with primary empty sella.

**Case report**

A 27-years-old man presented to our department in 2008 with impuberism, micropenis, hypoplastic scrotum, inguinal gonads. He presented an eunucoid habitus but the karyotype was 46xy and the Barr test was negative. Laboratory tests showed prepubertal levels of FSH, LH and testosterone. The IGF1 level was also normal. The MRI revealed an empty sella. He started treatment with testosterone-gel which was stopped 2 months later due to upper arms acne. In 2010, without any treatment, the evaluation reconfirmed the hypogonadotropic hypogonadism (LH = 1.2 mU/ml, FSH = 0.7 mU/ml, testosterone = 0.9 ng/ml). PRL level was slightly elevated (15.8 pg/ml, n < 15). But further tests showed thyrotrop and corticotrop deficiency (FT<sub>4</sub> = 7.44 pmol/l, n = 12-22 pmol/l and cortisol = 9.4 ng/ml, n = 60-230). Clinically, the patient didn't show symptoms of adrenal or thyroid hypofunction. The MRI revealed empty sella with compression of the pituitary gland. He received treatment with testosterone enanthate, prednisone and levothyroxinum.

**Conclusion**

Considering the possibility for the development in time of global pituitary hypofunction, when empty sella is confirmed, the early diagnosis of the deficits leads to proper replacement therapy avoiding potential life-threatening situations for the patient.

**P642****Giant invasive macroprolactinoma**

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**Background**

Apart from signs of hyperprolactinaemia, patients with macroadenomas with extrasellar extension generally seek medical attention due to mass effect. Macroprolactinomas, particularly in men, may occasionally exhibit a very aggressive clinical course.

**Case report**

Male 37 years old that went to a Neurology consultation complaining of progressively more frequent self-limiting episodes of restraint, silence and time and space disorientation in the last 3.5 years. CT scan revealed a large and infiltrative mass lesion centered to the skull base, associated with destruction of surrounding bone structures, including the limits of the sella turcica and sphenoid body, and compression of brain parenchyma (left temporal lobe) and bulge, with marked deformation of these structures. He was immediately sent to the emergency room of this hospital by the Imaging Center, to be examined by a neurosurgeon. No significant changes were detected on neurological examination, although he mentioned minor hearing impairment and lower visual acuity for objects in the right visual field. MRI favored the hypothesis of chordoma. He was evaluated by endocrinology and hormonal study revealed hyperprolactinemia (>2000 ng/ml). He had no signs or symptoms of hypothyroidism, hypocortisolism or hypogonadism. Treatment with dopamine agonist and an anti-epileptic was begun. Prolactin levels stabilized at 1.5 times the upper limit of normal. We also observed partial reduction (30%) of tumour volume, recovery of visual acuity and remission of seizures.

**Conclusion**

With this case, we stress the importance of hormonal evaluation in the differential diagnosis of tumors of the diencephalon-pituitary region. The clinical response, analytical and imaging data, 1.5 years after starting treatment, reinforces the diagnosis of invasive prolactinoma. The extent of the injury prevents, at present, its surgical resection. The good response to therapy reaffirms the concept long ago established that there are patients in whom surgical treatment is never an option.



## P643

### **Beaxarotene associated dyslipidaemia and central hypothyroidism in a patient with Sézary syndrome**

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#### Background

Sézary syndrome (SS) is a more aggressive leukemic variant of cutaneous T-cell lymphoma (CTCL). Beaxarotene (Targretin) is a synthetic retinoid analogue designed for the treatment of advanced stages of cutaneous manifestations of CTCL. Beaxarotene selectively suppresses thyrotropin secretion and up to 40% of patients develop reversible idiopathic central hypothyroidism. Severe mixed dyslipidemia may be present in up to 70%.

#### Case report

Seventy-year-old female who started development of skin changes on the trunk and limbs at 51 years of age, and 3 years later parapsoriasis in large plates was diagnosed. The evolution of the lesions led to deepening of etiological study, and 2 years later, CTCL in the form of SS, was diagnosed. Multiple treatments were tried without satisfactory clinical improvement. She began beaxarotene (Targretin) 300 mg/day in December 2002. Periodic laboratory surveillance, including lipid profile and thyroid function was performed. We detected maximum levels of triglycerides of 618 mg/dl, controlled with antidyslipidemic therapy, and fluctuations in the levels of TSH and free T<sub>4</sub> were mostly compatible with the diagnosis of central hypothyroidism. She was not treated for several years. Fluctuations in values of thyroid function and lipid profile were associated with changes in disease evolution and use of varying doses of beaxarotene. She was sent to the consultation of Endocrinology for diabetes mellitus, nodular goiter and assessing need for treatment with levothyroxine. There was need for treatment with levothyroxine and adjustment of oral antidiabetic treatment.

#### Conclusions

For the rarity of the disease and the endocrine-metabolic consequences of treatment it is important to expose this case. The evaluation of thyroid function and lipid profile should be performed before and during treatment with beaxarotene. Dyslipidemia is favored both by treatment with beaxarotene and untreated hypothyroidism.

## P644

### **Diffuse nodular hyperplasia of the adrenal cortex in a patient with renal cysts**

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Nodular hyperplasia of the adrenal as a cause of Cushing's syndrome is rare. Adrenal tumors, frequently causing syndromes such as hyperaldosteronism due to autonomous hormone secretion, have been described in patients with renal cysts. The aim was to describe the case of a female patient with diffuse adrenal hyperplasia, subclinical Cushing's syndrome and renal cysts.

A female patient aged 69 years presented with hyperplasia of the right adrenal cortex. In computer tomography evaluation a mass measuring 2 cm was observed in the right adrenal. ACTH was 9 pg/ml, serum cortisol 16.5 µg/dl and a dexamethasone suppression test with 0.5×4×2 mg dexamethasone was abnormal serum cortisol being 4 µg/dl after the test. Renin and aldosterone were 16 and 184 pg/ml respectively, in the supine position and 22 and 548 pg/ml respectively in the standing position. The mass was surgically excised and histology showed diffuse hyperplasia of all zones of the adrenal cortex, hyperplasia of the fasciculata zone being more prominent. In a subsequent evaluation, a year later, the dexamethasone suppression test with 0.5×4×2 mg dexamethasone remained mildly abnormal, serum cortisol being 2.1 µg/dl after the test. The patient had renal cysts in the right kidney.

In conclusion, an extremely rare case of a patient with diffuse hyperplasia of all zones of the adrenal, subclinical Cushing's syndrome and renal cysts is described. Renal cysts have been observed in patients with hyperaldosteronism and adrenal tumors. The case described is interesting as it is characterized by subclinical Cushing's syndrome, hyperplasia of all zones of the adrenal cortex and functional hyperaldosteronism.

## P645

### **A case of polymyositis and vasculitis induced by ovulation induction therapy with GnRH: case report**

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#### Background

GnRH is the first key hormone of reproduction. GnRH analogs are extensively used in IVF, and treatment of sex hormone-dependent cancers due to ability to bring about 'chemical castration'. Recent studies have demonstrated that GnRH and sex steroids exert not only hormonal effects but play an important role in the immune system modulation.

#### Case report

We report a case of vasculitis and severe polymyositis developed 5 days after GnRH administration in infertility patient. Symptoms included progressive and symmetric muscle weakness, myalgias, diffuse arthralgias, effort dyspnea. On physical examination muscular tenderness, tachycardia, normal size liver and spleen were found.

#### Results

Laboratory findings revealed elevated CK 198.6 µkat/l, increased CK-MB isoenzyme 6.05 µkat/l, elevated LDH 11.0 µkat/l, myoglobin 543.20 µg/l and elevation of troponin T. Moderate elevation of hepatic enzymes was detected. Positivity of ANA was found, while other antibodies were negative. Serum Ig demonstrated higher levels of IgE 374 IU/ml. All remaining findings were within normal range. Muscle biopsy demonstrated changes consistent with diagnosis of myositis and vasculitis. After treatment with prednisone 30 mg daily, aspirin and pentoxifyllin with rehabilitation an amelioration of muscle impairment was observed.

#### Conclusions

Autoimmune diseases could be induced by two mechanisms: firstly due to immunostimulating effect of GnRH and subsequent increased release of estrogens. Secondly, GnRH administration might unmask an underlying disease or might aggravate of subclinical disease. Nevertheless, our patient had neither previous history of autoimmune disease nor any symptoms of mild course of the autoimmune disease such as polymyositis or vasculitis.

## P646

### **Diabetes insipidus in a patient with breast carcinoma**

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#### Introduction

Central diabetes insipidus (CDI) is characterized by a deficient secretion of anti-diuretic hormone (ADH), in which a large volume of hypotonic urine is eliminated. Pituitary metastatic disease is rare, accounting for 1% of all excised pituitary tumors and generally involves the posterior pituitary. Neoplasms that more often originate pituitary metastases are breast and lung cancer. Breast cancer (BC) is associated with pituitary secondary involvement in 5.3 to 28% of cases. Case report

The authors describe the case of a 50-year-old woman, previously diagnosed with breast cancer, who underwent surgery, chemotherapy, hormone therapy and radiotherapy. During follow-up she developed polydipsia, polyuria, and nocturia. The analytical study revealed serum osmolality of 291 mosm/kg and urinary osmolality of 182 mosm/kg. Biochemical and hormonal workup was normal. Pituitary MRI showed a nodular lesion, gadolinium emphasized, in the pituitary stalk, suggesting a metastatic lesion. Diffuse brain metastasis were also evident. The patient was treated with desmopressin, and there was clinical and analytical improvement. During follow-up liver and bone metastasis were diagnosed, her condition deteriorated and she died shortly after.

#### Conclusions

A case of CDI in the context of the pituitary stalk metastasis of BC is described. Owing to high etiologic clinical suspicion, water deprivation/vasopressin tests were not undertaken. Although symptoms resulting from metastatic lesions and pituitary adenomas are similar, CDI is described in only 1% of the latter and it has been suggested that its presence is the most important criterion in distinguishing between the two entities. In the context of malignancy, the emergence of CDI should alert the clinician to the possibility of pituitary's metastatic involvement.

**P647****Ovarian hyperthecosis with type 2 diabetes mellitus and severe insulin resistance in postmenopausal woman**Vadim Gavan<sup>1</sup>, Andra Caragheorghopol<sup>1</sup>, Adrian Vaduva<sup>2</sup>, Crsitian Poalelungi<sup>2</sup>, Radu Lichiardopol<sup>3,4</sup> & Corin Badiu<sup>1,3</sup><sup>1</sup>National Institute of Endocrinology, Bucharest, Romania; <sup>2</sup>Cantacuzino Hospital, Bucharest, Romania; <sup>3</sup>University of Medicine and Pharmacy, Bucharest, Romania; <sup>4</sup>Institute of Diabetes, Nutrition and Metabolic Disorders, Bucharest, Romania.**Introduction**

Ovarian hyperthecosis is a rare cause of severe hyperandrogenism. Unlike PCOS, it is also described in postmenopausal women.

We report the case of a 67-year-old obese (BMI=37.5 kg/m<sup>2</sup>) and dyslipidemic woman, gravid 5 para 3, menopause at 49, with poor controlled type 2 diabetes mellitus (HbA1c=10%). She was in treatment with insulin for the last 12 years, currently on 168 U/24 h (1.71 U/kg)- indicating severe insulin resistance, complicated with stage III diabetic nephropathy, hypertension, ischemic heart disease with history of coronary artery bypass surgery and class III NYHA heart failure. She was admitted for severe hirsutism and balding, symptoms that developed progressively in the last 2 years. Physical examination revealed androgenic rash, male type alopecia, severe facial and thoracic hirsutism, deepening of the voice, acanthosis nigricans, mild ankle edema, dyspnea at minimal effort, BP=130/80 mmHg, HR=74/min. Biochemical profile: erythrocytosis (Hb=16.6 g/dl), renal failure (creatinine=2.9 mg/dl, BUN=159 mg/dl), hyperglycemia (Glyc=240 mg/dl), dyslipidemia (triglycerides=435 mg/dl). Basal total testosterone level was high (2.74 ng/ml), with normal plasma cortisol (15.7 µg/dl), and DHEAS (32.5 µg/ml). Mullerian inhibiting substance was in the fertile female range (1.27 ng/ml), β-hCG and CA125 were normal. A two days low dose dexamethasone suppression test indicated normal suppression of the adrenal axis (plasma cortisol=1.26 µg/dl), with no significant reduction (<40%) of the total testosterone (1.85 ng/ml), reflecting autonomous androgen secretion of ovarian source. Transvaginal ultrasound examination showed enlarged uterus (57/46 mm) and ovaries (left ovary 30/26 mm, right ovary 29/30 mm) for age. The patient was transferred to the gynecological department where total hysterectomy with bilateral anexectomy was performed. Two weeks after surgery the insulin requirement dropped by 39% (102 U/24). Total testosterone was under the detectable range of the laboratory.

**Conclusion**

Etiologic treatment of hyperandrogenism in cases of insulin-treated type 2 diabetes mellitus can lower the insulin resistance.

**P648****How differentiated can a poorly differentiated thyroid cancer be?****A rare case presenting with hyperthyroidism and secondary adrenal insufficiency**

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**Introduction**

Poorly differentiated thyroid cancers are usually aggressive tumors that lack many of the characteristics of differentiated thyroid tissue, including the ability to uptake and process iodine.

**Case report**

A 75-year-old male presented with weight loss, tiredness, asthenia, tremor, increased perspiration, tachycardia and bone pain in several locations. A hard and fixed thyroid nodule was palpable in the left lobe (FNA-«follicular tumor»). Laboratory and Imaging workup showed: TSH<0.004 µU/ml, fT<sub>3</sub>=7.3 pg/ml (1.8–4.2), fT<sub>4</sub>=1.6 ng/dl (0.8–1.9); thoracic and abdominal CT scan: nodule in the left lobe of thyroid gland with 57 mm; lytic lesion on right clavicle; multiples pulmonary nodules. The patient was treated with methimazole and then underwent total thyroidectomy. The pathology examination revealed a poorly differentiated thyroid carcinoma. A <sup>131</sup>I therapeutic activity of 108 mCi was administered. Post-therapeutic whole body scan showed metastatic lymph node involvement in the mediastinum, lung uptake and bone metastatic lesions in the basis of the skull, right orbit, several rib bones, left scapula, T10 and T11 vertebrae, right humerus, left iliac bone and left femur; some of these lesions did not have iodine uptake. Thyroglobulin=158 028 ng/ml (undetectable Tg-Abs). Head CT showed a supra-sellar mass with sellar invasion. Prolactin=82 ng/ml (n<20), basal/stimulated cortisol=8/17 µg/dl. After surgery, treatment with L-T<sub>4</sub> was started (100 µg/day); this dose was progressively decreased to 12.5 µg/day, as the patient developed atrial fibrillation along with persistently high fT<sub>3</sub> levels. The patient died from pneumonia 4 months after surgery.

**Conclusions**

This case illustrates two rare clinical manifestations of thyroid cancer: hyperthyroidism (functioning metastasis) and secondary adrenal insufficiency. Despite being considered as a poorly differentiated tumor and having an aggressive behavior, some metastasis seem to preserve the ability to produce/convert T<sub>3</sub>.

**P649****HDR syndrome or Barakat syndrome**

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HDR syndrome (hypoparathyroidism, deafness, renal dysplasia syndrome) is a rare clinical entity, which includes the simultaneous presence of hypoparathyroidism, sensorineural deafness and renal dysplasia. It is due to mutations of GATA3 gene in XP.10p and is inherited with the autosomal dominant type.

The aim was to describe the case of a patient with clinical symptoms and laboratory and imaging findings compatible with HDR or Barakat syndrome.

A male patient aged 51 years, Caucasian, with a history of chronic hypocalcemia, sensorineural deafness affecting both ears and renal dysplasia was admitted with hypertonism of body and extremities and loss of consciousness. On admission he was found to have severe hypocalcemia and mild renal insufficiency. He was properly managed therapeutically and recovered consciousness while clinical and laboratory findings improved.

He was found to have severe hypocalcemia, serum calcium being 3.5 mg/dl, severe hypoparathyroidism, PTH being 0.7 pg/ml, renal dysplasia with mild renal insufficiency and sensorineural deafness affecting both ears. Mild vitamin D insufficiency, 25(OH)vitamin D<sub>3</sub> being 17 ng/ml was also detected and serum magnesium was low.

In conclusion, the extremely rare case of a patient with HDR or Barakat is described.

**P650****Coincidental presence of acromegaly and nonclassical congenital adrenal hyperplasia in a patient with hirsutism: a case report**Arzu Gedik<sup>1</sup>, Mehmet Muhittin Yalcin<sup>2</sup> & Abdurrahman Comlekci<sup>1</sup><sup>1</sup>Endocrinology Department, Dokuz Eylul University Medical Faculty, Izmir, Turkey; <sup>2</sup>Aksaray Government Hospital, Aksaray, Turkey.

Hirsutism, development of terminal body hair in women in unexpected areas, may result from different etiologies; 4.3% being nonclassical congenital adrenal hyperplasia (NCCAH) and acromegaly being one of the rarest. We here present a severe hirsutism case with acromegaly and NCCAH.

**Case**

A 30-year-old woman with headache and visual field abnormalities was consulted by our clinic about her cranial imaging revealing a macroadenoma in pituitary localization. She had had secondary amenorrhea, infertility and progressive hirsutism for 3 years and had been on contraceptives irregularly and on regular cabergoline treatment for 2 years. On physical examination: blood pressure: 140/100 mmHg, pulse: 86/min, body mass index: 33.057 kg/m<sup>2</sup>. She had distinct hirsutism with a Ferriman-Gallwey score of 31 (n<8). She had no acromegaloïd or cushingoid features. On hormonal evaluation, prolaktin: <0.50 mg/ml (1.9–25), GH: 5.22 ng/ml (0.06–5), IGF1: 422 ng/ml (69–358), Total testosterone: 184 ng/dl (15–80), androstenedione: 4.58 ng/ml (0.3–3.5), 17-OH progesterone: 1 ng/ml (<2). Cortisol was suppressed below 1.8 µg/dl in low dose dexamethasone suppression test, but nadir GH level in oral glucose tolerance test was 1.56. There was a 14 mm macroadenoma in the pituitary and bilateral hemianopsia on visual field examination. She underwent transsphenoidal surgery with a presumptive diagnosis of acromegaly. Pathological specimen was 60% GH positive. After surgery acromegaly was under control but her symptoms persisted. The re-evaluation of the hormones revealed 17-OH progesterone: 3.2 ng/dl, which rised above 10 ng/dl with ACTH stimulation test. Thus, she was accepted as NCCAH. She is now on prednisolone 5 mg/day therapy and has started to have regular menses.

**Conclusion**

Hirsutism is a relatively uncommon but reported finding in acromegalics. We detected concomitant acromegaly and NCCAH in our patient. While evaluating for hirsutism, one should rule out all the possible underlying etiologies, especially endocrinopathies even though a distinct reason has already been detected.

**P651**

**Adrenal rest in a patient with late onset 21-hydroxylase deficiency**

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Adrenal rests, nodules of the adrenal, in patients with chronic elevation of ACTH levels have been previously described.

The aim was to describe the case of a patient with an adrenal tumor and late onset 21-hydroxylase deficiency.

A female patient, aged 76 years, presented with an incidentally discovered mass of the left adrenal measuring 1.2 cm. The patient was short with a height of 152 cm. Morning serum ACTH levels were 31 pg/ml, serum cortisol 16.8 µg/dl, serum aldosterone 14.7 pg/ml and serum renin 17 pg/ml, urine free cortisol 6.5 µg/day and 17(OH)progesterone 5 ng/ml. An intravenous ACTH stimulation test was performed. Cortisol levels before the administration of ACTH, 30 and 60 min later were 12, 13.7 and 14 µg/dl respectively. Serum 17(OH)progesterone levels before the administration of ACTH, 30 and 60 min later were 10, 50 and 50 ng/ml. The elevation of 17(OH)progesterone after the administration of ACTH was diagnostic of the presence of non classical 21-hydroxylase deficiency. The insufficient cortisol increase after the administration of ACTH was suggestive of partial adrenal insufficiency.

In conclusion, the rare case of a patient with non classical 21-hydroxylase deficiency is described. The patient presented in old age with an adrenal rest. It is possible that the chronic stimulation of the adrenal cortex from ACTH as a result of the partial cortisol insufficiency was involved in the pathogenesis of the adrenal tumor.

**P652**

**Long term evolution of a coincident ACTH-dependent and independent Cushing's syndrome**

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**Introduction**

The coexistence of Cushing's disease with an ACTH-secreting adrenal adenoma is not frequent.

**Case report**

A 32-year-old woman was observed in our Department in 2000 for obesity. Hypercortisolism was documented and the basal hormonal study and functional testing oriented towards a pituitary origin (ACTH 24.8 pg/ml). Pituitary magnetic resonance imaging (MRI) showed an asymmetric pituitary gland and a pituitary stalk deviation to the left. Adrenal computerized tomography (CT) revealed a nodule in the right gland sized 1.5 cm. Inferior petrosal sinus sampling demonstrated a center to periphery ACTH gradient > 3 before and after CRH. The patient abandoned the appointment and returned 6 years later complaining of weight gain, facial plethora and fatigue. The basal hormonal study and functional testing confirmed ACTH-dependent Cushing's syndrome (ACTH 11.4 pg/ml) and the pituitary MRI showed a right-sided microadenoma. The patient was submitted to transphenoidal resection in 2008 and histology confirmed an ACTH-secreting adenoma. However, hypercortisolism persisted. Pituitary MRI showed no adenoma. ACTH level 1 year after surgery was 7.6 pg/ml and showed no significant increase after CRH. Adrenal CT revealed a 2.5 cm adenoma on the right gland and a normal left gland. Adrenalectomy was performed in 2010 and histology revealed a cortical adrenal adenoma. There was resolution of hypercortisolism and the patient is on replacement therapy with hydrocortisone. **Conclusion**

Whether an adrenal adenoma coexisted with a pituitary adenoma from the beginning or an autonomous adrenal adenoma after ACTH-induced-hyperplasia developed after longstanding stimulation by a pituitary ACTH adenoma, we don't know. Investigations show that suppressed ACTH secretion in patients with Cushing's syndrome results in reduction of the ACTH receptor mRNA expression in nonneoplastic adrenocortical cells. However, the regulatory mechanism of ACTH-R expression might be different in adenoma and persistent expression of ACTH-R alone might play a role in the autonomous production of cortisol.

**P653**

**Maltose interference in the determination of blood glucose levels by test strips based on glucose dehydrogenase-PQQ method: a case report**

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**Background**

The different systems for monitoring capillary blood glucose levels may suffer interference from various substances.

**Case report**

Male patient, 48 years old, with history of hypertension, dyslipidemia and previous smoking. He presented also type 1 diabetes mellitus, with 37 years of progress, with diabetic retinopathy, kidney failure, in peritoneal dialysis since 2009, and ischemic heart disease (myocardial infarction in 2005). He was treated with insulin, angiotensin receptor antagonists, beta blockers, calcium channel blockers, darbepoetin, metolazone, loop diuretics, aspirin, proton pump inhibitors, folic acid. Because of glycemic instability, with frequent hypoglycemic events, he was proposed for therapy with insulin infusion pump. As an inpatient, at the time of therapy adjustment with insulin pump, he frequently referred symptoms suggestive of hypoglycemia. However, determinations of capillary glycaemia (CG) with test strips based on glucose dehydrogenase-PQQ method revealed persistently high glucose levels. At the 4th day of hospitalization, capillary glycaemia determinations were performed simultaneously with test strips based dehydrogenase-PQQ method and with test strips based glucose oxidase method. A significant difference, of about 100 units, was found between the two values (dehydrogenase-PQQ method CG= 147 mg/dl; glucose oxidase method CG=45 mg/dl).

**Conclusions**

Some sugars, such as maltose, interfere with blood glucose monitoring systems based on dehydrogenase-PQQ method. This interference occurs solely through the use of maltose in preparations administered parenterally or used in peritoneal dialysis. Maltose administered orally does not interfere with the determinations of the CG. Thus, CG determination by test strips based on glucose dehydrogenase method should not be used in patients on peritoneal dialysis using solutions containing isodextrin, which has maltose as an intermediate metabolite.

**P654**

**Type II polyglandular syndrome: a case report**

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**Introduction**

The combination of two or more, autoimmune, endocrine dysfunctions defines the presence of polyglandular syndromes.

**Case report**

Female patient, 43 years old, history of type 1 diabetes for 23 years and autoimmune hypothyroidism for 7 years, with no other relevant clinical history and without family history of autoimmune endocrinopathies. She was under therapy with insulin and levothyroxine. She came into the Endocrinology Department in May 2010 with frequent episodes of severe hypoglycemia during the previous month. She had no other new symptoms. Insulin therapy was then optimized until re-evaluation in 2 weeks. At that time she referred persistence of the episodes of hypoglycemia with increasing frequency. A new adjustment in treatment was made and, at the same time, levels of morning serum ACTH and cortisol were requested.

Meanwhile she developed, darkening of the skin, especially the creases and oral mucosa. She presented no other symptoms, namely, fatigue, weakness, nausea or vomiting. There was no weight loss or sings of hypotension. From the study requested, ACTH was 1098 ng/l and cortisol 2.5 µg/dl, no hyponatremia was found. Hydrocortisone therapy was initiated (10 mg + 5 mg + 5 mg/day) with hyperpigmentation improvement. No other hypoglycemic episodes were registered and there was the need to increase total insulin dose. Two weeks after the beginning of treatment serum ACTH was 238.6 ng/l and cortisol 2.9 µg/dl. Seven months after de beginning of treatment she was steel clinically well.

**Conclusion**

Autoimmune polyglandular syndrome type II is not a common disease, but it has life threatening consequences when the diagnosis is overlooked. It is defined by

the combination of chronic autoimmune adrenal insufficiency with autoimmune thyroid disease, type 1 autoimmune diabetes mellitus, or both. In patients with type 1 diabetes, a sudden drop in insulin requirement point out to early adrenal insufficiency.

## P655

### Impact of two novel mutations of calcium sensing receptor (*CaSR*) gene on calcium metabolism: two clinical case reports

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Disorders of calcium metabolism arise in a familial or sporadic setting. The resulting hypo- or hypercalcemia can induce serious clinical features. Calcium sensing receptor (*CaSR*) plays a key role in maintaining this balance and its molecular investigation is useful to determine the nature of a certain condition or to choose the appropriate therapeutic approach.

In the present study we reported two clinical cases. A 16-year-old patient had a mild hypercalcemia associated with normal-to-low urinary calcium excretion and normal-to-high parathyroid hormone (PTH) levels. Because of family negative phenotype, familial hypocalciuric hypercalcemia was originally excluded and bilateral inferior parathyroidectomy was realized. Conversely, a 54-year-old man showed a symptomatic hypocalcemia, low PTH levels, hyperphosphatemia and slight hypercalciuria. The evaluation of all family members revealed the same phenotype in the sister. The whole coding region of the *CaSR* gene of both probands and their first-degree relatives was sequenced. In the first patient a novel double mutation predicting a change from alanine to lysine was found in codon 423 (A423K) of exon 4, with genetic analysis being negative in the parents. The latter patient showed a heterozygous novel missense mutation resulting in a change from glutamic acid to lysine in codon 556 (E556K) of exon 6, his sister also being positive. In conclusion, we reported two novel mutations of the *CaSR* gene, a double inactivating mutation in codon 4 and the first activating mutation in codon 6. This study demonstrates the importance of genetic testing of *CaSR* to assist in management and therapeutic decision.

## Developmental endocrinology

### P656

#### Comparative levels of serum/plasma concentrations of hormones in Juvenile periodontitis patients of Pakistan

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Gums inflammation (gingivitis) if not treated on time and permitted to grow, ends into periodontitis in which alveolar bone that supports teeth is invaded and destroyed. Juvenile periodontitis is a genetic disorder and can occur without plaque and calculus. It is a disease of people below 30 years of age.

Subjects and methods

This cross sectional study involved a total of 160 subjects including 80 chronic periodontitis patients and 80 healthy controls between the ages of 18–25 years. A detailed personal history, past medical and dental history was taken. Patients of chronic periodontitis were selected using community periodontal index of treatment needs (CPTN).

Blood sample were drawn and serum/plasma concentrations of LH, GH, cortisol, triiodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>), TSH, ADH, parathyroid hormone (PTH), aldosterone, melatonin, calcitonin, insulin, cortisol, epinephrine, norepinephrine, estrogen, progesterone, testosterone, thymosins were measured by using standard ELISA method.

Results

The results of this study showed that serum/plasma concentrations of all the hormones were statistically significantly different in patients of Juvenile periodontitis.

The correlation between them and severity of Juvenile periodontitis was also evaluated and a highly significant positive correlation with the severity of periodontitis was found.

Conclusions

Under the influence of Juvenile periodontitis in otherwise healthy young population, the probability of being at hormonal imbalance had appreciably augmented.

## P657

### Zilpaterol hydrochloride (a $\beta$ -agonist) improve growth performance and carcass quality in castrated goat

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The aim of this study was to investigate the effect of Zilpaterol hydrochloride (a  $\beta$ -agonist) on growth performance and carcass quality in goats. 14 Mahabadi castrated male goats with 6 months old were selected and assigned in two groups. The animals in both groups were fed with finishing diet (2.35 Mcal ME/kg and 15.23% CP). The goats in treated group were received 0.2 mg/kg per live body Zilpaterol hydrochloride (Zilmax, Intervet South Africa) for 30 days with 3 days as withdrawn period. Data were analyzed by proc GLM in SAS. Zilpaterol hydrochloride decreased dry matter intake ( $P < 0.05$ ) while increased average daily gain ( $P < 0.05$ ) and total live body weight ( $P < 0.05$ ) at the end of feedlot period. As a result, this  $\beta$  agonist caused to improve feed efficiency. Zilmax increased hot carcass weight ( $P < 0.05$ ) and longissimus muscle area (LM) ( $P < 0.05$ ) and decreased back fat thickness ( $P < 0.05$ ). Carcass dressing percentage tended to increase ( $P = 0.064$ ) in treated group. Results suggest that Zilpaterol hydrochloride could improve feedlot performance and carcass quality in castrated goats.

## P658

### Disorders of sex development: current scenario in North East India

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Introduction

The understanding and approach to management of DSDs has undergone rapid changes in recent years with focus on early diagnosis followed by disclosure and decision making with family members. However in certain regions specially in developing countries, lack of awareness and the social environment contribute to late diagnosis and problems in management. The present study was done to assess the clinical presentation of DSDs attending an endocrine clinic at a tertiary care centre in North East India.

Methods

Retrospective analysis of patients with DSD presenting to the Endocrinology Department of Gauhati Medical College over a period of 5 years from Jan 2006 to Dec 2010. The presenting features, age at presentation, sex of rearing and any associated unusual features were recorded. The patients had been subjected to clinical examination, relevant hormonal estimation, imaging procedures and karyotype. Genetic studies for specific defects were not done due to lack of facilities.

Results

There were a total of 58 patients with DSD. Of these 33 were sex chromosome DSD (19 Turner, 14 Klinefelter). The age of presentation of Turner's ranged from 14 to 40 years with failure to attain puberty being the major reason for consultation. In patients with Klinefelter's age at presentation ranged from 17 to 30 years. There were 10 patients with 46XX DSD (simple virilizing 21 OH def in 3, pure gonadal dysgenesis in 3 and mullerian agenesis in 4). 46XY DSD was seen in 15 patients (3 partial androgen insensitivity, 1 complete gonadal dysgenesis and isolated hypospadias in 11).

Conclusion

Relatively late presentation of Turner pts seen due to lack of awareness. The small number of CAH pts may be due to social taboos or low incidence of consanguinity in this region.

## P659

### Cost-effectiveness analysis of conservative treatment strategy in patients with diabetic foot in Moscow

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Introduction

Recently there has been a steady increase in direct costs for treatment of patients with diabetic foot. Therefore, cost-effectiveness analysis of different treatment strategies of patients is important for healthcare organizations and is an additional argument when deciding on the allocation of financial resources.

Aim

A comparative analysis of cost-effectiveness of non-specialized and specialized medical care for patients with diabetic foot.

Research design and methods

In a retrospective study included medical documents of 89 patients with diabetic foot who were treated in specialized (40 patients) and non-specialized (49) medical facilities (inpatient and outpatient) in 2007. Neuropathic plantar foot ulcers 1A,2A,1B,2B,3B classes (University of Texas Diabetic Foot Wound Classification System) were determined in all of them. Patients in the comparison groups were comparable in age, sex, type and duration of diabetes ( $P > 0.05$ ). Was assessed direct costs and cost-effectiveness analysis in a group of outpatient and inpatient treatment. We have analyzed the effectiveness of non-specialized and specialized treatment (healing time, recurrence rate of diabetic foot at 1 year after healing) and calculated of direct costs and cost-effectiveness analysis in a groups. We calculated only the direct costs, which included the cost of hospitalization, TCC or half-shoe, medical and nursing time, diagnostic evaluation (bacteriological study, X-ray, and other), dressing materials, antimicrobial treatment, antiseptic solutions.

Results

Cost-effectiveness analysis confirmed that the outpatient specialized care compared with non-specialized more efficiently with both clinical and economic points of view. Economic analysis has shown that a specialized hospital care compared with non-specialized more cost-effective.

Conclusions

Our study shows that specialized outpatient and inpatient care for patients with diabetic foot is most advantageous.

## Diabetes therapy

### P660

#### Method of myocardial preconditioning during exercise tests in patients with type 2 diabetes mellitus after acute myocardial infarction

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Patients type 2 diabetes mellitus and MI need multimodality treatment including, besides of drug therapy, non-drug approaches. Repeated Treadmill exercise test is open second window of preconditioning. Ischemic preconditioning limits infarct size in the heart, increase level of physical activity.

Study aim

To study the effect of early started repeated Treadmill exercise tests in patients with type 2 diabetes mellitus and MI.

Design

Open, randomized study on 124 MI and type 2 diabetes mellitus patients, 52.3 ± 3.6 years old. Patients were on 5–7 day of MI and received a basic cardiovascular and dietary therapy with ACEI, aspirin, β-blockers and statins and were randomized to two treatment groups (62 patients in each) at 1:1 ratio. Group 1 (control (CG)) – received a standard drug and dietary therapy and standard physical rehabilitations; group 2 (training (TG)) – received a standard drug and dietary therapy and 10 repeated exercise tests twice a day. The follow-up period was 12 months.

Study results

Characteristics ST depression did not differ significantly in both groups at the beginning. However after 10 days in TG durations of the ST depression significantly changed. Times for onset of ischemic changes ( $228 \pm 94$  vs  $365 \pm 103$  s,  $P = 0.01$ ) and appearance of angina ( $282 \pm 153$  vs  $428 \pm 177$  s,  $P = 0.04$ ) were observed. In CG ST depression characteristics were not significantly changed ( $238 \pm 96$  vs  $265 \pm 99$  s) and appearance of angina ( $276 \pm 148$  vs  $293 \pm 153$  s) were observed.

Physical activity tolerance were increase significantly in TG (42%), and increase not significantly in CG (8%).

Patients in TG had significantly less duration hospitalizations  $15.1 \pm 2.3$  and  $18.6 \pm 2.2$  days in the comparison group.

Total of 48 hospitalizations occurred during the follow-up period, 15 in the TG and 33 in the CG. Training group patients had significantly less hospitalizations due to cardiovascular cause (7.5 vs 37%).

Conclusion

Repeated exercise tests in patients with type 2 diabetes mellitus and myocardial infarction can be started in their acute period. It decrease myocardial ischemia, decrease duration of hospitalizations and number of hospitalizations and cardiovascular events during 12 month after MI.

### P661

#### Effect of amino acid residues on N-terminal extension of GLP-1/IgG-Fc fusion protein

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone playing an important role in glucose-dependent insulin secretion and β-cell growth. However, GLP-1 is rapidly degraded by dipeptidyl peptidase IV (DPP-IV) *in vivo*. Many attempts have been made to develop long-acting GLP-1 analogs with chemical modification, amino acid substitution, and fusion protein technology. We previously showed that the N-terminal Ala- or Gly-extended GLP-1 and human immunoglobulin gamma heavy chain (IgG-Fc) fusion protein is resistant to DPP-IV *in vitro* and induces expression of insulin receptor substrate-2 in INS-1 cells (*Bull. Korean Chem. Soc.* 2009 **30** 2471–2474). In this study, we substituted a polar, aliphatic, charged, or aromatic amino acid (Ser, Leu, Lys, Asp, or Phe) for Ala or Gly, which was tethered on the N-terminus of GLP-1/IgG-Fc fusion protein, and compared the effects on the GLP-1 receptor-mediated signaling in INS-1 cells, *in vivo* half-life, and blood glucose level. The fusion proteins expressed in CHO-K1 cells were secreted into medium and purified by Protein A affinity chromatography. Our data show that all of the N-terminal-extended GLP-1/IgG-Fc fusion proteins by a single amino acid are resistant to DPP-IV. We will discuss the use of N-terminal-extended GLP-1/IgG-Fc fusion proteins for the treatment of insulin-resistant type 2 diabetes.

### P662

#### Evaluation of the global impact of incorporating a telemedicine system for type 1 diabetes patients with an insulin pump and real-time continuous glucose monitoring system

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Introduction

Telehealth systems have become an alternative method to control chronic illnesses like diabetes. The aim of this study was to evaluate a telehealth system in patients with type 1 diabetes who have an optimized treatment with an insulin pump and real-time continuous glucose monitoring system.

Research design and methods

Prospective, 1-year study of 15 patients with a continuous insulin infusion system and a real time glucose sensor. Three medical visits took place: prebaseline, baseline and at 6 months. Each month the patients sent by telecare platform information about glucose meter, glucose sensor and insulin pump. We adjusted the treatment and returned the information by e-mail. We evaluated psychological and metabolic variables, including HbA1c, hypoglycemia, hyperglycemia, number of controls, glucose variability: s.d., mean amplitude glycemic excursions (MAGE), mean of daily difference (MODD); low blood glucose index (LBGI), high blood glucose index (HBGI), blood glucose risk index (BGRi), GRADE and 8-isoprostanoid F2 α in 24-h urine samples.

Results

The mean age of patients at the start was  $40.46 \pm 8.40$  years and the mean evolution of diabetes of  $21.92 \pm 6.26$  years. There was a reduction in HbA1c ( $7.50 \pm 0.49$  vs  $6.97 \pm 0.59$ ;  $P < 0.05$ ), an increase in the number of self-monitoring blood glucose controls per day ( $5.19 \pm 1.97$  vs  $6.18 \pm 1.97$ ;  $P = 0.034$ ), and an improvement in variability: MODD ( $67.3 \pm 44.40$  vs  $53.1 \pm 40.69$ ;  $P < 0.05$ ) and MAGE ( $136.3 \pm 44.40$  vs  $102.4 \pm 40.69$ ;  $P < 0.05$ ). Psychological results: improvement in quality of life ( $92.38 \pm 17.46$  vs  $86.92 \pm 18.07$ ;  $P = 0.012$ ), satisfaction with the treatment ( $34.2 \pm 6.4$  vs  $31.5 \pm 7.5$ ;  $P = 0.02$ ) and less fear of hypoglycemia ( $35.6 \pm 10.1$  vs  $31.5 \pm 10.9$ ;  $P = 0.018$ ).

Conclusion

Telemedicine resulted in an improvement in metabolic control, glucose variability and quality of life of patients with type 1 diabetes treated with sensor and insulin pump.

**P663****Evaluation of adolescents type 1 diabetic patients after transition from paediatric to adult care**

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**Objective**

Our objective in this study was to identify the type of clinical care received by young type 1 diabetic patients who have made the transition from paediatric to adult care, and to assess the metabolic status of the patients. The research aimed to develop a sustainable and coordinated approach to facilitating the transition between diabetes services for adolescents and reveal from the perspective of the adolescents living with type 1 diabetes their experiences surrounding the process of transition.

**Material and methods**

We evaluate all the patients transferred to our adult unit during the last year. 23 type 1 diabetic patients were analysed. A questionnaire was used to evaluate the opinion of the patients concerning the transitional process.

**Results**

Twenty-three type 1 diabetic patients (63%F/36%M) with mean evolution of diabetes 9.5 years (6–14 years). Mean BMI was 23.68 kg/m<sup>2</sup>, having 44.4% of the patients BMI higher than 25 kg/m<sup>2</sup>. Mean HbA1c was 7.58% (6.5–8.6%). The patients were treated with MDI (18.18% with NPH and rapid insulin and 81.8% with glargina/Detemir and rapid insulin). 9% had incipient nephropathy. No other chronic complications were found. The patients were quite satisfied with the transitional process.

**Conclusions**

Transition marks a critical phase for young, diabetic patients as they may frequently switch from one physician or centre to another. The individual optimization of therapy, established during paediatric care, provides the decisive groundwork for disease control in young adults. It's important to prepare, coordinate and evaluate transitional processes between paediatric and adult units.

**P664****Clinical effectiveness of a bolus calculator in patients with type 1 diabetes mellitus treated with insulin pump**

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**Objective**

Calculation of accurate insulin boluses is one of the major problems related to intensive insulin regimens. A bolus calculator (BC) incorporated into the insulin pump estimates the dose of insulin to be administered at the meal and makes calculation easier and more precise.

The aim of the present study was to assess the efficacy of a BC on glycaemic control of patients with DM1 on CSII.

**Material and methods**

We enrolled 20 DM1 patients >18 y and treated for >12 months with CSII (Minimed 722, Medtronic). They received an infrared-linked glucometer (Contour Bayer), being glycemic values directly transmitted to the pump to be used by the BC, with possibility to download all the recorded data.

Data evaluated baseline and after 3 months using the BC were: HbA1c, daily insulin dose (Basal and bolus), number of bolus/day and acute complications. Glycemic liability was evaluated using MAGE and SD from SMBG information, and quality of life (DQOL). A satisfaction questionnaire was also evaluated.

**Results**

After 3 months, significant changes were observed ( $P < 0.05$ ) in weight (71.8 ± 8 vs 72.9 kg) and metabolic control (HbA1c 7.7 ± 1.1 vs 7.5 ± 1%), with no differences found in parameters related to glycemic liability. Patients were satisfied with the BC and found this option easy to use and accurate.

**Conclusions**

In a group of patients treated with CSII, the addition of a BC achieved an improvement in glycemic control with decrease of HbA1c levels and weight, confirming the patients a high level of satisfaction.

**P665****Evaluation of long term outcomes of intensive treatment with s.c. insulin infusion (CSII) in type 1 diabetic patients**

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**Introduction**

Several studies have shown that treatment with insulin pump is an effective therapy for selected type 1 diabetes. Nevertheless, most of them analyse results in short period of time. The objective of this study is to evaluate long term effects on quality of life (DQOL) and metabolic control of CSII.

**Material and methods**

We did a longitudinal study of type 1 diabetic patients previously treated with multiple daily insulin (MDI) therapy who initiated insulin pump and maintained it for longer than 3 years. Initially we recruited 105 patients and after we excluded pregnant and patients using combined sensor-pump systems. Finally we analysed in 69 patients: diabetes duration, complications, cause of initiating CSII, total daily insulin requirements, hypoglycaemias, HbA1c, DQOL, physical evaluation, number of controls and number of medical visits.

**Results**

The criteria for CSII initiation were: 21.9% hypoglycaemic events, 37.5% poor metabolic control and 40.6% glycemic liability. After 3 years, 79.64% continue using insulin pump, 12.2% dropped out and 8.16% withdrawn following medical reasons.

	Basal	6 month	12 month	24 month	36 month
HbA1c	8.11 ± 2.2	7.6 ± 1.3*	7.8 ± 0.9*	7.2 ± 2.6*	8.2 ± 0.97
Severe hypoglycemic events/year	0.43	0.06	0	0	0.03
Hypoglycemic events/week	4.5 ± 3	3.3 ± 2.8	2.1 ± 1.7*	2.8 ± 2.2*	2.7 ± 1.6
Ketoacidosis with hospitalary admission	0	0	0	0	0
Sensibility factor	37.6 ± 17.8	45.3 ± 19.04	40.7 ± 18.9	38.8 ± 18.4	41.3 ± 10.3
CHRatio	1.05 ± 0.5	0.88 ± 0.44	0.95 ± 0.46	0.97 ± 0.44	1.03 ± 0.29
Autoanalysis/day	3.6 ± 2.5	3.1 ± 1.99	3.8 ± 1.9	3.7 ± 2.2	3.2 ± 1.4
Visits/month	–	0.62 ± 0.22	0.28 ± 0.14	0.30 ± 0.11	0.30 ± 0.11
DQOL	92 ± 16.15	80.82 ± 19.2*	82.12 ± 19.1*	82.5 ± 14.0*	82.5 ± 14.0*

Long term evaluation of CSII. \* $P < 0.05$ .

**Conclusions**

CSII achieve a good metabolic control during the first 2 years and an improvement in DQOL that is maintained during all the evaluated period of time.

**P666****Experience with the use of a real time continuous glucose monitoring system during the simultaneous pancreas kidney transplant in patients with diabetes**

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**Introduction**

Simultaneous pancreas kidney transplant (SPK) is a prime line treatment option in patients with type 1 diabetes and chronic kidney failure. The glucose control during perioperative period can be a prognostic factor for transplant. Actually, the utility of the continuous glucose monitoring is not well defined. Our objective was to study the utility of a continuous glucose monitoring system during the simultaneous pancreas kidney transplant.

**Material and methods**

We installed a real time continuous glucose monitoring system (Guardian, Medtronic) to patients transplanted the last 2 years in Carlos Haya Hospital. The system was maintained since 6–10 h before until 10–15 days after transplant. Capillary glucose controls were done (2–4/day) to calibrate the system. Alarms were programmed for hypo and hyperglycemia. The data apored by the system were used for the perioperative glucose control and afterwards were discharged using carelink-pro1 program.

**Results**

Twenty patients received a simultaneous pancreas–kidney transplantation. The medium age was 38.08 (29–53) years and diabetes duration was 21.66 (17–28) years. 83.3% of the patients were in hemodialysis before the transplant (medium of time, 15.1 months). During the monitoring period, mean of capillar glucose was  $127.9 \pm 34.8$  mg/dl (median 121 mg/dl). Interstitial mean glucose from sensor was  $127.3 \pm 32.2$  mg/dl (median 124 mg/dl). A good correlation between capillar and interstitial glucose was observed ( $R=0.93$ ,  $P<0.05$ ). AUC below 70 was  $0.12 \pm 0.2$  and AUC below 140 was  $9.9 \pm 10.2$ .

Medical staff in operating room, intensive care and nephrology departments displayed good acceptance and satisfaction with the system. Principal advantages were: avoid doing capillar glycemia hourly and prevent hyper/hypoglycemia. Alarms played an important role in detecting hyperglycemia in these patients for an intensive early treatment.

**Conclusions**

Real time continuous monitoring could be an effective and useful tool for intensive glycemic control during peritransplant period with good correlation with capillar glucose data.

**P667****Unacylated ghrelin improves insulin sensitivity in the early phase of obesity**

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In Europe more than 50 million adults are diabetic. Of these, 90–95% have type 2 diabetes (T2D). It is also recognized that an even larger population is insulin resistant and it is thought that treatment at this early phase of the metabolic syndrome may prevent the occurrence of overt diabetes and associated pathologies. We are in the process of developing unacylated ghrelin (UAG) analogues as therapeutics for T2D. Towards this goal, we have used a mouse protocol in which we pretreat mice with the analogues, then initiate a short-term high fat diet (HFD) to induce insulin resistance but not diabetes. Twelve week old C57BL/6 mice ( $n=10$ ) were fed either normal chow (12% kcal from fat), or a HFD consisting of 41% kcal from fat, for 2 weeks. We assessed animal weight, food intake, fat mass, fed and fasting plasma glucose concentrations, and glucose tolerance (glucose tolerance tests) during the study, then insulin sensitivity (insulin tolerance tests, ITT) at the end of the study. Body weight was significantly increased by the HFD during the study period, and fat mass was markedly increased by ~2.5-fold compared with animals on control diets. This occurred despite a decrease in food intake in this group. The animals on the HFD became glucose intolerant, and insulin resistant. Infusion of UAG and analogues had no consistent effects on glycemia or glucose tolerance in this short-term model. Importantly, though, insulin sensitivity was significantly improved, as assessed by ITT, in agreement with our preliminary data in obese diabetic *ob/ob* mice. UAG agonists may be of use as therapeutics in the treatment of insulin resistance in metabolic syndrome.

**P668****Antihyperglycemic action of creatine compared to glibenclamide in type II diabetic patients**

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Possible involvement of creatine in the regulation of glycaemia in diabetic patients has been implicated.

The aim of this study was to investigate the short-term effect of creatine on the glycaemic profile during standard meal in comparison to the oral sulfonylurea hypoglycaemic agent glibenclamide, in type II diabetic patients.

In a 14-day symmetrically randomized crossover trial, recently detected type II diabetic patients received either creatine (3 g) or glibenclamide (3.5 mg) for five successive days, followed by two days of washout, and crossover to the opposite treatment. Glucose, insulin, c-peptide, and creatine were measured before, and at 60, 90, 120, 180 and 240 min after a standard meal.

Both creatine and glibenclamide decreased glucose concentrations versus basal glucose (–15, 60, 90, 120, 180, and 240 min; mmol/l):  $12.6 \pm 0.83$  vs  $12.2 \pm 0.56$  vs  $12.1 \pm 0.57$ ,  $15.3 \pm 0.63$  vs  $13.5 \pm 0.70^a$  vs  $13.0 \pm 0.57^a$ ,  $14.8 \pm 0.57$  vs  $13.8 \pm 0.59^a$  vs  $13.4 \pm 0.46^a$ ,  $14.6 \pm 0.61$  vs  $12.3 \pm 0.49^b$  vs  $12.4 \pm 0.61^a$ ,  $12.8 \pm 0.76$  vs  $10.0 \pm 0.40^c$  vs  $10.3 \pm 0.41^c$ , and  $11.4 \pm 0.67$  vs  $8.3 \pm 0.40^c$  vs  $8.5 \pm 0.36^c$  ( $^aP<0.05$ ;  $^bP<0.01$ ;  $^cP<0.001$  versus basal glucose). Treatment with both creatine and glibenclamide increased insulin and c-peptide concentrations after 120 and 240 min ( $P<0.05$  and  $P<0.01$ ).

Short-term peroral administration of creatine and glibenclamide, at the applied doses, elicits similar glucose lowering action. Relative efficacy of creatine in comparison to glibenclamide remains to be established.

**P669****Impact of a DPP4-inhibitor on liver and heart lipid content and cardiovascular risk in type 2 diabetic patients**

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**Background**

Increased liver and cardiac fat are common in patients with type 2 diabetes mellitus (T2DM) and associated with increased risk for liver fibrosis and cardiovascular events. The effect of a DPP-4 inhibitor vildagliptin on the fat content of the liver and heart has not been evaluated.

**Methods**

A total of 40 patients, 20 males and 20 females, treated at our diabetes metabolic unit of the Medical University of Vienna with gliptins underwent magnetic resonance spectroscopy before and 6 months after start of therapy with vildagliptin twice a day 50 mg as standard add on to metformin. MRT was performed with 3 Tesla Siemens MRT.

**Results**

A total of 40 patients have been included, 10 patients have finished the study (5 males, 5 females). The mean age was  $55.9 \pm 7.2$ , weight  $88.3 \pm 17$ , BMI  $31.6 \pm 5.1$  kg/m<sup>2</sup> and duration of diabetes  $6 \pm 4$  years. After 6 months mean weight loss was  $5.1 \pm 2.3$  kg and  $-7 \pm 3$  cm in waist circumference. Systolic and diastolic RR improved significantly:  $151.3 \pm 2.6/94.2 \pm 10$  to  $122 \pm 14.4/80.1 \pm 8.9$  mmHg ( $P=0.04$ ,  $P=0.05$ ) respectively. HbA1c sunk significantly ( $8.3 \pm 1.5$  to  $6.5 \pm 0.4\%$ ,  $P=0.004$ ). Before therapy patients had increased liver fat  $15.3 \pm 9.1\%$ , women significantly higher than men ( $30.2 \pm 2.5$  vs  $15.3 \pm 3.5$ ,  $P<0.05$ ) which significantly decreased in the 10 finished patients to  $8.8 \pm 4.1$ ,  $P=0.0004$ , followed by a decrease of cardiac fat of  $1.4 \pm 0.4\%$ . The cardiac ejection fraction greatly improved ( $51.4 \pm 2.3$  to  $67.1 \pm 0.5\%$ ,  $P<0.0001$  as well as the myocardial mass decreased for  $10.1 \pm 2.4$  g.

**Conclusion**

Vildagliptin is very well tolerated and leads already after 6 months of therapy to significant decrease of liver and cardiac fat, improves the heart function, blood pressure and leads to weight loss where most of the other oral antidiabetics fail. These preliminary data could indicate vildagliptin as first line therapy of fatty liver with a special focus on cardiovascular function improvement.

**P670****Self-monitoring of blood glucose in type 2 diabetes mellitus: effect on glycaemic control in non-insulin-treated patients**

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**Introduction**

The efficacy of self monitoring of blood glucose (SBMG) on glycaemic control in non-insulin-treated (NIT) type-2-diabetes-mellitus (T2DM) is still controversial. In this paper we evaluated the influence of SBMG on glycaemic control in patients with T2DM with poor glycaemic control treated with oral antidiabetic agents.

**Methods**

Thirty NIT-T2DM patients with poor glycaemic control and HbA1c > 7% under treatment with oral hypoglycaemic agents attending our Diabetes Clinic were

randomized as follows: *SMBG Group* (patients currently using SBMG without a previous training to interpret the result); *Control Group* (patients who were asked to increase the dosage at visit 1 for a better control of diabetes). The patients were evaluated after 3 and 6 months and HbA1c and fasting plasma glucose (FPG) were monitored. 15 subjects (7 females and 8 males) aged  $65 \pm 7.7$  years were randomized in the SMBG group and 15 (6 females and 9 males) aged  $62.7 \pm 9.7$  years in the control group.

#### Results

HbA1c levels significantly dropped after 3 ( $7.7 \pm 0.7\%$ ) and 6 ( $7.5 \pm 0.7\%$ ) months from baseline ( $8.4 \pm 0.6\%$ ) in the SMBG Group ( $P < 0.001$ ); HbA1c levels significantly dropped after 3 ( $8.0 \pm 1.1\%$ ) and 6 ( $7.7 \pm 1.0\%$ ) months from baseline ( $8.5 \pm 1.0$ ) also in the control group ( $P < 0.05$ ) but without a significant difference between the two Groups when compared at the same time (3 or 6 months) of control. The FPG progressively decreased in both Groups in a significant way from baseline in SMBG group just after 3 months, while in the control group 6 months were necessary for reaching significance; no significant difference between the two groups was found when compared each other at 3 and 6 months.

#### Conclusions

The SMBG is as effective as increasing oral antidiabetic therapy dosage in improving the glycaemic control in NIT-T2DM subjects with previous poor glycaemic control even without a detailed training for interpreting the results.

## P671

### Insulin resistance of multiple metabolic pathways in patients with type 1 diabetes on long term continuous s.c. insulin infusion therapy

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#### Objective

The aim of the present study was to determine whether insulin resistance is important in well controlled, uncomplicated patients with type 1 diabetes on long term CSII, compared to matched healthy controls.

#### Research design and methods

We studied 8 patients (4 men and 4 women) with type 1 diabetes on stable continuous subcutaneous insulin pump therapy and 8 healthy controls, matched for age, gender and BMI. Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp studies with infusion of  $[6,6\text{-}^2\text{H}_2]$  glucose.

#### Results

Endogenous glucose production (EGP) did not differ in the basal state between patients and controls. However, EGP was less suppressed during clamp conditions in patients compared to controls (64 vs 79%,  $P = 0.01$ ), indicating decreased hepatic insulin sensitivity. During the clamp, glucose disposal rate was  $\sim 38\%$  lower in patients compared to controls ( $24.4 \pm 2.5$  vs  $39.7 \pm 5.6 \mu\text{mol.kgLBM}^{-1}.\text{min}^{-1}$ ,  $P = 0.04$ ). Accordingly, the rate of infusion of glucose was  $\sim 51\%$  lower in patients ( $17.7 \pm 2.8$  vs  $39.7 \pm 5.7 \mu\text{mol.kgLBM}^{-1}.\text{min}^{-1}$ ,  $P = 0.02$ ). Finally, NEFA levels were  $\sim 2.5$  times higher in patients during steady state clamp conditions ( $150 \pm 26$  vs  $58 \pm 4 \text{ pmol/l}$ ,  $P = 0.01$ ), reflecting decreased insulin sensitivity of lipolysis.

#### Conclusions

Insulin resistance is a prominent feature of patients with type 1 diabetes on long term and stable treatment with CSII. Insulin resistance involves multiple unrelated metabolic pathways in these patients.

## P672

### Factors related with the adherence of physicians to diabetes guidelines in type 2 DM patients in Turkey

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#### Background

Guidelines for management of diabetes mellitus (DM) provide standardization of care and treatment. National clinical practice guidelines on DM have been

developed in 2006 by The Society of Endocrinology and Metabolism of Turkey and reviewed annually.

#### Methods

One hundred and eighty physicians evaluated previous 12 months' medical records of their type 2 DM patients with special emphasis on whether the patients were followed consistently with national guidelines. This report depends on the analysis of 6032 visits of 1790 patients. Adherence to guidelines was scored to reach a total adherence score of 0–10 for three dimensions of adherence (taking medical history (HISTORY), physical examination (PHY\_EXAM) and laboratory evaluation (LAB\_EVAL)). Total adherence score was calculated by multiplying the mean of HISTORY, PHY\_EXAM and LAB\_EVAL scores by 10 (range: 0–100).

#### Results

Mean age was  $58.7 \pm 10.9$  years and 62% were women. The mean duration of DM was  $7.7 \pm 7.5$  years. Total adherence score didn't show any relationship with gender and age, but LAB\_EVAL score was better in male patients (6.84 vs 6.53 out of 10;  $P = 0.021$ ) and differed between age groups (40–49 years was the group with the lowest general adherence score). All of the adherence dimensions were related with duration of DM; for 0–5 years of DM, total adherence score and HISTORY, PHY\_EXAM and LAB\_EVAL scores were significantly lower than groups with longer duration of DM (all  $P < 0.05$ ). PHY\_EXAM, LAB\_EVAL and total adherence scores were better in patients with chronic complications (all  $P < 0.05$ ). PHY\_EXAM and LAB\_EVAL scores were significantly higher in university hospitals versus state hospitals ( $P < 0.05$ ). With the mean score of 84.5, total adherence of endocrinologists was significantly better than of both family physicians and internists ( $P < 0.005$ ).

#### Comment

We suggest that in order to overcome barriers to increase the adherence to guidelines, factors related with patients and physicians should be studied and understood well.

## P673

### Effects of estradiol + drospirenone on sexual function in postmenopausal women with type 2 diabetes mellitus

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#### Introduction

Sexual dysfunction is more prevalent in postmenopausal women with diabetes mellitus.

#### Objective

The aim of the study was to prospectively evaluate the effects of oral estradiol + drospirenone on sexual function in postmenopausal women with type 2 diabetes mellitus.

#### Methods

The study included 86 postmenopausal women with type 2 diabetes mellitus who were randomized into two groups: 44 women received oral 17 $\beta$ -estradiol + drospirenone daily and 42 women received no treatment as served as a control group. Sexual function was evaluated with a detailed 34-item questionnaire, a sexual function questionnaire.

#### Results

After 6 months a high probability of sexual dysfunction was present in 26.19 vs 45.2% before the treatment ( $P < 0.001$ ). There were no significant changes in the control group. The highest improvement was achieved in libido score: from  $12.5 \pm 7.3$  to  $18.7 \pm 5.67$  in hormonal therapy group and decreased from  $13.2 \pm 8.5$  to  $5.7 \pm 6.43$  in the control group ( $P = 0.000$  and  $P = 0.000$ , respectively). There was no association between probability of sexual dysfunction and HbA1c.

Conclusions: Hormonal therapy provided significant improvement in sexual function in postmenopausal women with type 2 diabetes mellitus.

Keywords: sexual function, diabetes mellitus, postmenopausal women.



## P674

### Effect of sitagliptin monotherapy on serum total ghrelin levels in type 2 diabetic patients

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#### Background

Sitagliptin, unlike the some major antihyperglycaemic drugs, is not associated with weight gain and has neutral effects on body weight. It is unclear whether sitagliptin treatment alters serum ghrelin levels in people with type 2 diabetes. The aim of the present study was to assess the effect of sitagliptin on serum ghrelin levels in patients with type 2 diabetes mellitus.

#### Methods

Forty-four type 2 diabetic subjects were randomly assigned to receive sitagliptin (100 mg/day;  $n=28$ ) or medical nutrition therapy (MNT) ( $n=16$ ) for 12 weeks. Changes in anthropometric variables, glycemic control, insulin resistance, lipid parameters and total ghrelin levels were evaluated at baseline and following 12 weeks of treatment.

#### Results

Significant decreases in body weight and body mass index were observed over the entire study period in both of the treatment groups. HbA1c and postprandial plasma glucose (PPG) levels were statistically significantly decreased in the sitagliptin group compared with baseline values, while they were unchanged in the MNT group. There was a significant decrease in total ghrelin in sitagliptin group ( $P=0.04$ ) compared with baseline values but not in MNT group ( $P=0.46$ ) at the end of the 12 weeks. Percentage change in total ghrelin levels was not statistically different between the sitagliptin and MNT groups ( $-9.3 \pm 21.8$  vs  $21.4 \pm 53.3$ ,  $P=0.082$ ).

#### Conclusions

In this study of patients with type 2 diabetes, treatment with sitagliptin was associated with a significant decrease in serum ghrelin levels. These results suggest that weight neutral effect of sitagliptin might be associated with the suppression of fasting serum ghrelin levels.

## P675

### UK National Exenatide Guidelines (NICE:CG87) compared to clinical outcomes at a district general hospital's diabetes department

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#### Objectives

The aim of the audit was to determine how our current clinical practice differs from that of the national standard set out in the UK by NICE guidance CG87 in regards to the use of exenatide.

#### Material and methods

We measured weight and HbA1c at baseline and then again at 6 months from the date exenatide was started. The UK NICE guidelines recommends exenatide should only be considered in patients with a BMI  $\geq 35.0$  kg/m<sup>2</sup> with a HbA1c  $\geq 7.5\%$  (adjusted for ethnicity). We analysed data from all patients who had been followed up by the Diabetes Nurse Specialist for at least 6 months from initiating exenatide therapy.

#### Results

Complete data was available for 27 patients. 59% of patients were started on exenatide in a non-EMEA licensed combination with other diabetic drugs. 85% of patients were commenced on exenatide with a HbA1c  $\geq 7.5\%$ , but only 62% of patients had a BMI  $\geq 35.0$  kg/m<sup>2</sup> (adjusted for ethnicity). At 6 month follow up only 22% of patients had both a reduction of at least 1% in HbA1c and a weight loss of at least 3% of initial body weight.

#### Conclusions

According to UK NICE guidance, the target to continue exenatide beyond 6 months will only apply to 22% of our patients (~1 in 5). If targets were to be relaxed, so that exenatide could be continued in patients who achieved EITHER a reduction of at least 1% in HbA1c OR a weight loss of at least 3% of initial body weight then 65% of our patients would have been able to continue exenatide therapy beyond 6 months. These 'relaxed' targets might decrease patient dissatisfaction from discontinuing therapy and provide more freedom to clinicians in order to continue exenatide therapy.

## P676

### Remission of diabetic nephropathy in type 2 diabetic Asian population: role of tight glucose and blood pressure control

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#### Background

Asian has higher prevalence of diabetic nephropathy and end-stage renal disease as compared to Caucasian. No study to date has evaluated whether multifactorial intervention was associated with remission of microalbuminuria in type 2 diabetic Asian population. We evaluated the effect of tightly controlling multiple factors on the remission of diabetic nephropathy in type 2 diabetic Chinese with microalbuminuria.

#### Materials and methods

A total of 587 type 2 diabetic patients with microalbuminuria were collected in a longitudinal cohort study. Cohort members received intensified treatment to meet the following ADA recommended goals: HbA1c  $<7\%$ , SBP  $<130$  mmHg, DBP  $<80$  mmHg, LDL-C  $<100$  mg/dl, TG  $<150$  mg/dl, HDL-C  $>40$  mg/dl for men and  $>50$  mg/dl for women. Remission of microalbuminuria was defined as shift of albumin-creatinine ratio (ACR) from microalbuminuria to normoalbuminuria.

#### Results

During the 4.5 year period, 210 (35.8%) patients achieved remission to normoalbuminuria. A significant association was found between remission of microalbuminuria and the achievement of ADA goals, including HbA1c  $<7\%$  (hazard ratio (HR) = 1.345; 95% confidence interval (CI): 1.010–1.792;  $P=0.04$ ), and systolic blood pressure  $<130$  mmHg (HR = 1.516; 95% CI: 1.100–2.089;  $P=0.01$ ). Intensive SBP control ( $<120$  mmHg) was significantly associated with remission of microalbuminuria (HR = 2.076; 95% CI: 1.347–3.198;  $P<0.001$ ).

#### Conclusion

The remission of diabetic nephropathy could be achieved under multifactorial intervention. Therapeutic focus on remission by tight glycemic and blood pressure control should be considered in Asian population with diabetes and microalbuminuria.

## P677

### Comparative study of combination therapy between metformin plus thiazolidinedione (TZD) and metformin plus DPP-4 inhibitor in type 2 diabetes

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The pathogenesis of type 2 diabetes is explained by increment of insulin resistance and dysfunction of insulin secretion. If improvement of insulin resistance is not adequate in the early stage of diabetes especially in this country with more severe basal insulin secretion defect from the very beginning, diabetes rapidly progress to more aggressive stage with relative insulin deficiency. The most proper method of improving insulin resistance by prescription is metformin from the first and adding TZD class in terms of improvement of insulin resistance when the target of glucose control doesn't reach by metformin alone. The next step when the target of glucose control doesn't reach by improving insulin resistance prescription with metformin and TZD is not certain. Adding the other prescription that has different action or insulin is one of options and it is determined by degree of glucose control, insulin secretory capacity of  $\beta$  cell or economic status of patient in practice. The DPP-4 inhibitors that increase insulin secretion by glucose dependent manner also relieve insulin resistance because they improve first phase insulin secretion defect and prevent late hyperinsulinemia. When we can't reach the target below 7% of HgA1c with metformin plus TZD combination that is best in terms of relieving insulin resistance in early diabetes, switching the TZD to the DPP-4 inhibitors that improve insulin secretory dysfunction can be the next useful step to attain glucose control goal. So we compared the effect of glucose control when we switched the TZD to the DPP-4 inhibitor in patients who can't reach the HgA1c below 7.0% with metformin plus TZD. We also observed the change of insulin resistance index HOMA-IR and  $\beta$  cell function index HOMA- $\beta$ CF between treatments. Total 84 patients were enrolled and mean age was  $53 \pm 11$ . The decrement of HgA1c 6 months after switching was  $0.47 \pm 0.61$ . Homa-IR was improved in 57% and mean change was

$-0.24 \pm 0.97$ . HOMA- $\beta$ CF was increased in 43% and mean change was  $-2.7 \pm 11.6$ . So metformin plus DPP-4 inhibitor can be a good treatment option in improving insulin resistance as well as glucose control as compared to Metformin plus TZD therapy.

## P678

### Insulin analogue management: subjective and objective assessment of life quality in patients previously managed with human insulin

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#### Introduction

By using insulin analogue, it is expected to improve glycemic regulation in poorly controlled type 2 diabetes (t2DM). The aim of the study was to examine the quality of glycemic control and quality of life (QoL) of patients managed with insulin analogues, previously treated with human insulin.

#### Materials and methods

The pilot study included 30 t2DM outpatients, divided into two groups (15 subjects) – the first group was treated with human insulin, while the second group was managed with insulin analogues during at least one year, but previously treated with human insulin. The following parameters were observed: sex, age, occupation, t2DM and insulin use duration, examinees estimation of insulin therapy effects, GHQ<sub>12</sub> score, and HbA1c levels. Obtained data were analyzed by SPSS 17.0.

#### Results

The mean t2DM duration and duration of insulin therapy management was  $5.5 \pm 3.5$  and  $3.5 \pm 3$  years respectively. Twenty (66.6%) subjects assessed overall effects of insulin therapy as grades 3/4 of 5. The mean HbA1c levels were 9.1 and 7.2% respectively ( $t=4.869$ ,  $P<0.01$ ). HbA1c levels and subjective grades of insulin effects negatively correlated ( $\rho=-0.568$ ,  $P<0.01$ ). GHQ<sub>12</sub> score was  $9 \pm 3.5$  and did not statistically differ between groups ( $Z=-1.481$ ,  $P>0.05$ ). GHQ<sub>12</sub> score and subjective grades of insulin-analogue effects negatively correlated ( $\rho=-0.730$ ;  $P<0.01$ ).

#### Conclusion

Insulin analogue improves glycemic control of t2DM patients. The subjective assessment of the insulin therapy effects correlated with the objective QoL parameter – GHQ<sub>12</sub> score.

## P679

### Twelve months exenatide treatment in type 2 diabetes and severe obesity

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#### Aim

To assess the effectiveness, safety and tolerability of Exenatide twice daily treatment of type 2 diabetes and obesity.

#### Material and methods

Data analysis of obese type 2 diabetic patients initiated on Exenatide therapy in our Endocrine clinics who complete 1 year of treatment.

#### Results

We included 42 patients, 30 women (71.4%) and 12 men (28.6%), mean ( $\pm$ s.d.) age  $57 \pm 10$  and  $9 \pm 7$  years diabetes evolution. At baseline, mean HbA1c was  $7.45 \pm 1.4\%$ , and mean weight  $105.8 \pm 16$  kg with body mass index (BMI)  $41.1 \pm 5.7$  kg/m<sup>2</sup>, 57% of them being morbidly obese patients. 95.2% included Metformin in their basal treatment, alone or in combination with others oral antidiabetic drugs. Seven patients were receiving insulin therapy, stopped at the moment of Exenatide initiation. We did not include any modifications in previous lifestyle plans. After 12 months of treatment mean HbA1c reduction was  $0.8 \pm 1.8\%$  with 69% of patients reaching HbA1c  $<7\%$ . Mean weight loss was  $7.6 \pm 8$ ,  $4.5 \pm 5$ ,  $5.9 \pm 7$  and  $7.6 \pm 8$  kg at 3, 6 and 12 months respectively with a BMI reduction of  $2.83 \pm 2.9$  kg/m<sup>2</sup>. All of these differences were statistically significant ( $P<0.01$  Wilcoxon test). We did not find any significant change in blood pressure, lipid profile or renal function. Adverse effects occurred in 31% of patients, mainly mild to moderate gastrointestinal ones that conditioning

Exenatide withdrawal in one patient. Hypoglycaemia was reported in three patients, being all of them on concomitant sulfonylurea treatment.

#### Conclusions

In our experience, Exenatide provides glycemic control and a substantial weight loss that occurs early in treatment being an excellent option in diabetic patients from whom weight gain is a main concern.

## P680

### New opportunity of treatment women with postmenopausal osteoporosis and diabetes mellitus type 2

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#### Purpose of the study

Research dynamic of parameters of mineral metabolism, during 12 months therapy with Strontium ranelate (in dose – 2 g/day) at women with postmenopausal osteoporosis and diabetes type 2 (D2).

#### Materials and methods

Forty-two women (age  $65.9 \pm 3.2$  years) in postmenopausal period with verified DM2 (DM2 longer from 1 to 12 years, all patients have standard on per oral therapy with ant diabetes drug – gliclazide, metformin or rosiglitazone) and postmenopausal osteoporosis (DEXA – T-score less –2.5).

#### Clinical results

11.9% of patients have dyspeptic phenomena (regress was during 1–3 days. Nobody of the patients finish treatment in early stage of treatment). Bone mineral density at DEXA T-score grow on  $21.5 \pm 2.86\%$  ( $P=0.05$ ), lumbar spine L 1 – 16.5%, L2 – a 18.4%, L3 – 23%, femoral neck – 24.87%, Wards – 16.7%, Troch. – 27.3%, Total hip – 23.35% ( $P=0.05$ ).

Calcium, and alkaline phosphates are in normal range, on background of therapy negative dynamic is not revealed. Serum C- terminal peptide cross link before treatment raised at 33%. And after one year treatment with Strontium ranelate was note regress on 33.5% ( $r=0.05$ ).

88.1% of women had pain syndrome (in spine or in the hip), after 2 month of the therapy 2.4% mark its regress, 11.9% after 4 months, 23% after 6 months, 33% after one year treatment of Strontium ranelate. Nobody of the patient had hypoglycemia. Nobody have nonvertebral and vertebral fracture risks.

#### Conclusions

Strontium ranelate, being effective in reducing both nonvertebral and vertebral fracture risks. Strontium ranelate is a new first treatment of postmenopausal osteoporosis and diabetes type 2.

## P681

### Effects of lifestyle changes on incidents of type II diabetes in Tehran adult population

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#### Introduction

Studies show that a change in lifestyle can prevent or delay progression to diabetes in those with glucose intolerance. This study aimed to survey the effects of lifestyle changes on incidents of type II diabetes in Tehran adult population.

#### Materials and methods

This was a community based interventional study. Non-diabetic participants of Tehran Lipid and Glucose Study aged  $\geq 20$  years (6437 in control group and 2931 in intervention group) were followed for a mean duration of 3.5 years. The changes in lifestyle include an improvement in diet, increase in the level of physical activity and quitting of smoking. Mantel-Cox method was used to compare the incidents of diabetes between the groups. The Cox proportional hazard was used to obtain the proportional risk of incidents of diabetes in the intervention group.

#### Results

After an average period of 3.5 years follow up 58% of subjects completed the follow-up examination. The mean age of the population was  $43 \pm 11$  years and there were 41% males. The percentage of increase in weight, waist circumference, serum triglyceride and blood glucose in the intervention group was lower than controls (0.6 vs 3.3% for fasting glucose, 5.4 vs 10.6% for 2-h glucose, 1.2 vs 6.4% for triglyceride, 4.5 vs 5.7% for waist circumference ( $P<0.01$ ) and 2.5 vs 3.2% for weight ( $P<0.01$ )). Incidence of diabetes was 12.2/1000 person-year in

control group and 8.2/1000 person-year for case group. Relative risk of developing diabetes was significantly lower in the intervention groups after adjusting for confounding factors (hazard ratio 0.34, 95% CI: (0.25–0.47),  $P < 0.001$ ). Number needed to treat to prevent one case of diabetes was 25, 5.7, and 6.7 respectively for the whole population, those with impaired glucose tolerance, and those with body mass index  $\geq 25$  kg/m<sup>2</sup>.

#### Conclusion

This study showed that after changes in lifestyle for 3.5 years, there was more than 65% reduction in incidents of diabetes in the Tehranian adult population.

### P682

#### The addition of acipimox in patients with type 2 diabetes with triglyceride level over 150 mg/dl in spite of fenofibrate therapy

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Patients with type 2 diabetes have an increased risk of premature coronary heart disease (CHD). One of the underlying reasons for this increased risk is atherogenic dyslipidemia. Current management strategies focus on the initial use of statin or fibrate therapy (the latter approach indicated in patients with pronounced hypertriglyceridemia).

American Diabetes Association recognizes serum triglycerides as a surrogate for atherogenic triglyceride-rich lipoproteins and suggests a target of  $< 150$  mg/dl. This target goal of TG is often very difficult to achieve in spite of fenofibrate treatment. So we tried to evaluate the effect of combination with acipimox, which is an analog of nicotinic acid. Acipimox is also believed to improve glucose control by enhancing insulin sensitivity unlike the case of nicotinic acid. We selected seventeen patients with type 2 diabetes whose glycemic control was stable for 6 months, but elevated serum TG level  $> 150$  mg/dl with fenofibrate monotherapy. In this study acipimox 250 mg capsule was administered twice daily for 2 months, and we measured lipid profile and glycated hemoglobin, creatine kinase and myoglobin concentration before and after the addition of acipimox.

#### Results

1. One female patient was withdrawn because of myalgia in both thigh muscle after 3 days of acipimox combination treatment, but her serum CK and MB concentration were normal.
2. Sixteen patients were completed, and serum TG level was decreased significantly from  $328.3 \pm 35.29$  to  $192.4 \pm 30.59$  mg/dl, and total cholesterol decreased significantly also. But other lipid parameters were not changed significantly.
3. There were no side-effects such as myositis, rhabdomyolysis and liver dysfunction.

#### Conclusion

This preliminary study showed clearly that in patients with type 2 diabetes who have elevated TG level in spite of fenofibrate monotherapy we can decrease serum TG level significantly with the addition of acipimox.

### P683

#### Monitoring thyroid function in diabetic patients on treatment with glucagon-like peptide 1 analog

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#### Background and aims

Incretin mimetics are a new class of pharmacological agents with antihyperglycemic actions that mimic the actions of incretin hormones originating in the gut, such as glucagon-like peptide (GLP)-1. The thyroid gland exhibit a strong GLP-1 receptor expression in rodents but only low receptor levels in humans. GLP-1 receptors activation stimulates calcitonin secretion and promotes the development of C cell hyperplasia and medullary thyroid cancer in rodents. We have monitored the thyroid function in diabetic patients in treatment with exenatide.

#### Materials and methods

Thirty-three patients with type 2 diabetes mellitus were recruited for this study. The subjects were between 40 and 64 years. Patients were screened initially with a questionnaire detailing their medical history, hereditary or familial medullary thyroid carcinoma. The level of serum TSH, calcium and calcitonin in the blood were measured. Neck ultrasound was performed. The patients followed therapy

24 months with exenatide (10 µg injected s.c. twice daily, 60 min before breakfast or dinner).

#### Results

Baseline characteristics of subjects: 23 patients were not diagnosed with thyroid disease, 8 patients present nontoxic goiter, and 2 patients Hashimoto's thyroiditis. Median value of TSH was  $3.39$  µIU/ml of calcitonin  $8.62 \pm 1.24$  ng/l, of total calcium  $9.08 \pm 0.32$  mg/dl, and of ionized calcium  $4.52 \pm 0.22$  mg/dl. The level of TSH, calcium and calcitonin was similar before and after 24 months with exenatide treatment in patients with type 2 diabetes. The nodules remain stable in size.

#### Conclusion

The clinical study with exenatide has not revealed an increase in calcitonin levels during treatment. The surveillance of thyroid nodules is indicated. The study limit of low numbers of patients, requires further validation.

### P684

#### Impact of structured testing on level of glycemic control

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#### Introduction

Clinical trials have shown that improved glycemic control reduces the incidence and progression of diabetes complications. One of the most important steps in diabetes treatment is to educate patients how to manage self monitoring blood glucose (SMBG) and adjust their life habits with their diabetes. A new diabetes management concept of structured testing is supported by Accu-Chek 360 View.

#### Objectives

Assess the impact of structured testing on glycemic levels and attitudes toward SMBG.

#### Methods

Multicenter, observational study involved 40 patients with diabetes, over 3 months from B&H patients were type 1 and type 2 diabetes with HbA1c level  $> 8.0\%$ , average age of 51.9 with a mean duration of diabetes of 8.2 years. After providing basic information on glycemic control to the patients, the Accu-Chek 360° View form was provided to patients who performed 7-point glucose monitoring for three consecutive days, at baseline, month 1, month 2 and at the end of the study.

#### Results

HbA1c was significantly reduced with 1.7%. The decrease of HbA1c was from 9.7% at baseline to 8.0%, at the end of the study ( $P = 0.001$ ). There were no significant changes in lipid status and systolic and diastolic blood pressure from baseline to study end. Improvement in the average blood glucose level at all 7 times of assessment was achieved. The mean blood glucose values were decreased from 9.6 to 8.1 mmol/l. Moreover structure testing was associated with positive attitudes towards SMBG. The frequency of blood glucose testing increased from 16 times per week to 25 times per week.

#### Conclusions

Structure testing with use of Accu-Check 360 View tool was effective in improving diabetes control. Accu-Check 360 View tool can be used as an educational and motivational tool for strengthening patients empowerment in order to change lifestyle and to improve glucometabolic health.

### P685

#### Insulin therapy of diabetes mellitus type 2 and risk of development cardiovascular mortality

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#### Purpose of work

Research influence of insulin therapy in the form of combine therapy with analogue of prolonged insulin – glargin and per oral anti diabetes treatment with (glibenclamide) on dynamics of risk of development of coronary (CHD) heart disease and death rate from cardiovascular disease (CVD) at diabetes mellitus type 2 (DM 2).

**Materials and methods**

During the study was researched dynamic of risk of development CHD and death rate from CVD on several parameters: atherogenicity index (AI (rate <4.0), relation of total cholesterol (TCh) to LDH (TCh/ LDH (rate ≤5.5), relation LDL/LDH (rate ≤3.5) and index of 10-ctfs fatal risk of development CVD – SCORE (Systemic Coronary Risk Evaluation, rate <1%). Level of compensation of DM 2 valued of parameters of fasting glucose (FPG), postprandial glucose (PPG) and HbA1c. Duration of observation of 6 month.

**Results**

A total of 20 patients (12 female/8 male) 44–71 years old, Middle age  $55 \pm 1.7$  years; duration of disease  $7.4 \pm 1.2$  years) with decompensate DM 2 on background of reception of maximum dose glibenclamid. AI are source made  $4.52 \pm 0.03$ , relation of TCh/ LDH 5.52.

LDL/LDH  $3.67 \pm 0.03$ , SCORE-4% that testified to high risk of development CHD and death rate At addition to treatment of Glargine at 22 O'clock in total dose  $0.40 \pm 0.02$  ED/kg/ 24 h, on background of improvement of carbohydrate metabolism through 6 months – decreased FPG with  $10.5 \pm 0.5$  up to  $6.3 \pm 0.2$  mmol/l ( $P < 0.01$ ), PPG from  $13.6 \pm 0.6$  up to  $7.9 \pm 0.3$  mmol/l ( $P < 0.01$ ) and HbA1c from  $10.1 \pm 0.5\%$  up to  $7.3 \pm 0.2$  (rate 4–6%,  $P < 0.05$ ) was observed positive dynamics of all risk factors: AI has decreased with  $4.52 \pm 0.03$  up to  $3.10 \pm 0.02$  ( $P < 0.02$ ); TCh/LDH from  $5.52 \pm 0.10$  up to  $4.17 \pm 0.21$  ( $P < 0.001$ ); LDL/LDH with  $3.60 \pm 0.07$  up to  $2.70 \pm 0.09$  ( $P < 0.001$ ); SCORE from  $4.0 \pm 0.25$  up to  $1.60 \pm 0.071\%$  ( $P < 0.05$ ).

**Conclusions**

Accordingly to progressive of DM 2 recommended to a thicket and earlier to begin the combined therapy by the prolonged analogs of insulin glargine with sulfonilurea drug that leads to stabilization of diabetes and will protect from progress core decease and decrease in risk of coronary death rate from CVD.

**Diabetes complications****P686****Diabetic retinopathy in type 1 diabetes mellitus patients with depression**

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**Aim**

To define the frequency of diabetic retinopathy course in type1 diabetes mellitus (T1DM) patients with and depression; to study the relation between depression and diabetic retinopathy severity.

**Materials and methods**

One hundred and thirty-three T1DM patients aged  $40.27 \pm 12.17$  years (56 males and 77 females) without exacerbation of chronic and acute inflammatory diseases and verified mental disorders were included into the study. The duration of T1DM before the moment of inclusion into the study was  $12.16 \pm 3.65$  years. The hospital scale of anxiety and depression was used for detection of depression. The depression was estimated at indicators of 8-21 scores. Statistical processing of the material was performed using STATISTICA 6.0.

**Results**

For definition of frequency of development of diabetic retinopathy in patients with depression the study group was divided into subgroups: patients without diabetic retinopathy, patients with non-proliferative diabetic retinopathy, and patients with diabetic preproliferative and proliferative retinopathy. In T1DM patients with depression the frequency of diabetic preproliferative and proliferative retinopathy was 55.5% and was significantly higher than in patients without depression (44.5% ( $\chi^2 = 5.60$ ;  $P = 0.018$ )). There were no differences among subgroups of patients with non-proliferative retinopathy and diabetic preproliferative and proliferative retinopathy ( $\chi^2 = 2.81$ ;  $P = 0.094$ ). For analysis of the frequency of laser coagulation execution there were singled out the following subgroups: T1DM patients with diabetic retinopathy without laser coagulation and with laser coagulation. In patients with depression the frequency of execution of retina laser coagulation is 70% that is higher to compare patients without depression (30%) ( $\chi^2 = 3.90$ ;  $P = 0.048$ ).

**Conclusions**

In T1DM and depression patients the frequency of diabetic retinopathy is higher to compare patients without depression.

**P687****The frequency distribution of PPAR $\gamma$ 2 Pro12Ala polymorphism in type 2 diabetic, cardiac patients and healthy subjects of Bangladeshi population**

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The prevalence of diabetes is increasing worldwide. According to the recent World Health Organization (WHO) estimates, more than 180 million people worldwide have diabetes. This number is likely to be more than the double by 2030. It is estimated that the developing countries will bear the brunt of diabetes epidemics in the 21st century. It is well known now that increased rates of other chronic diseases, such as hypertension, obesity, cardiovascular diseases are highly associated with type 2 diabetes (T2D). Besides other risk factors, genes play a crucial role in progression of type 2 diabetes. In this study, the Pro12Ala polymorphism of PPAR $\gamma$ 2 gene was studied in type2 diabetic, cardiac patients and healthy people of Bangladesh. Here, 62 diabetic patients, 73 diabetic and cardiac patients and 48 control subjects were investigated. PCR and RFLP analysis were used for identification of individual genotype. In type2 diabetes patient 46 samples (74.19%) were identified as homozygous Pro/Pro genotype and 16 samples (25.80%) as heterozygous Pro/Ala genotype. In cardiac patients those also have type 2 diabetes 63 samples (88.73%) were homozygous for Pro/Pro genotype and 8 samples (11.26%) were heterozygous for Pro/Ala genotype. In the control group, 26 samples (54.16%) were Pro/Pro homozygous, 21 samples (43.75%) Pro/Ala heterozygous and 1 sample was Ala/Ala homozygous genotype. Previous studies showed that, Pro12 allele is the risk factors for onset of diabetes and Ala12 allele is the protective factor for diabetes. In control subject this Pro12Ala polymorphism was significantly higher than patients thus proving Ala12 allele play the protective role. Most significant finding of this study is Pro12 allele was significantly higher in cardiac patients having type 2 diabetes further emphasizes the fact that, diabetes increases the risk of heart disease and stroke.

**P688****The risk factors for cardiovascular disease in type 2 diabetes patients**

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**Introduction**

Diabetes mellitus is equivalent of coronary artery disease (CAD), plenty of patients with CAD already has diabetes or prediabetes. Diabetes originate as result of interactions between diferent factors, like genetic and environment. The risk factors for type 2 diabetes are: positive family history for type 2 diabetes, obesity, age over 45 years, previous glucose intolerance or impaired fasting glucose, previous gestational diabetes or birthweight of children over 4 kg in women, hypertension (> 140/90 mmHg), HDL cholesterol <0.9 mmol/l, and/or tryglicerides > 2.8 mmol/l, polycystic ovary syndrome. Primary characteristics of CAD in diabetic patients are: younger age than in patients without DM, rapid progression, and diffuse and extended changes on blood vessels.

**Aim**

The aim of our study was to determinate presence of risk factors for cardiovascular disease in type 2 diabetes patients in Kragujevac, and the difference depending on gender.

**Matherial and method**

The study included all patients with type 2 diabetes patients in Kragujevac, we collected demographic data about patients, and about risk factors related to this disease.

**Results**

This examination registered 3108 type 2 diabetic patients, 1248 (40.1%) men and 1860 (59.9%) women. The prevalence of obesity in this population was 36.77%, in 40.4% women versus men 31.3% ( $P < 0.001$ ), hypertension 55.5%, women 59.7% versus men 49.2% ( $P < 0.001$ ), hyperlipidemia 23.5%, women 24.4% versus men 22.1% ( $P = 0.076$ ), smoking 10.88%, women 8.3% versus men 14.7% ( $P < 0.001$ ), alchocol consumers 3.76%, women 0.38% versus men 8.8%,  $P < 0.001$ , birthweight of children over 4 kg in type 2 diabetic women 7.47%. Positive family hystory for diabetes was in 28.1%, women 29.2% versus men 26.4% ( $P = 0.052$ ), positive family hystory for CAD was in 24.3%, women 27.0% versus men 20.27% ( $P < 0.001$ ).

**Conclusion**

The prevalence of obesity and hypertension is significantly higher in women, the prevalence of smoking and alchocol consuming is higher in men with type 2 diabetes in Kragujevac.

**P689****Study of endothelial dysfunction in diabetes. assesment of ANP leves in patients without vascular disease**

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**Background**

Endothelial dysfunction may have deleterious effect cardiovascular pathology in diabetic patients.

Atrial natriuretic peptide (ANP) exerts beneficial effects on the cardiovascular system in part by exerting antioxidant activity. Recently it was reported that ANP is able to exert an antihypertrophic effect in myocytos at least in part due to inhibition of NADPH oxidase and superoxido generation.

It seems that hyperglucemia acute is accompanied by a rapid increase in circulating ANP and in this metabolic interaction, ANP might counteract the renal sodioum-retaining effect of hyperglucemia and hyperinsulinemia.

Given that oxidant stress is a key cause of endothelial dysfunction in diabetes we investigated whether there is a relation between ANP levels and the presence of vascular disease and his relation with the HbA1c.

**Methods and patients**

We analysed 21 diabetic patients, 8 woman and 13 men, with ages (X + DS) 61.3 ± 12.6 and 8.4 ± 9 years of diabetes evolution. Two were diabetic Type 1 and 19 type 2. The control group were 34 subjects. ET1 and ANF were measured by RIA.

**Results**

There were no statistically significant differences between ANP levels in the group of diabetic patients depending on the HbA1c group (ANP: 9.24 ± 5.30 and 12.04 ± 7.79 pg/ml in patients diabetic with HbA1c < 9 y HbA1c > 9% (NS).

However ANP levels was increased in the both groups of patients with diabetes without vascular disease compared with healthy controls (ANP: 5.84 ± 3.96 pg/ml)  $P < 0.05$ ;  $r = 0.43$ .

**Conclusion**

ANP levels was increased significantly in diabetic patients without vascular disease compared to controls but not directly related to HbA1c levels.

Elevation of ANP levels may precede vascular complications associated with diabetes and offers a protective actions on endothelium-dependent and independent vasodilatation. This may represent new, more effective means of treating vascular disease specifically in the setting of diabetes.

**P690****Obese diabetics and left ventricular hypertrophy**

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**Introduction**

Type 2 diabetes mellitus (DM) is independent risk factors for micro and macrovascular disease. So, it has been associated with abnormalities of cardiac function and left ventricular hypertrophy (LVH). Type 2 diabetic individuals, particularly obese women, had higher heart rates, greater left ventricular wall thicknesses and greater cardiac mass than subjects without diabetes. The objective of the present study was to investigate association between waist circumference-body mass index (BMI) and left ventricle mass (LVM)-left ventricle mass index (LVMI) in patients with type 2 DM and without known cardiac disease.

**Methods**

The study group comprised of 65 patients, 22 men and 43 women with mean age 58.6 ± 10.09, with type 2 diabetes. Patients with presence of renal failiure or heart failure of any stage, known cardiac diseases, pulmonary diseases, anemia, were excluded. DM was defined by fasting plasma glucose levels 126 mg/dl or by specific treatment. BMI was calculated by standard formula and abdominal circumference was measured. Two-dimensional echocardiograms of the LV were performed. LVM was calculated by the formula introduced by Devereux and Reicheck and was indexed for body surface area to obtain LVMI. LVH was diagnosed when LVMI was > 134 g/m<sup>2</sup> in men and > 110 g/m<sup>2</sup> in women.

**Results**

In all patients, there were significant correlation between abdominal circumference and LVM; between BMI and LVM; between BMI and LVMI. According to sex, were significant relations between BMI and LVM ( $P = 0.03$ ), BMI and LVMI ( $P = 0.007$ ), only in woman. These parameters were not associated in men.

**Conclusion**

In diabetic patients, high BMI and abdominal circumference were associated with increasing LVM and LVMI, especially in women. So, in obese diabetics these parameters are important risk factors, for heart disease.

Keywords: diabetes, hypertrophy, left ventricle, BMI, risk factors.

**P691****Psychoemotional status and quality of life of T1DM patients**

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**Aim**

To estimate QOL and intensity of depression symptoms in T1DM patients.

**Materials and methods**

Total 53 T1DM patients (26m, 27f), mean age (M ± σ) 33.6 ± 2.7 years (20.00–54.00) and 35 of the control group (17m, 18f), mean age 34.8 ± 2.9 years (22.00–50.00) were provided with the questionnaire of Medical Outcomes Study 36-Item Short-Form Health-Survey, Beck depression inventory and Test for estimation of subjective control level.

**Results**

The patients with HbA1c < 7% the parameters of Role-Physical, Role-Emotional, Mental Health ( $P < 0.05$ ) were higher than in patients with HbA1c ≥ 7%. The negative correlation of expressiveness of depression symptoms with GH ( $r = -0.54$ ;  $P < 0.05$ ) were obtained. The expressiveness of depression symptoms is lower in HbA1c < 7% T1DM patients to compare with HbA1c ≥ 7% Me 21.8 (11.3; 45.2) vs Me 11.3 (8.1; 11.3), ( $t = -2.053$ ;  $P = 0.057$ ). By the scale of 'Total internality' the average level was 73%, the high level – 27%, the low level – 0%; by the scale of 'Internality of achievements' the average level consisted 36.5%, the high level – 63.5%, the low level – 0%; 'Internality of failures' the average level – 54.5%, the high level – 45.5%, the low level – 0%; 'Internality of family relations' the average level was 54.5%, the high level – 27.3%, the low level – 18.2%; 'Internality of industrial relations' the average level was 82%, the high level – 18%, the low level – 0%; 'Internality of interpersonal attitudes' the average level – 27.2%, the high level – 27.2%, the low level – 43.8%; 'Internality of health and disease' the average level was 64%, the high level – 36%, the low level – 0%.

**Conclusion**

The evident depression in 38% of patients testifies to the unfavorable emotional state at T1DM. Higher physical, emotional, psychological health and less expressiveness of depression symptoms in compensation patients underline the urgency of achievement of aim levels of T1DM compensation.

**P692****Features of the lipid exchange among the patients suffering from diabetes with various degree of carbohydrate exchange compensation**

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**Research objective**

To estimate interrelation of carbohydrate exchange compensation with indicators of blood lipids.

**Methods**

Four hundred and seven patients suffering from diabetis of 1 and 2 types at the age of 47.20 (35.63<sup>25th</sup>–57.42<sup>75th</sup>) have been examined. The disease experience – 10.90 (6.40<sup>25th</sup>–18.46<sup>75th</sup>) years. All the examined patients have been divided into 3 groups. In the first group are patients with the glycated hemoglobin level < 7.5%; in the second – 7.6–10.5%; in the third – more than 10.5%. Average age and the diabetes experience didn't differ authentically among patients of the three groups. Among the given patients blood lipids have been defined. The range of reference values for triglycerids (TG) has made 0.20–1.89 mmol/l; lipids of high density (HDL) – 1.04–1.55 mmol/l; lipids of low density (LDL) – 0.10–4.00 mmol/l; very low density (VLDL) – 0.10–0.70 mmol/l.

**Results**

It is proved that at a diabetes the levels of triglycerids among the examined of the first group have made 1.17 (0.47<sup>25th</sup>–1.63<sup>75th</sup>) mmol/l and were authentically lower in comparison with patients of the third group 1.79 (1.05<sup>25th</sup>–2.48<sup>75th</sup>) mmol/l ( $U = 3333.00$ ;  $P = 0.0002$ ). HDL and VLDL levels among patients of 1 group have made 2.83 (1.76<sup>25th</sup>–3.20<sup>75th</sup>) mmol/l and 0.55 (0.34<sup>25th</sup>–0.78<sup>75th</sup>) mmol/l, accordingly. HDL and VLDL levels were authentically lower against indicators of patients of group 3 (3.45 (2.55<sup>25th</sup>–4.8<sup>75th</sup>) mmol/l and 0.81 0.81 (0.49<sup>25th</sup>–1.12<sup>75th</sup>) mmol/l) ( $U = 7609.50$ ;  $P = 0.01$  and  $U = 7315.00$ ;  $P = 0.03$ , accordingly). At carrying out of the correlation analysis direct authentic correlation between glycated hemoglobin levels and TG ( $r_s = 0.20$  is determined;  $P = 0.000001$ ); glycated hemoglobin levels and VLDL ( $r_s = 0.17$ ;  $P = 0.0007$ ).

**Conclusions**

At a diabetes, as aggravation of carbohydrate metabolism disorder, metabolic lipid imbalance progress: increase in TG HDL and VLDL levels.

**P693****Diabetic foot with risk for ulceration associated with diabetic retinopathy in type 2 diabetes**

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**Aim**

To define impact of diabetic retinopathy as a risk factor at peoples with type 2 diabetes and diabetic foot.

**Material and methods**

One hundred hospitalized patients with type 2 diabetes, screened for diabetic foot and diabetic retinopathy for 1 year. Clinical examination and laboratory investigations were evaluated.

**Results**

From 100 patients, 53% were female and 47% male, duration of diabetes 10.47 ± 4.77 years. Mean HbA1c was 8% ± 1.2%. HbA1c < 7% had 18%, HbA1c 7–8% had 43% and HbA1c > 8% had 49% of patients. At visit 1, risk score for diabetic foot ulceration is: low risk (0) 29%, medium risk (1) 35%, high risk (2) 18% and very high risk (3) 18%. Retinopathy was present with 68% – 53% non-proliferative and 15% proliferative. According the risk score at visit 1 retinopathy had: in score 0 – 15% non-proliferative and 0% proliferative, score 1 – 18% non-proliferative and 1% proliferative, score 2 – 11% non-proliferative and 6% proliferative, and score 3 – 9% non-proliferative and 8% proliferative. After 12 months risk score for diabetic foot was: 0 – 17%; 1 – 39%; 2 – 19% and 3 – 27%. Diabetic retinopathy was present after 12 months 72% of which 51% non-proliferative and 21% proliferative. According the risk score after 1 year diabetic retinopathy were present: in score 0 – 6% non-proliferative and 0% proliferative, score 1 – 22% non-proliferative and 3% proliferative, score 2 – 10% non-proliferative and 7% proliferative, and score 3 – 13% non-proliferative and 11% proliferative.

**Conclusion**

Association between risk score for foot ulceration and diabetic retinopathy was present. Group with risk score 0 and 1 have more non-proliferative retinopathy and group with score 2 and 3 have more proliferative retinopathy (Cross tabulation. Kruskal Wallis test  $P < 0.01$ ).

Keywords: diabetic foot, score, diabetic retinopathy.

**P694****Type 2 diabetes mellitus and testosterone: a meta-analysis study**

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**Introduction**

Type 2 diabetes mellitus (T2DM) is often associated with male hypogonadism. The role of testosterone replacement therapy (TRT) in T2DM has not been completely clarified. To systematically analyse the relationship between androgen levels and T2DM by reviewing and meta-analyzing available prospective and cross-sectional studies. In addition, a specific meta-analysis on the metabolic effects of TRT in available randomised clinical trials (RCTs) was also performed.

**Methods**

An extensive Medline search was performed including the following words 'testosterone', 'type 2 diabetes mellitus' and 'males'. Out of 742, 37 articles were included in the study. In particular 28, 5, and 3 were cross-sectional, longitudinal, and interventional studies, respectively. A further unpublished RCT was retrieved on [www.clinicaltrials.gov](http://www.clinicaltrials.gov).

**Results**

T2DM patients showed significantly lower T plasma levels in comparison with non-diabetic individuals. Similar results were obtained when T2DM subjects with and without ED were analyzed separately. Meta-regression analysis demonstrated that aging reduced, while obesity increased, these differences. However, in a multiple regression model, after adjusting for age and BMI, T2DM was still associated with lower total T (TT) levels (adj.  $r = -0.568$ ,  $P < 0.0001$ ). Analysis of longitudinal studies demonstrated that baseline TT was significantly lower among patients with incident diabetes in comparison to controls

( $-2.8$  (3.57;  $-0.59$ ),  $P < 0.001$ ). Combining the results of RCTs, TRT was associated with a significant reduction of fasting plasma glucose, HbA1c, fat mass, and triglycerides.

**Conclusions**

T2DM can be considered independently associated with male hypogonadism. Although only few RCTs have been reported, TRT seems to improve glycometabolic control as well as fat mass in T2DM subjects.

**P695****Early diagnostics of diabetic peripheral neuropathy manifestations in patients with diabetes mellitus**

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**Introduction**

Purpose of this research was the study of peculiarities of early diabetic peripheral neuropathy (DPN) manifestations in patients with diabetes mellitus depending on diabetes manifestation age, indicators of antibody level of S100B.

**Description of methods**

Sixty-two patients with diabetes mellitus (DM) at the age of 21.4 to 75.2 (the average age was 47.7 ± 14.1 years) including 34 males (54.8%) and 28 females (45.2%) with longevity of DM more than 1 year (disease duration is 13.2 ± 8.4 years) were examined. S100B-antibodies were determined with use of CanAg S100 EIA reactive (CanAg Diagnostics AB, Sweden). The average value for the control group ( $N = 30$ ) was 54.00 ± 15.60 ng/l. The laboratory data were presented in the form of mean ± S.D., nonparametric – median, 25th and 75th percentiles.

**Results**

The patients were divided into 4 groups by the disease manifestation age. The first group was represented by the patients with DM manifestation age before 14 years (9 individuals), the second group was composed of patients with disease manifestation age 14–20 years (14 individuals), the third group was represented by the patients with manifestation age 20–30 years (16 individuals), the fourth group was composed of patients with disease manifestation age after 40 years (23 individuals). DM longevity in patients fallen ill before 14 years was significantly higher (Me = 20.17 (12.00; 23.50)) versus disease longevity in patients fallen ill after 40 years (Me = 10.00 (6.00; 15.00)); ( $U = 122.50$ ;  $P = 0.02$ ). In spite of the smaller disease longevity, the S100B level in patients fallen ill after 40 years was significantly higher (Me = 31.11 (26.94; 35.03)) versus patients fallen ill before 14 years (Me = 24.00 (21.06; 30.37)); ( $U = 60.50$ ;  $P = 0.04$ ).

**Conclusion**

The additional early laboratory predictor of DPN in patients at the age of 40 years regardless disease longevity is the increased level of S100B.

**P696****Changes in GLUT1 expression under metformin treatment in rat models of severe and mild streptozotocin diabetes mellitus**

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**Introduction**

Metformin improves hyperglycaemia via mechanisms which include activation of AMP-activated protein kinase (AMPK). Recent findings indicate that some metabolic actions of metformin occur also by AMPK-independent mechanisms.

**Objective**

To study the action of metformin on expression of GLUT1 glucose transporter in rat models of severe and mild streptozotocin diabetes mellitus.

**Materials and methods**

Severe diabetes mellitus in rats was induced by single injection of streptozotocin 50 mg/kg, i.v. To induce mild diabetes mellitus with hyperlipidaemia, rats were fed by high-fat chaw for 2 weeks, then two i.p. injections of streptozotocin (35 and 30 mg/kg) followed with a week interval. Rats were treated with metformin (100 mg/kg daily, *per os*) for 6 weeks while monitoring parameters of carbohydrate and lipid metabolism. GLUT1 mRNA and protein expression in kidneys, heart and liver were studied by means of real time RT-PCR and immunohistochemistry correspondingly.

## Results

In severely diabetic rats, metformin treatment decreased glucose concentration, glycated haemoglobin % and improved glucose tolerance. In mildly diabetic rats, only decrease in glycated haemoglobin % was observed in the end of the metformin treatment. Streptozotocin diabetes provoked increase of both GLUT1 gene and protein expression in kidneys of both severely and mildly diabetic rats. Metformin treatment produced normalization of the GLUT1 gene and protein expression levels in severely diabetic rats, in mildly diabetic rats only GLUT1 protein expression was normalized. In the liver, severe (but not mild) diabetes triggered an increase in GLUT1 protein expression, which was normalized by metformin. GLUT1 protein expression was increased in the hearts of severely and mildly diabetic rats. Metformin normalized the parameter only in severely diabetic rats.

## Conclusion

Metformin decreases GLUT1 hyperexpression in kidneys, induced by streptozotocin severe diabetes mellitus and mild diabetes mellitus with hyperlipidaemia. Therefore, it is prospective for treatment of diabetic nephropathy.

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## P697

### The study of DM late complications prevalence and risk factors contributing to their progression in urban and rural diabetics of Uzbekistan

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DM is a major cause of blindness, cardio-vascular risk the highest in the diabetics. More than 40% of all trauma-unrelated lower extremity amputations are performed because of diabetic foot syndrome (DFS) and lower extremity gangrene, 35–40% of all deaths due to uremia fall to the diabetics.

#### Aim

To study DM late complications prevalence and risk factors contributing to their progression.

#### Materials and methods

A total of 1000 and 1141 patients with type 2 diabetes mellitus, urban and rural residents, respectively, underwent general clinical examination; arterial pressure, carbohydrate and lipid metabolism parameters were measured. A special questionnaire was filled up for each patient.

#### Results

The study established higher DM late complications prevalence in urban diabetics as compared with those rural ( $P < 0.001$ ). Incidence of macroangiopathies of lower extremity vessels, DFS and myocardial infarction (MI) was found higher in males than the one in females, while ischaemic heart disease (IHD) prevalence was registered higher in females regardless of region. Increase in incidence of DM late complications with the disease duration was found in both regions ( $P < 0.001$ ). Prevalence of macroangiopathies, diabetic polyneuropathy and DFS upon the decompensated disease was the highest both among urban and rural residents. The smokers had higher prevalence of diabetic retinopathy, macroangiopathies of lower extremity vessels, MI and IHD regardless of region. Age contributes to progression of DM late complications too.

#### Conclusions

DM late complications incidence is higher in the urban diabetics, than in the countrymen. Sex, age, disease duration, compensation stage and bad habits are found the risk factors of DM late complications progression.

Keywords: epidemiology, diabetes mellitus, late complications, urban and rural populations, risk factors.

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## P698

### Illuminating diabetic neuropathy: novel systems for *in vivo* monitoring and microenvironmental investigations in a murine model

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#### Introduction

Major advances in the research and treatment of diabetes-related neuropathic phenomena are hampered due to its complicated pathophysiology and the scarcity of valid experimental models. The aim of this study was to establish novel systems facilitating monitoring and dissection of processes central to the development of diabetic neuropathy.

#### Methods

In a non-invasive *in vivo* model, two-photon microscopy is used to detect and assess mechanoreceptors (Meissner's corpuscles) in whole footpads of mice

carrying a neuronal genetic tracer. This technology offers a potent tissue penetration capacity coupled by superb resolution. The illustrated *ex vivo* system simulates the native microenvironment of the nerve ending by a unique co-culture of primary sensory neurons and freshly harvested skin from control or diabetic mice. Innovative high-throughput tools were used to quantify the growth of hundreds of neurons in a non-biased manner.

#### Results

Qualitative and quantitative alterations in Meissner's corpuscles in an intact footpad of diabetic animals were detected *in vivo*, the results corroborated with parallel histopathological studies comparing control and diabetic animals. The *ex vivo* assays demonstrated that neurons from either control or diabetic mice have similar basal growth and that both show substantial growth enhancement upon exposure to skin-derived cues. However, the ability of diabetic skin to support such growth was dramatically diminished.

#### Conclusions

A non-invasive imaging of genetically traced Meissner's corpuscles by two-photon microscopy offers a robust model to assess diabetes-induced distal axonal degeneration. The degeneration was found to correlate with target-organ malfunction rather than intrinsic neuronal factors. Moreover, diabetic neurons preserve their tentative ability to respond to skin-derived cues, thus may fully regenerate.

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## P699

### Gilbert syndrome and diabetes: a perfect union?

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#### Introduction

Gilbert syndrome is one of the most common inherited diseases, with a prevalence of 5–7%. It is caused by a mutation in UGT1A1 gene, which is in turn responsible for a deficiency in bilirubin glucuronidation. It courses with unconjugated hyperbilirubinemia, and jaundice may occur in adolescence, after fasting, exercise, or in menses, and also with certain drugs. Individuals with Gilbert syndrome seem to have a reduction in the prevalence of micro- and macrovascular complications due to the anti-oxidant effects of bilirubin.

#### Clinical case

A 20-year-old male patient, smoker, with type 1 diabetes mellitus diagnosed at the age of 6 and long standing poor metabolic control. He had a history of episodes of mild jaundice that had never been valued by the patient. Despite the poor compliance to diet and insulin therapy, with persistent poor glycemic control, he had no target organ complications. The patient was hospitalized in the Endocrinology Department for significant glycemic instability. A1c on admission was 10.7%. During hospitalization, gastroenterological advice was requested for persistent unconjugated hyperbilirubinemia (total bilirubin 28.5 mg/l, direct bilirubin 5.1 mg/l) without hemolytic disease or liver dysfunction. The diagnosis of Gilbert syndrome was then made. The patient's microalbuminuria was 12.65 mg/24 h (<30 mg/24 h). Retinopathy was excluded.

#### Discussion

It has been recently documented a lower prevalence of vascular complications in patients with diabetes mellitus and Gilbert syndrome, as well as reduced markers of oxidative stress and inflammation. Bilirubin appears to have an inhibitory effect on NADPH oxidase activity, which may be an important source of increased superoxide production in diabetic vascular tissues. It is possible that sustained hyperbilirubinemia inhibits oxidative stress and prevents vascular complications in patients with both diseases.

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## P700

### Influence of metabolic disorders in children with diabetes mellitus type 1 on changes of QT interval

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Diabetes mellitus disturbs cellular metabolism of all organs and systems, including myocardium. The disturbances in cardiomyocytes lead to changes of QT and QTc intervals prolongation. Research: to evaluate values of intervals QT and QTc in children with diabetes mellitus type 1 (DM1) and to reveal interrelation of the given changes with the duration of disease, age, sex, indices of metabolic control.

**Materials and methods**

QT and QTc were evaluated on ECG in 168 children with DM1 (middle age  $13.56 \pm 0.92$  years, duration of disease  $5.01 \pm 0.89$  years) and in 60 sex- and age-matched healthy children. Level of HbA1c in children with DM1 made up  $9.66 \pm 0.5\%$  ( $N < 7.5\%$ ;  $P < 0.0005$ ).

**Results**

Increases of values of intervals QT and QTc are established at children with DM1 in comparison with control group ( $363.51 \pm 8.5$  ms and  $421.13 \pm 12.5$  ms,  $352.97 \pm 15.1$  ms and  $392.73 \pm 13.0$  ms respectively,  $P < 0.0001$ ). It is established that values of QTc in girls with DM1 are higher than in boys ( $425.8 \pm 14.8$  vs  $416.24 \pm 14.8$  ms,  $P < 0.002$ ). 21.6% of girls and 15.79% of boys with DM1 have QTc > 440 ms. At the same time values of HbA1c are higher in boys than in girls ( $412.88 \pm 11.35$  and  $377.66 \pm 8.76$  mkmol/l respectively,  $P < 0.001$ ). The difference in pulse rate between girls and boys is insignificant for the group studied. Connection of QTc with age ( $r = 0.334$ ,  $P < 0.0003$ ) and level of HbA1c ( $r = 0.37$ ,  $P < 0.0001$ ) was established. Feedback connection of QT with pulse rate was revealed ( $r = -0.48$ ,  $P < 0.0001$ ). Reliable correlation between QTc and values of body mass index, level of cholesterol, duration of the disease and values of systolic and diastolic blood pressure was not established. Conclusions. The increase of QT and QTc is noticed in children with DM1. Age, level of glycemic, pulse rate are the factors defining values of QTc. In girls QTc has higher values more often than in boys.

**P701****Receptor activator of nuclear factor kappa  $\beta$  ligand and osteoprotegerin levels in the patients with diabetic nephropathy**

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**Aim**

The aim of this study was to investigate the relationship between the clinical and laboratory findings of metabolic bone disease regarding the levels of osteoprotegerin (OPG) and the receptor activator of nuclear factor kappa B ligand (RANKL) in the patients with diabetic nephropathy.

**Material and method**

This study includes 61 type 2 diabetic patients and age-, sex-matched 20 healthy subjects. Diabetic patients were divided into three groups according to a modification of Mogensen's clinical classification (stage III, stage IV, stage V). In addition, diabetic patients were further divided into two groups according to glomerular filtration rate (GFR) as follows: group 1: GFR < 60 ml/min per 1.73 m<sup>2</sup>, group 2: GFR > 60 ml/min per 1.73 m<sup>2</sup>. The serum OPG and RANKL levels were determined by ELISA according to manufacturer's manual.

**Results**

The serum iPTH, P and RANKL level in diabetic patients was found significantly higher than in control group ( $P < 0.05$ ). There were no significant differences in the serum OPG levels and RANKL/OPG ratio among the groups. No significant differences were determined between levels of OPG, RANKL, and RANKL/OPG ratio in diabetic nephropathy stages. The serum RANKL levels were significantly higher in the group 1 diabetics than in the group 2 diabetic patients ( $P < 0.05$ ), whereas there were no significant differences in serum OPG level and RANKL/OPG ratio between the group 1 and group 2 diabetics. In addition, RANKL levels and RANKL/OPG ratio were found to be correlated positively with ALP levels in group 1 diabetic patients ( $P < 0.05$ ).

**Conclusion**

Our results suggest that increased serum RANKL levels might play role in development of the metabolic bone disease in patients with diabetic nephropathy.

**P702****Matrix  $\gamma$ -carboxyglutamate (Gla) protein (MGP) in type 2 diabetes**

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**Objective**

Matrix  $\gamma$ -carboxyglutamate (Gla) protein (MGP) is a marker of athero-sclerosis reflecting the degree of inhibition of ongoing calcification processes in the

vascular wall. Osteoprotegerin (OPG) and adiponectin (ADP) exerts anti-inflammatory and anti-atherosclerotic effects. We aimed at determining in type 2 diabetes patients the relationship between serum MGP, OPG, ADP levels and the HbA1c, HOMA insulin-sensitivity or B-cell function.

**Research design and methods**

Serum MGP, OPG and ADP were determined in 37 adult patients with type 2 diabetes using an ELISA method. Insulin sensitivity and  $\beta$ -cells secretion were measured using HOMA model.

**Results**

There were no significant correlations between serum OPG, MGP or ADP and glycated hemoglobin HbA1c. Significant correlations were found between insulin sensitivity (HOMA-S log) and serum MGP-log or serum ADP-log, and also between  $\beta$ -cells function (HOMA-B log) and serum MGP-log, or serum ADP-log (Table)

**Table 1.**

	MGP-log	ADP-log	OPG-log
HOMA-B log	$P = 0.03$ , $r = 0.35$	$P = 0.01$ , $r = -0.31$	$P = 0.26$
HOMA-S log	$P = 0.02$ , $r = -0.36$	$P = 0.004$ , $r = 0.34$	$P = 0.88$
hs-CRP log	$P = 0.0009$	$P = 0.10$	$P = 0.19$

No correlations were found between serum MGP and abdominal perimeter ( $P = 0.42$ ) or body mass index ( $P = 0.12$ ). Serum levels of MGP and OPG were inversely correlated ( $P = 0.02$ ).

**Conclusion**

Serum MGP was positively correlated with  $\beta$ -cells function ( $P = 0.03$ ) and hsCRP ( $P = 0.0009$ ), and inversely correlated with HOMA-insulin sensitivity ( $P = 0.02$ ). In type 2 diabetic patients, the increased levels of plasma MGP may counteract vascular calcification, but also an increased inflammation which may reflect either the vascular remodeling and/or a mechanism loading to increased resistance of insulin.

**P703****Diabetic patients with autonomic neuropathy do need erythropoietin therapy at the early stages of renal failure**

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**Introduction and aims**

Diabetic neuropathy may trigger the development of anemia in patients even before the onset of advanced renal failure. Autonomic neuropathy with subsequent renal denervation, combined with damaged erythropoietin producing fibroblasts in renal cortex, may have contributed to the early development of anemia in the patients with diabetes. Proliferative retinopathy, autonomic neuropathy, microalbuminuria, and moderate renal failure, suggesting that, factors other than CKD can contribute to anemia development.

**Methods**

We studied 35 diabetic patients with type II diabetes, ages 47–78, with anemia, but without overt renal failure. We measured erythropoietin levels, hemoglobin (Hb) levels, serum creatinin (Cr), microalbuminuria, glomerular filtration rate (GFR), low density lipoproteins (LDL), glycosylation of Hb (HbA1c), glycose levels. We also examined the patients for the incidence of cardiovascular disease (CVD), diabetic retinopathy and macular edema. We used questionnaires scoring the quality of life in these patients. Obesity and lifestyle had taken in thought. We treated the whole anemic group with darbepoetin and we measured the new results.

**Results**

Twenty from 35 patients were found anemic, (mean Hb of 10.6 g/dl), with decreased production of erythropoetin, which means blunted erythropoetin response to anaemia, mild retinopathy (OR, 5.3, 95% CI, 2.3–12.6) Cr levels < 1.87 mg/dl. Mild microalbuminuria was found to 18 of the 20 anemic patients. GFR was between 110 and 88 ml/h per 1.73, glucose levels between 92 and 213 mg/dl, HbA1c levels > 5.5% to all patients, LDL levels > 200 mg/dl for 18 patients, 6 patients had mild heart failure (ER < 60%), 3 had a stroke in their medical history. Low scores measuring quality of life were obtained from all the anemic patients. Thirteen patients were obese and had sedentary lifestyles. After treating the whole anemic group of diabetic patients with darbepoetin in order to keep Hb > 12 g, diabetic retinopathy and macular edema have been shown to respond to therapy. Most of the patients achieved a more active life and improved quality of life scores. Creatinine levels remained unchanged also proteinuria. The EF increased in 8 patients, sympathetic neuropathy improved in 50% of the patients, LDL levels were measured < 180 mg/dl to 45% after the darbepoetin treatment.



#### Conclusions

The cause of early anaemia in patients with diabetes, is not only the renal nephropathy. As diabetes progresses, the basement membrane of glomeruli thickens as a result of glycosylation, leading to increased intrarenal pressure. Factors other than CKD contributed to anaemia development. Autonomic neuropathy maybe showing the need of erythropoietin treatment, as hyperglycemia affects the function of nerves and muscles acutely and possibly all other tissues aswell. Therefore, erythropoietin responses to anaemia in diabetes may also be disturbed. Determining the causes of early anemia in evidence of the need for early anemia screening and treatment in patients with diabetic and early stages of renal failure.

#### P704

##### **Beta estrogen receptors inhibition improves wound healing in diabetes** I R Botusan<sup>1,3</sup>, V G Sunkari<sup>1</sup>, J Grunler<sup>1</sup>, S Lindberg<sup>1</sup>, O Savu<sup>1,4</sup>, K Steffensen<sup>2</sup>, K Brismar<sup>1</sup> & S B Catrina<sup>1</sup>

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Diabetic wounds represent a medical and economical burden with a high need to develop more efficient therapies.

Estrogens have a positive effect on cutaneous wound healing. Their decreased levels could count for impaired wound healing in elderly. The biological estrogen's effects are mediated by two nuclear receptors subtypes (ER $\alpha$  and ER $\beta$ ) and they were differently implied for the wound healing benefic processes. However, the estrogen effects on diabetic wounds have not yet been studied.

Here, we investigate the role of estrogen receptors subtypes on the wound healing rate in diabetes.

#### Methods

Diabetes was induced with streptozotocin in knock out for alpha (ERKO) or beta (BERKO) estrogen receptors mice and in wild type controls. Wounds were performed on the dorsum of animals, and evaluated by photos taken every second day. At the end of the experiment, wounds biopsies were analyzed for granulation, dermal regeneration (hematoxylin eosin), angiogenesis (isolectin staining), markers for inflammation and recruitment of endothelial precursor cells (EPC) (qRT-PCR). *In vitro*, migration and angiogenesis assays were evaluated on primary dermal fibroblasts and endothelial cells in the presence of specific estrogen subtype receptors modulators.

#### Results

Wound healing rate in diabetic BERKO, but not diabetic ERKO mice was significantly faster than in diabetic controls (50% wound closure at  $3.4 \pm 0.3$  days ( $P < 0.05$ ),  $4.5 \pm 0.5$  days respectively  $4.7 \pm 0.5$  days). This correlated with levels of granulation tissue and with recruitment of EPCs in the wounds of BERKO mice. Agonists for both specific estrogen receptors significantly increased fibroblasts migration rate, while angiogenesis was accelerated only by blocking the beta estrogen receptor.

#### Conclusion

In diabetes, BERKO mice display an accelerated wound healing when compared to ERKO or control mice. Moreover, beta estrogen receptor inhibition leads to better recruitment of EPC and angiogenesis suggesting the use of specific ER modulators for therapeutic trials.

#### P705

##### **Diabetes mellitus and risk of fracture**

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#### Objective

To examine association between diabetes properties (type, duration, treatment regimen etc.) and risk of any fracture in diabetic population.

#### Methods

We analyzed local trauma clinic data for 3 sequential years from 2662 diabetic persons who were entered The National Register of diabetes mellitus patients (133 subjects with type 1 diabetes mellitus (T1DM) and 1995 ones with T2DM).

#### Results

Fracture risk was higher in T1DM people compared to T2DM ones (relative risk 1.67; 95% CI 1.22–3.40). In T1DM subjects, fractures of foot and hand were more frequent ( $P = 0.028$ ). We haven't found in T2DM population any significant influence on fracture risk of gender, age, body mass index, diabetes duration and use of any antidiabetic drugs. Presence of diabetic retinopathy, nephropathy or macroangiopathy (atherosclerosis of coronal, cerebral and/or lower limb arteries) was associated with two-fold higher risk of all fractures while diabetic neuropathy was not (RR 1.77; 95% CI 0.96–3.25). Among 149 T2DM patients who were interviewed via telephone, fracture risk was increased in current smokers (RR 3.1, 95% CI 1.08–8.88) and daily coffee drinkers ( $> 7$  cups a week) compared with rarely drinkers ( $< 1$  cup a week: RR 10.9; 95% CI 1.18–101.13). Elevation of risk was also observed for women with short period of lactation (1–5 months: RR 3.06; 95% CI 1.02–9.16). The risk was decreasing along either with prolongation of breastfeeding and for women with two or more children whereas this reduction wasn't statistically significant.

#### Conclusion

These results indicate that main fracture risk factors in diabetic population are diabetic complications, suggesting that fracture prevention strategy should be a consideration in the treatment of diabetes. Influence of lifestyle factors are also significant and should be took into account for vulnerable group definition.

#### P706

##### **Health illiteracy as a challenge in contemporary endocrinology**

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#### Introduction

According to American Medical Association Health literacy means a group of skills, including reading and basic math skills that allow smooth functioning in the health care system. Despite the universality of schools in Europe, the problem of secondary or functional illiteracy did not only exist but seems to be growing. This is also a great ethical problem, because the illiteracy makes, e.g. fictive so basic patients right as informed consent. The reasons for this are 'external' to the endocrinology or closely linked to it. 'External' are the quality of the education system, the omnipresence of the images in interpersonal communication, overwhelming tendency of lawyers to write more and more difficult texts and ...internet. This is a useful medium for teaching us a very selective reading, often only the headlines. 'Internal' reasons means limited efficacy of our treatment especially for diabetes, arteriosclerosis and Alzheimer's disease. Especially in the elderly, this also applies to thyroid disease and/or electrolyte imbalance. Important problem is also hidden nature of the phenomenon, which illustrated our small study. In our Department of Endocrinology, we investigated consecutive patients who are willing to participate and did not declare some problems with reading. The ability to read and understand was examined based on 2 texts: health impact of salt, patient's information about one medicament and commonly used pictograms.

#### Results

About 30% of patients had some difficulties with these texts. Surprising the most difficult task was the interpretation of pictograms (25%) and most easy the consideration about salt (4%).

#### Conclusion

The quantitative extrapolation of our date would be ineligible, but our observation maybe could stimulate other studies and more wide reading examination, at least before we give our patients to read medical information or card of his/her fundamental rights.

#### P707

##### **Adrenocortical hypofunction in patients with diabetes mellitus type 1**

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This work aimed at gathering own data on adrenal response to low-dose Synacthen test, CRH test and about periferal metabolism of cortisol in autoimmune type 1 diabetics with onset in adults. Seventy diabetics were investigated; age  $44 \pm 10$  years (mean  $\pm$  s.d.), age at diagnosis  $28.5 \pm 10$  years, disease duration  $15 \pm 8$  years, BMI  $24.5 \pm 2.7$  kg/cm<sup>2</sup>, HbA1c  $7.2 \pm 1.2\%$ . The study was approved by the Ethical Committee. Adrenal reserve was tested

by low-dose Synacthen test, pituitary function was tested by CRH test and peripheral metabolism of cortisol was evaluated by suppression of cortisol endogenous production by dexametazone administered orally and followed by cortisone acetate (25 mg) administration. We evaluated serum ACTH, serum cortisol, salivary cortisol, aldosterone, DHEA, cortisone during these tests, cortisol binding globulin, adrenal autoantibodies, thyroid function and metabolic parameters of diabetics. We have found a subnormal response in 25% of patients (<500 nmol/l) of the serum cortisol during low-dose Synacthen test, accompanied by significantly decreased stimulated values of aldosterone and salivary cortisol. Basal serum cortisol, aldosterone, were significantly reduced, while ACTH, cortisol binding globulin and salivary cortisol did not differ. The CRH test displayed the low response in serum cortisol and ACTH as well in group of these patients. As compared with group of patients with sufficient response to Synacthen, the course of cortisol after cortisone acetate administration was delayed and significantly different from cortisol response in diabetics with hypocorticalism. The results indicate that the disorder of adrenocortical function occurs in all adrenocortical zones, on pituitary level and peripheral adrenal metabolism could be change as well. These result may contribute to better understanding latent adrenal insufficiency adaptation in diabetics type 1. The study was supported by grant no. NT 11 277 of the IGA MZCR.

## P708

### Effect of oxytocin on the blood glucose level in rat

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#### Background

Oxytocin is a nonapeptide hormone synthesized in the supraoptic and paraventricular nuclei of the hypothalamus. Oxytocin has an effect on uterine smooth muscle contraction, blood pressure, memory, learning capability, nursing, sexual and feeding behavior. Oxytocin and vasopressin stimulate the release of insulin and glucagon from the rat pancreas. Multi organelle effects of oxytocin make it as important hormone in body.

#### Objective

The aim of the present study was to investigate the effect of exogenous oxytocin injection on blood glucose level in rat.

#### Material and methods

Eight adult female rats were divided into 3 groups, one as control and two experimental models have been used to study the effects of oxytocin hormone on the blood glucose level. The rats received 0.2 IU/kg oxytocin via intrapleural (IP) in treatment 1 (T1) and the same volume of normal saline solution in control group. Blood glucose level measured 20 min after injection by collecting the blood from tail. For treatment 2 (T2), animals survived for four month with condition of standard humidity and temperature and they were used for second experimental model. The rats received 0.4 IU/kg oxytocin via IP and the same volume of normal saline solution for control group. Blood glucose level measured same as first treatment.

#### Results

The results showed that blood glucose level in T1 ( $99.45 \pm 4.86$ ) and T2 ( $68.08 \pm 4.86$ ) were significantly lower compared to the control group ( $170.2 \pm 4.86$ ) ( $P < 0.05$ ). Also blood glucose level in T1 ( $99.45 \pm 4.86$ ) was significantly higher than T2 ( $68.08 \pm 4.86$ ) ( $P < 0.05$ ).

#### Conclusions

These *in vivo* results suggest that oxytocin dose has significant effect on the blood glucose level. With respect to results of this study with increasing the dose of oxytocin in animals the level of blood glucose decreased.

## Diabetes (epidemiology, pathophysiology)

### P709

#### To develop non-invasive cytochemical diagnostic technique and monitoring of compensation of diabetes mellitus type 2

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#### Aim

To develop non-invasive cytochemical diagnostic technique and monitoring of compensation of diabetes mellitus type 2.

#### Materials and methods

Material for cytological and cytochemical analyses was oral swab cheek in 22 healthy people and 33 sick ones with type 2 diabetes mellitus at sub compensation stage. The group under study was homogenous in age (45–65 years), sex (men) and biorhythmic type. Experiments were performed on human subjects and local Ethical Committee approval was obtained. Oral swabs were fixed in spirit-acetone and were McManus dyed. For glycogen identification in mucous cells computer morphodensitometry was used. Moreover, blood glucose level was determined. For mathematical data processing correlation analysis and Student's criteria were used. The research results reveal dramatically reduced glycogen optical density in mucous cells in the patients on the second day in hospital. In type 1 transient cells it reaches  $0.081 \pm 0.66$  vs  $0.32 \pm 0.008$  units in norm ( $P < 0.01$ ), in type 2 transient cells it reduces to  $0.073 \pm 0.004$  vs  $0.2 \pm 0.007$  units in norm ( $P < 0.01$ ) and in surface cells density reduces to  $0.054 \pm 0.007$  vs  $0.011 \pm 0.006$  units in norm ( $P < 0.05$ ). Glycogen optical density in mucous cells significantly increases on the 6 and 12 days of treatment by drugs of metformin and sulfanilureal groups ( $P < 0.01$ ), but does not reach the norm. Strong correlative connections are revealed between glycogen concentration in buccal epithelial cells, blood glucose level and authentic changes of differentiation and cornification indexes of oral swab epithelial cells.

#### Conclusion

In this way, suggested noninvasive histochemical method allows to diagnose and monitor the compensation level of metabolic processes in the type 2 diabetes mellitus and reduce the period of its control and risk of its complication development.

## P710

### Continuous glucose monitoring enhances the diagnostic value of mixed meal test in reactive hypoglycemia

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#### Background

Clinical reactive hypoglycemic events are defined as the coexistence of plasma glucose <60 mg/dl and autonomic and/or neuroglycopenic symptoms occurring in fed conditions. The diagnosis is based on tests aiming to simulate the causative triggers (i.e. mixed meal test). In clinical practice, however, a high percentage of symptomatic patients results negative to such diagnostic approach, leading to the impression of an inadequate sensitivity of this diagnostic tool. Based on this background, aim of the present study was to evaluate the diagnostic value of continuous glucose monitoring (CGM) associated to standard mixed meal test in the diagnosis of reactive hypoglycemia.

#### Methods

Thirteen subjects referring to our Center for symptoms suggestive for hypoglycemia were included in this study. All these subjects underwent i) mixed meal test (MMT); ii) CGM for 5 days for the evaluation of plasma or interstitial glucose level, respectively. We considered as positive the presence of symptoms and low glucose concentrations (<60 mg/dl for MMT and <70 mg/dl for CGM).

#### Results

Mean plasma glucose and the glycaemic nadir during MMT ( $79.9 \pm 9.6$  and  $55.3 \pm 15.3$  mg/dl) were similar to those during CGM ( $92.2 \pm 11.2$  and  $53.9 \pm 7.6$  mg/dl). Four patients were positive to both MMT and CGM while 9 patients were negative to MMT but positive to CGM. The sensibility of CGM turned out to be 44%, the specificity 100% and the predictive positive value 80%.

#### Conclusion

The present study supports that CGM could be a relevant adjunctive tool in the diagnosis of reactive hypoglycemia. In fact, CGM is likely to be a diagnostic tool able to detect hypoglycaemic events during real life that are not adequately triggered by a standard MMT.

## P711

### Vitamin D and gestational diabetes

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#### Introduction

There is an increasing evidence of vitamin D role in maintaining normal glucose homeostasis. Gestational diabetes (GD) is related to insulin resistance and vitamin D deficiency has been linked with GD risk in some studies.

**Aims**

To assess whether vitamin D levels are related with gestational diabetes in a group of healthy pregnant women.

**Subjects**

The whole group included 845 pregnant women recruited from April 2009 to April 2010 initially selected to study vitamin D (25OHD) status. We also selected a subgroup (OGTT group) of 273 with abnormal O'Sullivan test who were performed an oral 100-g glucose tolerance test (OGTT) according to the American Diabetes Association (ADA) recommendations. Sixty-two of them were diagnosed of GD (GD+ group) and in 190 the OGTT was normal (GD- group). The 21 remaining didn't complete the test.

**Methods/measurements**

25OHD were measured at weeks 11–23 (D1) and 24–28 (D2) in the whole group and basal levels of glucose and insulin (minute 0 of the curve) in the OGTT group. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) index. We compared 25OHD levels between GD+ and GD-. Pearson correlation analysis was used to test for univariate linear relationship between 25OHD and insulin, glucose and HOMA-IR.

**Results**

i) Median serum 25OHD concentration (ng/ml): weeks 11–13: DG+: 24.07 (6.07–39.70); DG-: 22.49 (4.53–55.56); ( $P=0.175$ ); weeks 24–26: DG+: 23.2 (4.5–39.70); DG-: 25.60 (4–52.40); ( $P=0.27$ ). ii) The relationship between 25OHD and glucose ( $r=0.12$ ), insulin ( $r=0.047$ ) and HOMA-IR ( $r=0.23$ ) was not statistically significant.

**Conclusions**

i) Vitamin D levels were insufficient in both periods of pregnancy; ii) vitamin D levels were similar in women with and without gestational diabetes; iii) we didn't find correlation between vitamin D levels and the parameters of glucose metabolism studied.

**P712****The frequency of Cushing's disease among obese, type 2 diabetes mellitus patients: a single center experience in Turkey**

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**Background**

Obesity and diabetes mellitus (DM) are the features of Cushing's disease (CD). Studies indicated different frequencies of CD among obese, type 2 DM patients. The frequency of CD among obese, type 2 DM have not been studied in Turkey. Therefore, we aimed to assess the frequency of CD among obese ( $BMI > 30 \text{ kg/m}^2$ ) type 2 DM patients that had not classical morphological findings of CD.

**Patients and methods**

Two hundred consecutive obese ( $BMI > 30 \text{ kg/m}^2$ ) type 2 DM patients included into the study (172 female (86%), 28 male (14%), mean age  $51.7 \pm 8.5$ ). All patients underwent 1 mg low dose dexamethasone suppression test (LDDST). Patients with 0800 h cortisole level  $> 1.8 \mu\text{g/dl}$  underwent further investigation for diagnosis of CD.

**Results**

LDDST filed to suppress cortisole levels in 19 (9.5%) of 200 patients. After 2 days 2 mg dexamethasone suppression test (DST) cortisole levels suppressed to  $< 1.8 \mu\text{g/dl}$  in 16 of 19 patients with unsuppressed cortisole levels. Patients with suppressed cortisole levels after 2 days 2 mg DST underwent repeat LDDST after 3 months. Cortisole levels suppressed to  $< 1.8 \mu\text{g/dl}$  in all of these patients with LDSST. With further investigation, CD diagnosed in 3 (1.5%) patients with unsuppressed cortisole levels after 2 days 2 mg DST (2 pituitary CD and one adrenal Cushing).

**Conclusion**

According to our study results the frequency of CD is higher in type 2 DM patients with  $BMI > 30 \text{ kg/m}^2$  than in general population. We advise LDDST as screening test in all obese type 2 DM patients.

**P713****Resting energy expenditure in impaired glucose metabolism**

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**Introduction**

Indirect calorimetry is the most frequently used method to measure resting energy expenditure (REE), but the great expense of equipment precludes its widespread use. The best known REE calculation formula is the 'Harris and Benedict Formula'. The aim of this study is to assess the impact of glucose metabolism status on REE.

**Methods**

Data from 110 subjects with impaired glucose tolerance (IGT), 108 subjects with impaired fasting glucose (IFG) and 72 control subjects with normal glucose tolerance (NGT) were analyzed. Their age, sex, weight, height, body mass index (BMI), waist-hip circumferences, glucose levels during oral glucose tolerance test and fasting insulin levels were recorded. The degree of insulin resistance was determined with the homeostasis model assessment of insulin resistance (HOMA-IR). Fat mass values were calculated by a formula based on Quetelet index, which uses sex, weight and height measurements. For REE calculation, the Harris-Benedict equation was used.

**Results**

No significant differences in age, sex, BMI, fat mass, and HOMA-IR levels were determined between IGT, IFG and NGT groups. Waist circumference and waist to hip ratio of IGT group were higher than NGT group. There was no significant difference between three groups in terms of REE.

**Conclusion**

It is well known that diabetic patients have an increased REE than the normal population. But in this study, we concluded that impaired glucose metabolism patients who have increased risk for development of diabetes had any difference from NGT controls in terms of REE measurements.

**P714****A rare case: Shwachman-Diamond syndrome presenting with diabetic ketoacidosis**

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**Introduction**

Schwachman-Diamond syndrome (SDS) is usually manifested with exocrine pancreatic insufficiency, short stature, and chronic neutropenia. SDS associated with osteoporosis and diabetes mellitus (DM) presenting with ketoacidosis is quite rare.

**Case**

A 29-year-old woman was admitted to emergency room with symptoms of frequent urination and confusion. She had no history of diabetes mellitus or any other disease. On her examination, she had no orientation and cooperation. She appeared dehydrated. Lab findings were as follows: Hg: 15.5/ $\mu\text{l}$ , WBC: 1200/ $\mu\text{l}$  (NEU: 100/ $\mu\text{l}$ ), glucose: 483 mg/dl, total protein: 5.5, albumin: 2.2, calcium: 5.6 mg/dl, phosphorus: 0.1 mg/dl, pH: 6.94,  $\text{HCO}_3^-$ : 4.3 mmol/l. At urinalysis, ketonuria was +3. Patient was hospitalized and treated with i.v. fluid and electrolyte replacement and insulin infusion. Stool analysis revealed rare protein fibers. Her serum amylase and lipase were determined at relatively lower levels. Her bone survey was performed and osteopenia (L2 T score: -1.65, Z score: -1.63 and trochanter of femur T score: -2.29, Z score: -1.95) was detected. 25OH vit D was 6, PTH was 99. Her HbA1c was 11.4%, C-peptide was 1.8 (0.4–4) and anti-insulin antibody was positive. Pancreatic atrophy was detected on abdominal computed tomography. Because she had neutropenia, findings of malabsorption and pancreatic atrophy, the patient was considered as SDS. Genetic analysis was performed for monosomy 7. So the patient was diagnosed as SDS. Pancreatic enzymes and insulin replacement was administered. After clinical and laboratory improvement, she was discharged and she had cyclic neutropenia attacks in her follow-up.

**Conclusion**

Despite DM and SDS rarely coexists, the probability of this condition must be considered in diabetic patients whose clinical features fit. Genetic analysis should be done to support diagnosis and patients should be followed up to late stages in order not to overlook complications such as osteoporosis and malnutrition.

**P715****Autoimmune markers in patients with type 1 diabetes mellitus**

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**Introduction**

Type 1 diabetes mellitus (DM) is frequently associated with other autoimmune diseases. In this study, we aimed to evaluate thyroid autoantibodies, celiac antibodies and antiparietal antibody in patients with type 1 DM.

**Methods**

Thyroid antithyroid peroxidase (anti-TPO) and antithyroglobulin (anti-Tg) antibodies, antiinsulin, antiislet cell and anti-glutamic acid decarboxylase (anti-GAD) antibodies, antiparietal antibody, antiendomysium, antigliadin IgA and IgG antibodies were obtained from the records in patients diagnosed and followed with type 1 DM in our clinic. Also, thyroid functional status and serum vitamin B12 levels were evaluated. In available patients, thyroid ultrasonography (US) findings were recorded.

**Results**

One hundred and sixty-one patients with type 1 DM were included in the study. Antibodies related to type 1 DM were obtained in 117 patients and 63 (53.80%) had positive anti-GAD, 4 (3.42%) had positive antiinsulin, 13 (11.11%) had positive antiislet cell antibodies. Thyroid functions were normal in 138 (85.71%) patients, 18 (11.18%) had hypothyroidism and 5 (3.11%) had thyrotoxicosis. Anti-TPO antibody was positive in 55 (34.16%), Anti-Tg was positive in 31 (19.25%) patients, and in 58 (36.02%) patients at least one of these antibodies was positive. Thyroid US was performed in 113 patients, 59 (52.21%) had normal thyroid US findings, 49 (43.37%) had chronic thyroiditis with or without nodules, 18 (15.93%) had solitary or multiple nodules. Celiac antibodies were positive in 15 (14.42%) of 104 patients. Among 77 patients with available antiparietal antibody, it was positive in 9 (11.69%) and negative in 68 (88.31%) patients. Forty-six (28.57%) patients had vitamin B12 levels lower than 250 pg/ml.

**Conclusion**

Autoantibodies related to various diseases may be observed with high frequency in patients with type 1 DM. It seems reasonable to screen for concomitant thyroid diseases, celiac disease and vitamin B12 deficiency by measuring specific antibodies in a patient newly diagnosed with type 1 DM.

**P716****Prevalence of non-alcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes**

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**Introduction**

Non-alcoholic fatty liver disease (NAFLD) is a key component of the insulin resistance, the metabolic syndrome and type 2 diabetes mellitus (DM2). The aim was to study the prevalence of NAFLD in women and men with DM2.

**Methods/design**

This is a retrospective, cross-sectional, observational study. We analyzed data from in-hospital patients: 96 women (mean age 63.2 ± 11.4 years) and 80 men with DM2 (mean age 59.0 ± 10.7 years). Body weight, height, BMI, waist circumference, systolic and diastolic blood pressure were analyzed as well as laboratory parameters such as fasting plasma glucose (FPG), HbA1c, serum lipids, liver enzymes (ASAT, ALAT, GGT). The liver shape, size and sonographic pattern were documented by abdominal sonography.

**Results**

7.6% of DM2 women had ALAT >40 IU/l and 32.5% - GGT >35 IU/l; respectively 11.2% of DM2 men had ALAT >40 IU/l and 35.0% - GGT >35 IU/l. 2/3 of the women and 40% of the men with DM2 had liver steatosis at sonography. Between group differences (with/without NAFLD) were significant only for ALAT in women with DM2 and for BMI ( $P=0.027$ ), diastolic blood pressure ( $P=0.031$ ), and GGT ( $P=0.044$ ) in men. If a diagnostic threshold for detecting NAFLD of 40 IU/l is set the sensitivity of ALAT is around 15% in women and 29% in men and that of GGT - around 20% in women and around 33% in men with DM2.

**Conclusion**

This study documented a high prevalence of sonographic signs for liver steatosis in women and men with diabetes type 2. An epidemiologic survey of the prevalence of NAFLD is needed.

**P717****The fed to fasting transition differentially affects PEPCK protein expression in pericentral and periportal murine hepatocytes**

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Hepatic glucose metabolism is zoned. Glycolysis is mainly localized in hepatocytes surrounding the hepatic vein (pericentral zone) whereas gluconeogenesis is mainly carried out by hepatocytes surrounding the portal vein (periportal zone). Expression of the gene encoding the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK, PCK1) is reduced upon feeding, an effect mainly controlled by insulin. PEPCK is considered a peri-portal enzyme. However, the differential effects of feeding and fasting and thus changes in plasma insulin concentrations on PEPCK zonation are unknown. In the present study, laserdissection microscopy, immunohistochemistry and primary periportal and pericentral hepatocytes were used to assess the effects of feeding and fasting on PEPCK gene and protein zonation. The reduced hepatic *Pepck* mRNA concentrations in fed mice resulted in reduced PEPCK protein expression in pericentral but not in periportal hepatocytes. In alloxan-induced type 1 diabetic mice, which lack insulin, *Pepck* mRNA expression and PEPCK protein levels did not increase upon fasting, confirming the regulatory role of insulin. Nevertheless, PEPCK was still zoned along the liver acinus suggesting that other factors than insulin mediate PEPCK zonation. In conclusion, our data show that the feeding to fasting response affects PEPCK protein expression and this is mediated by insulin. This transition mostly affects pericentral hepatocytes. However, not insulin but other yet to be determined factor(s) regulate PEPCK zonation along the liver acinus.

**P718****Disturbed subjective sleep characteristics in adult patients with type 1 diabetes mellitus**

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**Introduction**

Sleep restriction and/or impaired sleep quality negatively influence glucoregulation. The aim of this study was to assess subjective sleep parameters in patients with type 1 diabetes, to relate sleep parameters to long-term glycaemic control, and to assess possible risk factors for impaired sleep.

**Research design and methods**

We studied 99 adult patients with type 1 diabetes (55M, 44F, duration of diabetes 26.9 ± 1.2 years) and 99 age-, gender-, and BMI- matched non-diabetic controls. Subjective sleep characteristics were assessed by validated questionnaires, i.e. Pittsburgh Sleep Quality Index (PQ4I), Epworth Sleepiness Scale (ESS), and the Berlin Questionnaire (BQ). Glucoregulation was assessed by HbA1c-values. Clinical parameters were obtained from medical charts. Depression was assessed by the Hospital Anxiety and Depression Scale (HADS). Peripheral polyneuropathy was assessed by neurological examination and quantitative sensory testing. Results

Thirty-five percent of the patients with type 1 diabetes had subjective poor sleep quality compared to 20% of the control subjects ( $P=0.021$ ). A higher proportion of the patients with type 1 diabetes were at increased risk for obstructive sleep apnoea (OSA) (17.2 vs 5.1%,  $P=0.012$ ). There was no significant association between individual sleep characteristics and HbA1c-values. On logistic regression analysis, HADS depression score, presence of polyneuropathy, habitual snoring, and other sleep disturbances, i.e. hypoglycaemia, were independently associated with poor sleep quality.

**Conclusion**

Adult patients with longstanding type 1 diabetes have disturbed subjective sleep quality and a higher risk for OSA, compared to control subjects. Subjective sleep disturbances are part of the complex syndrome of longstanding type 1 diabetes.

**P719****Prevalence of depressive syndrome in type 2 diabetes. Sociodemographic, clinical and biochemical differences**

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**Background**

Depressive syndrome is a common psychiatric disorder in type 2 diabetic patients (T2DM), with negative outcomes in metabolic control and risk of developing chronic complications among these patients.

**Aims**

i) To know the prevalence of depressive syndrome in a group of 113 T2DM seen consecutively in an endocrinologic outpatient clinic. ii) To analyze if there are any sociodemographic, clinical or biochemical differences between T2DM with positive screening for depressive syndrome and/or antidepressant treatment and T2DM who do not meet these criteria.

**Materials and methods**

One hundred and thirteen T2DM (68.1% men, age  $59.5 \pm 8.3$  years, diabetes duration  $11.2 \pm 8.4$  years) were evaluated for depressive symptoms using the Beck Depression Inventory Questionnaire (BDI). Sociodemographic (gender, ethnic group, marital status, studies), clinical (smoking, alcohol consumption, type of hypoglycaemic drugs, BMI, waist circumference, known microvascular or macrovascular complications related to T2DM, hypertension, antidepressant drugs) and biochemical (HbA1c, lipid profile) data were obtained from the patient's medical history. A screening in BDI > 13 and/or taking antidepressant therapy was considered positive for depressive syndrome.

**Results**

We found a positive screening for depression in 54.9% T2DM evaluated. Only 50% of these patients positively screened were treated with antidepressants. Moreover, we found that the greater the duration of T2DM, the higher the risk of depression existed ( $P < 0.001$ ). As in the general population, we found more risk for depression in females ( $P < 0.001$ ), smoking ( $P < 0.01$ ) and being single ( $P < 0.02$ ). Having diabetic retinopathy was more related to depressive syndrome than any other complication related to T2DM ( $P < 0.03$ ).

**Conclusions**

There is a high prevalence of depressive syndrome in T2DM and it's often undertreated. However, we did not find any differences in metabolic control.

**P720****Diagnostic criteria for glucose homeostasis abnormalities in obese patients: are there any differences?**

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**Introduction**

The course of type 2 diabetes mellitus (DM) is insidious and, before its development, other glucose homeostasis abnormalities can be detected: impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and elevation of HbA1c.

**Aims**

To compare the prevalence of glucose homeostasis abnormalities in obese patients, using diagnostic criteria of American Diabetes Association (ADA) 2009, ADA 2010 and International Diabetes Federation (IDF) 2005.

**Methods**

Patients were 39.9 (10.6) years old and had a body mass index (BMI) of  $45.5$  ( $5.4$ )  $\text{kg/m}^2$ . Mean fasting glycaemia was  $99.1$  ( $20.1$ )  $\text{mg/dl}$ , mean glycaemia at 120' of OGTT was  $147.5$  ( $52.0$ )  $\text{mg/dl}$  and mean A1c was  $5.8$  ( $0.8$ )%. Considering ADA 2009 criteria, prevalence of isolated IFG was  $8.4\%$ , isolated IGT was  $17.5\%$ , IFG associated with IGT was  $4.9\%$  and DM was  $20.3\%$ . When the criteria  $A1c \geq 6.5\%$  was added to the previous criteria, the percentage of DM rose to  $23.1\%$ . On the other hand, when considering each criteria of DM alone, prevalence of DM was  $10.5\%$  by fasting plasma glucose  $\geq 126$   $\text{mg/dl}$ ,  $18.9\%$  by plasma glucose at 120' of OGTT  $\geq 200$   $\text{mg/dl}$  and  $14.0\%$  by  $A1c \geq 6.5\%$ . Finally, using the criteria of IDF 2005, prevalence of IFG decreased to  $2.1\%$  and IFG combined with IGT decreased to  $3.5\%$ .

**Conclusions**

OGTT alone was the best method for diagnosing diabetes in obese patients. Criterion  $A1c \geq 6.5\%$  increased the prevalence of diagnosed DM.

**P721****Glycated hemoglobin as diagnostic criteria for diabetes and prediabetes in obese patients**

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**Introduction**

Recently, the American Diabetes Association has proposed glycated hemoglobin (HbA1c) as a diagnostic criteria for diabetes ( $HbA1c \geq 6.5\%$ ) and prediabetes ( $5.7\% \leq HbA1c \leq 6.4\%$ ).

**Aims**

To compare HbA1c with fasting glucose and oral glucose tolerance test (OGTT) as diagnostic criteria for diabetes and prediabetes, in obese patients; to calculate its sensitivity and specificity when compared with standard diagnostic criteria.

**Materials and methods**

We studied 417 obese patients (73 men) without previous diagnosis of diabetes or prediabetes with mean age =  $41.8 \pm 12.4$  years, BMI =  $46.6 \pm 7.2$   $\text{kg/m}^2$  and waist circumference =  $124.7 \pm 16.1$  cm. They were submitted to a fasting blood collection for glucose and HbA1c assessments, followed by a classic 75 g OGTT. Patients were divided in diagnostic groups (normal values, prediabetes and diabetes) according to HbA1c or OGTT, and we studied the concordance degree between groups.

**Results**

The mean fasting glucose =  $95.2 \pm 19.6$   $\text{mg/dl}$ , 2nd hour glucose =  $127.8 \pm 54$   $\text{mg/dl}$  and HbA1c =  $5.73 \pm 0.78\%$ . According OGTT criteria, 232 patients (55.6%) showed normoglycemia, 135 (32.4%) prediabetes (impaired fasting glucose IFG = 49; impaired glucose tolerance IGT = 53; IFG + IGT = 33) and 50 (12%) diabetes. Based on HbA1c criteria, 221 patients (53%) had normal HbA1c, 151 (36.2%) prediabetes and 45 (10.8%) diabetes. Comparing both criteria classifications, there was a significant difference ( $P < 0.001$ ) between diagnostic groups. HbA1c as diagnostic criteria for diabetes and prediabetes showed, respectively, a sensitivity of 50 and 45.2% and a specificity of 55.6 and 40.4%. Comparing both diagnostic methods, no significant difference for gender was found.

**Conclusion**

Diabetes and prediabetes are highly prevalent in the obese population, by either HbA1c or glucose criteria. However, each method identifies different groups of patients with abnormal control of carbohydrate metabolism. Comparing with the standard criteria for diabetes and prediabetes (based on glucose values), HbA1c shows low sensitivity and specificity as diagnostic criteria for carbohydrate dysmetabolism in the obese population.

**P722****Impact of INSR His1085 and IRS1 Gly972Arg genetic variability on lipididaemia in patients with metabolic syndrome and type 2 diabetes**

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**Introduction**

The metabolic syndrome is increasingly recognized as a powerful predictor of patient risk for developing cardiovascular and cerebrovascular complications. It is viewed as the phenotypic confluence of atherogenic dyslipidaemia, hypertension, central obesity and insulin resistance (with or without type 2 diabetes) resulting from dysregulated gene expression and lifestyle behaviours.

**Aim**

Because insulin resistance is a core defect in type 2 diabetes and the metabolic syndrome and may cause the atherogenic dyslipidaemia, the aim of the study was to establish a possible correlation between INSR His1085 (C/C, C/T, T/T) and IRS1 Gly972Arg (G/G, G/A, A/A) genetic variability and levels of triglycerides, high-density lipoprotein cholesterol (HDL-C) and small dense low-density lipoprotein (LDL) particles.

**Methods**

We enrolled 214 patients (125 men and 89 women) average age of  $66 \pm 10$  years with average duration of type 2 diabetes and metabolic syndrome of 9 years (5–13). Genomic DNA was extracted from peripheral blood leukocytes while analysis of His1085 and Gly972Arg gene mutations was performed with PCR restriction fragment length polymorphism method (PCR-RFLP). Lipid parameters were determined on an automatic analyzer using original reagents for measurement.

**Results**

We did not found statistical significance between carriers of examined genotypes and levels of lipid parameters.

**Conclusion**

Our study has shown that the INSR His1085 (C/C, C/T, T/T) and IRS1 Gly972Arg (G/G, G/A, A/A) genetic variability is not associated with different lipid profile of the examinees.

**P723****High prevalence of impaired glucose metabolism in young adult patients with Williams syndrome**

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**Introduction**

Williams syndrome (WS) is a rare, multisystemic genomic disorder. While the natural history of the disease in childhood is quite well-known, few descriptions of the adult WS medical complications have been published.

**Method**

Twenty-two young adult patients with WS (13 females, 9 males, age: 29.2 ± 5.4 years) were studied. A 75 g oral glucose tolerance test (OGTT) was performed in all but one with known diabetes mellitus (DM). β-Cell function was estimated with HOMA-B% and with insulinogenic index at 30 min ( $\Delta I/\Delta G_{30}$ ). The insulin sensitivity was assessed with HOMA-IR, QUICKI and composite insulin sensitivity Matsuda index (ISI).

**Results**

Impaired glucose tolerance (IGT) was diagnosed in 12 subjects and DM in one, thus showing that 59% of this population had impaired glucose metabolism. ICA and GAD antibodies were absent. Logistic regression showed that the presence of impaired glucose metabolism was not associated with BMI ( $P=0.267$ ,  $B=0.118$ ), age ( $P=0.977$ ,  $B=0.004$ ), family history of diabetes ( $P=0.653$ ,  $B=0.747$ ), which are known risk factors for DM. WS subjects with IGT were more insulin-resistant than WS with normal glucose metabolism (HOMA-IR 2.3 ± 0.9 vs 1.0 ± 0.6,  $P=0.008$ ; QUICKI 0.34 ± 0.02 vs 0.39 ± 0.04,  $P=0.002$ , ISI 3.2 ± 1.4 vs 7.4 ± 2.8,  $P=0.006$ ). WS subjects with normal glucose tolerance compared to healthy adults matched for gender, age and BMI did not show any difference in β-cell function, insulin sensitivity, insulin levels during OGTT, but showed higher glucose levels at 120' after glucose load.

**Conclusion**

Given the high prevalence of impaired glucose regulation, adults with WS should be screened for diabetes or IGT. The reasons of this high prevalence of impaired glucose metabolism are not known. We excluded autoimmunity and usual risk factors for DM. Hemizygoty for a gene mapping to the WS chromosome region is likely the factor responsible for the high frequency of diabetes and IGT in WS.

**P724****TCF7L2 gene variants and plasma C-peptide concentrations in diabetes type 2**

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**Aims/hypothesis**

Several data have shown the association between diabetes type 2 (DM2) and transcription factor 7-like 2 (TCF7L2) gene polymorphisms, in several and different ethnical populations. Although the pathophysiology is not completely clear, it seems that beta and alpha cell functions are abnormal. Up to these days there is no clinical data showing association between TCF7L2 gene polymorphism allele rs7903146 T and beta cell insulin and C-peptide contents in DM2 patients.

**Methods**

The present study evaluated the association of rs7903146 TT genotype (high risk) and rs7903146 CC genotype (low risk) with fasting plasma glucose (G), insulin (IN), C-peptide (C-Ppt), in DM2 patients carriers of TCF7L2 gene polymorphisms. Population characteristics: age (58 ± 5) years; BMI (29 ± 3) kg/m<sup>2</sup>, gender (80 F/50 M). The genetic analysis by ABI-TaqMan genotyping assays. Statistics (Kruskal-Wallis one way ANOVA).

**Results**

According to rs7903146 genotypes, the wild type (CC) was present in 43% and the risk-conferring genotypes (CT e TT) were present in 47.1 and 9.9% respectively. The results between groups TT×CT×CC were respectively: glucose (mmol/l) (6.35 ± 1.16×6.88 ± 1.66×7.00 ± 1.22) NS; C-peptide (ng/ml) (2.5 ± 0.2×2.6 ± 0.6×3.6 ± 0.9)  $P<0.04$  insulin (μIU/ml) (8.75 ± 13×11.65 ± 7×10.95 ± 6.9) NS.

**Conclusion**

C-peptide plasma concentration was lower in DM2 patients carriers of TCF7L2 gene polymorphism rs7903146 TT genotype. There were no differences on insulin and glucose plasma concentrations among no-risk (CC), medium-risk (CT) and high risk (TT) DM2 carriers.

**P725****Risk factors of conversion from IGT and IFG to diabetes type 2**

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**Objective**

To determine the predictive risk factors for the development of type 2 diabetes mellitus (DM) in patients with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) during the 8.5 follow up.

**Research design and methods**

This is an 8-year prospective study in a randomly selected urban population including 2838 subjects aged ≥ 40 years living in Krakow. Seven hundred and six had IGT (387) or IFG (319) based on WHO criteria and 264 of them attended the follow-up assessment eight years later. 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.

The prevalence of type 2 diabetes in examined population of 264 people with previously (1998–2000) found IGT or IFG was 17%. Diabetes type 2 was found in 22% of people with previously diagnosed IFG and in 13% of people with previously found IGT. Among people with diagnosed diabetes, 51% had newly diagnosed diabetes during the control study, in 49% participants diabetes was diagnosed before in the period between the baseline and control study. In the studied population, statistically significant predictive factors of the progression to type 2 diabetes were fasting glycemia (RR 3.27;  $P<0.001$ ), fasting hyperinsulinemia (RR 2.1;  $P<0.05$ ), insulin resistance measured as HOMA IR index (RR 1.99;  $P<0.05$ ), WHR, (RR 1.67,  $P<0.01$ ) and family history of type 2 diabetes (RR 2.21;  $P=0.01$ ).

**Conclusion**

In the studied population, important predictive factors of the progression to type 2 diabetes were fasting glycemia, fasting hyperinsulinemia, insulin resistance measured as HOMA IR index, WHR, and family history of type 2 diabetes.

**P726****Effects of the ghrelin gene products on glucose metabolism in C2C12 muscle cells**

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Type II diabetes is a clinical disorder of glucose and fat metabolism, caused by an inability of insulin to promote sufficient glucose uptake into adipose tissue and striated muscle and to prevent glucose output from the liver. Skeletal muscle is an important element for the maintenance of glucose homeostasis because it can increase, after stimulation, glucose uptake from the blood. In previous experiments we found that acylated ghrelin (AG), unacylated ghrelin (UAG), obestatin (Ob) exert antiapoptotic and protective effects on β-cells and human pancreatic islets; moreover they enhance insulin secretion and stimulate glucose uptake in β-cells and human pancreatic islets. The aim of this study is to gain further insight on the metabolic role of AG, UAG and Ob in peripheral tissues, in particular on a muscle cell line (C2C12). Cells were grown in DMEM containing 10% FCS. At 80% confluence, cells were switched to a 2% heat-inactivated horse serum in DMEM, for differentiation of myoblast into myotubes. During this time, medium was changed every 24 h, and treatments began on day 5. All the experiments were conducted on myotubes. Gene expression was evaluated by RT-PCR or real time PCR, while the activation of specific signaling pathways by western blotting. To assess the effects of the peptides, we performed metabolic

studies: free fatty acid (FFA) and glucose uptake, respectively using QBT Fatty acid uptake kit and [<sup>3</sup>H]-2-deoxyglucose (with liquid scintillation counting). In C2C12 AG, UAG and Ob increased expression of GLUT-4 and CD36, which binds long-chain fatty acids and facilitates their transport into cells. These effects involved PI3K/Akt and AMPK phosphorylation. These findings indicate that AG, UAG and Ob promote glucose and FFA uptake in C2C12 muscle cells and suggest that they may exert beneficial effects *in vivo* in disorders such as insulin resistance and type II diabetes.

## P727

### Effects of Ramadan fasting on resting energy expenditure in T2DM

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#### Introduction

Ramadan fasting may alter fuel metabolism and energy expenditure by inducing changes in the meal timing, sleep-wake patterns and physical activity. It could affect blood glucose through disrupting energy balance. Evaluation of resting energy expenditure (REE) could help to recommend best nutrition advices to diabetics for maintaining acceptable metabolic control.

#### Methods

Thirteen type 2 diabetics who were fasted in Ramadan were compared with 18 non fasting patients and 15 fasted healthy volunteers. Well controlled diabetics were allowed and uncontrolled patients were advised to avoid fasting. REE, body mass index (BMI), HbA1c and fructosamine were measured in the first and last week of Ramadan and one week later. REE decreased significantly in all three groups during Ramadan. (13.5% ( $P=0.002$ ), 10.2% ( $P=0.007$ ) and 9.5% ( $P=0.007$ ) in fasting and non fasting diabetics and fasting healthy subjects respectively). HbA1c and fructosamine decreased significantly in non fasting diabetics ( $P=0.008$ ), because baseline HbA1c was higher in this group. Since HbA1c was in acceptable range in fasting diabetics, it didn't change significantly. BMI did not change significantly in any of the groups.

#### Conclusion

Ramadan fasting reduces REE, and this reduction in diabetic and healthy subjects is merely a consequence of fasting itself and is not affected by BMI or HbA1c changes. REE reduction in non fasting diabetic patients may be the result of close supervision during study and lifestyle changes brought about by alterations in everyday life of people in Ramadan. So, reduced REE may call for specific diet plans for diabetic patients who are willing to fast, and still maintain good metabolic control during Ramadan.

Keywords: Diabetes Mellitus, Resting Energy Expenditure, Ramadan Fasting, blood glucose, HbA1c.

## P728

### Chronic supplementation of $\beta$ -hydroxy- $\beta$ methylbutyrate (HM $\beta$ ) impairs glucose homeostasis in rats under normal conditions

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#### Aim

HM $\beta$  is a metabolite of leucine commonly used by athletes and in medical practice in order to increase muscle mass and/or minimize protein degradation. We have previously shown that chronic intake of HM $\beta$  increases GH synthesis and secretion in rats, which might account to its anabolic effect. However HM $\beta$ -treated rats, although normoglycemic, exhibited hyperinsulinemia, suggesting an apparent insulin resistance (IR) state. The present study attempted to investigate this possibility.

#### Methods

Rats were treated with HM $\beta$  (320 mg/kg BW) or vehicle, by gavage, for 4 weeks, during which the body weight was monitored. After this period, part of the animals was subjected to glucose tolerance test (GTT), and the remainder was sacrificed by decapitation for removal of the soleus and extensor digitorum longus (EDL) muscles to evaluate the GLUT4 content (GLUT4/g tissue), dry/wet weight ratio and cross-sectional area (CSA). Data were analyzed by unpaired Student's *t*-test. The HM $\beta$  chronic administration resulted in IR, as shown by the GTT ( $P<0.001$ ). No alterations were observed in the animals' weight gain and in the dry/wet weight ratio of soleus and EDL muscles between groups. However, it was

found a significant reduction of CSA and total GLUT4 content ( $P<0.05$ ) in soleus muscle of the HM $\beta$ -treated rats.

#### Conclusion

The chronic administration of HM $\beta$  induces an apparent IR state in a stage in which the hyperinsulinemia seemed to account for normoglycemia. However the reduction of GLUT4 content and CSA in soleus muscle strongly indicates that the repercussions of the IR state are taking place, which might limit the recognized beneficial effects of HM $\beta$ , at least in rats under normal conditions.

## P729

### Serum levels of fetuin-A correlate with bone turnover biomarkers in patients with type 2 diabetes mellitus

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#### Objective

Fetuin-A, which is a blood protein produced by liver and promotes bone mineralization, is an independent risk factor for type 2 diabetes. Whether Fetuin-A levels relate to the bone metabolism in type 2 diabetic patients is still not clear. Our study aimed to evaluate the relationship of fetuin-A to the markers of bone metabolism and bone turnover in male and female type 2 diabetic patients.

#### Participants and methods

Eighty type 2 diabetic patients, 40 males and 40 post-menopausal females (matched for age, body mass index (BMI) and duration of diabetes) were studied. Fetuin-A (using commercially available ELISA test) together with metabolic, bone, lipid, renal, and liver function parameters and sex hormones were measured in all participants.

#### Results

Levels of Fetuin-A did not differ significantly between male and female diabetic patients. Fetuin-A correlated significantly negatively with blood levels of C-telopeptide ( $r=-0.33$ ,  $P<0.04$ ) and positive with estradiol ( $r=0.44$ ,  $P<0.009$ ) among female group. In males, a significant positive correlation was observed between fetuin-A and the blood levels of bone alkaline phosphatase (ALP) ( $r=0.33$ ,  $P<0.03$ ). Controlling for age, BMI, fatty liver index (FLI), blood protein, albumin, calcium, gamma glutamyl-transferase (GGT) and C-reactive protein (CRP) did not alter the significance of these correlations.

#### Conclusions

Our results suggest significant correlations of fetuin-A levels with markers of bone turnover and bone metabolism in type 2 diabetic patients independent of age and BMI. More studies are needed to determine whether fetuin-A levels could indicate or predict risk of osteoporosis among them.

## P730

### The stress influence on the diabetes mellitus occurrence in the territory of northern Kosovo and Metohija in the period of 2000–2002

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The paper shows the data on diabetes mellitus occurrence in the population of northern Kosovo and Metohija (local inhabitants and displaced person). The research comprises results of 42 495 examinations in Unit of Internal Diseases of Medical Centre in Kosovska Mitrovica in the period of January 2000–December 2002 (14 000 examinations on average, annually). Diabetes was diagnosed and classified on the basis of the latest internationally recognized criteria in 280 580, that is 542 persons in the period studied. A particularly high occurrence of diabetes in the population examined was recorded in 2001 (4.05%). Recently diagnosed patients with diabetes make up around 30% of patients with diabetes examined in this period. Such high percentage of recent patients with diagnosed diabetes, particularly in 2001, indicates stress influence and change of live environment as highly probable causes of this disease.

Keywords: Diabetes mellitus, stress.

**P731****Prevalence of a gestational diabetes and its risk factors estimation**

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The medical and social importance of a problem of a gestational diabetes (GD) is caused by higher probability of occurrence of the complicated current of pregnancy and delivery. Frequency of a GD varies within 1.5–13% from total pregnancies, essentially increasing in risk groups.

**Materials and methods**

One hundred and fifty-six pregnant women in different periods of gestation were examined. The estimation of GD's risk factors was spent to all women. Low risk was revealed in 7 women, medium – in 43 pregnant women, high – in 62 patients. 75-g OGTT was spent between 24 and 28 weeks' gestation to women at low and medium GD risk. Women at high risk of GD were screened using the 75-g OGTT at first antepartum visit.

**Results**

GD was diagnosed in 10/156 (15.6%) in women at high risk for GD. Fasting glucose level in women with GD was  $5.4 \pm 1.7$  vs  $4.8 \pm 0.4$  mmol/l in women without GD ( $P=0.293$ ). Two-hours glucose level in women with GD was  $8.3 \pm 1.4$  vs  $5.5 \pm 1.2$  mmol/l in women without GD ( $P=0.001$ ). GD was diagnosed in 42/146 (28.7%) women at low and medium risk for GD. Fasting glucose level in GD group was  $4.9 \pm 0.6$  vs  $4.6 \pm 0.6$  mmol/l in women without GD ( $P=0.005$ ). Two-hours glucose level in GD group –  $8.9 \pm 0.9$  vs  $5.8 \pm 1.2$  mmol/l in women without GD ( $P=0.001$ ). 45.2% of GD women had parents with diabetes versus 26.3% women without GD ( $P=0.024$ ). Other risk factors for GD statistically didn't differ among groups.

**Conclusion**

GD prevalence at pregnant women has made of 15.6%. In group with high risk for GD should be included pregnant with singular risk factor – burdened heredity on a diabetes.

**P732****Relationship between diabetes and pancreatic cancer**

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**Background**

Pancreatic cancer (PC) is rare, representing 6.8% of mortality in our country. Diabetes mellitus (DM) is often associated. Main hypothesis include: PC triggers DM; DM is a risk factor for PC; diabetes treatment modifies cancer risk.

**Objective**

To evaluate DM and PC association.

**Methods**

Evaluation of patients with PC (2006–2009), divided into: group A (without DM) and group B (with DM). Parameters evaluated: age, gender, BMI, hypertension, dyslipidaemia, hyperuricaemia, smoking, alcoholism and pancreatitis; location, tumor size and invasion at diagnosis; treatment and survival. Group B was subdivided according to DM treatment and duration (subgroup B1  $\leq 1$  year, subgroup B2  $> 1$  year).

**Results**

A total of 132 patients evaluated with PC: 70 male (53%), 62 female (47%). Group A: 59.8% ( $n=79$ ); at diagnosis:  $67.8 \pm 14.6$  years old, BMI  $25.2 \pm 3.9$  kg/m<sup>2</sup>, hypertension 38.0%, dyslipidaemia 17.7%, hyperuricemia 2.5%, alcoholism 80.8%, tumor size 4.1 cm. Group B: 40.2% ( $n=53$ ); at diagnosis:  $66.7 \pm 12.2$  years old, BMI  $26.0 \pm 4.7$  kg/m<sup>2</sup>, hypertension 69.8%, dyslipidaemia 50.9%, hyperuricemia 17.0%, alcoholism 53.8%, tumor size 3.4 cm. There was statistical difference for: hypertension (OR=3.78), dyslipidaemia (OR=4.82), hyperuricemia (OR=7.86), alcoholism (OR=3.60) and tumor size. There was no statistical difference ( $P>0.05$ ) for smoking, pancreatitis, tumor location, invasion and mortality. Diabetes treatment in group B: 58.5% on oral agents, 56.6% on insulin. Subgroup B1: 72.0%; subgroup B2: 28.0%. Diabetes duration didn't influence tumor invasion, but influenced tumor size (subgroups B1 4.55 cm; B2 2.99 cm,  $P=0.01$ ). Overall survival was  $15.3 \pm 1.9$  months. There was no survival difference between: groups A and B (18.5 vs 11.7 months), treatment with insulin or oral agents (16.9 vs 13.5 months), subgroups B1 and B2 (15.4 vs 15.8 months).

**Conclusions**

Diabetes may be a consequence of pancreatic cancer since most patients had diabetes for  $< 1$  year. There was no difference in survival between groups,

irrespective of hypoglycemic therapy. Relationship between DM and PC is complex and needs further investigation.

**P733****Vaspin circulating levels in severely obese women with type 2 diabetes mellitus (T2DM): early changes following restrictive bariatric surgery**

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**Introduction**

Vaspin, identified as a novel insulin-sensitizing adipokine, is suggested to be dysregulated in T2DM patients with loss of association between circulating vaspin levels, BMI and insulin resistance (IR). The aim of this study was to examine whether restrictive bariatric surgery in severely obese women with T2DM leads to a realignment of vaspin and metabolic factors early following surgery and before complete resolution of diabetic status.

**Methods/design**

Therefore 9 women with T2DM and BMI  $> 35$  kg/m<sup>2</sup> underwent restrictive bariatric surgery. Anthropometric and biochemical assessments were performed prospectively at baseline and 4 weeks post surgery. An age-matched control group of 13 non-diabetic women with BMI  $< 25$  kg/m<sup>2</sup> was included in the study. (Data shown as mean  $\pm$  s.e.m.;  $P < 0.05$  was considered statistically significant).

**Results**

Circulating vaspin in controls correlated with HOMA-IR and fasting insulin ( $P < 0.05$ ), whereas vaspin serum levels in obese T2DM subjects did not correlate with IR indices and BMI. Obese T2DM subjects had almost fourfold higher circulating vaspin levels compared with controls ( $0.42 \pm 0.15$  vs  $0.11 \pm 0.03$  ng/ml;  $P < 0.01$ ). One month post bariatric surgery vaspin decreased by 48% compared to baseline (post-op levels:  $0.22 \pm 0.06$  ng/ml,  $P < 0.05$ ); still twofold higher than controls. Obese T2DM women 4 weeks post surgery significantly reduced BMI, percent body fat, HOMA-IR, fasting insulin and fasting glucose ( $P < 0.05$ ), however vaspin remained uncorrelated with these post surgery reductions.

**Conclusion**

Despite a 48% reduction, circulating vaspin remained uncorrelated with fat loss and IR improvement in obese T2DM women early after restrictive bariatric surgery. Further studies need to examine whether vaspin regains correlative status at later time-points post bariatric surgery following T2DM resolution. Supported by EFSD New Horizons research grant.

**P734****Does anxiety influence food preference and insulin resistance in healthy men?**

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**Introduction**

We tested our hypotheses that higher anxiety/depression scores can be connected with food preference of carbohydrates and higher insulin resistance.

**Methods**

We examined 42 healthy men (average age  $43.5 \pm 7.4$  years, average BMI  $27.4 \pm 5.7$  kg/m<sup>2</sup>). Study protocol included filling in the self-assessing scores of anxiety (SAS) resp. depression (SDS), carbohydrate-craving questionnaire (CCQ); assessment of 3-days diet records, hyperinsulinemic euglycemic clamp on two levels of insulinemia (1 and 10 mU/kg per min) with calculation of glucose disposal resp. metabolic clearance rate of glucose for both insulin levels (M1, M2 resp. MCR1 a MCR2) and citalopram challenge test (infusion of 0.3 mg/kg of citalopram with measurement of prolactin levels in minutes: –30, –15, 0, 15, 30, 45, 60, 90, 120, 150 and with calculation of area under the curve for prolactin levels. Student's *t*-test, Kolmogorov-Smirnov test and Spearman's correlation coefficient were used for statistical analyses.



## Results

We observed positive correlations between SAS resp. SDS scores and intake of monosaccharides in food:  $r=0.44$ ,  $P=0.02$ ; resp.  $r=0.4$ ,  $P=0.03$ , but negative correlation between MCR2 and SAS resp. SDS in the whole studied group:  $r=-0.38$ ,  $P=0.04$ ;  $r=-0.04$ ,  $P=0.03$ , whereas in the subgroup of insulin sensitive subjects MCR2 and SAS correlated positively:  $r=0.55$ ,  $P<0.05$ . We did not find any relationship between response in citalopram challenge test and either food preference of carbohydrates or parameters of insulin resistance.

## Conclusions

Subjects with higher depression/anxiety scores prefer more carbohydrates in the food. In general, subjects with higher depression/anxiety scores are more insulin resistant in comparison with those less anxious/depressive while the subjects from insulin sensitive subgroup seem to be more anxious than those insulin resistant. The study was supported by VZ MSM 0021620814.

## P735

### Insulin restores salivary secretion in diabetic rats: involvement of Na<sup>+</sup>/glucose cotransporter SGLT1

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## Introduction

Considering the high capacity of water transport of Na<sup>+</sup>/glucose cotransporter SGLT1, the aim of this study was to investigate the potential role the SGLT1 in luminal membrane of ductal cells of salivary glands upon the non-stimulated salivary secretion of diabetic rats. The present study also investigates the presence of glucose transporter GLUT1 in salivary glands of rats.

## Methods

Non-stimulated salivary secretion and SGLT1/GLUT1 protein (western blotting and immunohistochemistry) in parotid and submandibular glands were analyzed in non-diabetic and non-treated or insulin-treated (3, 6 and 9 U/day) diabetic Wistar rats.

## Results

Diabetes increased SGLT1 protein in luminal membrane of ductal cells, which was accompanied by ~80% reduction ( $P<0.001$ ) in salivary flow. Insulin treatment (6 and 9 U) decreased the SGLT1 protein expression in ductal cells of glands of diabetic rats, as compared to non-treated diabetic rats. These treatments were also able to revert ( $P<0.05$  and  $P<0.01$  respectively for 6 and 9 U) the diabetes-induced decrease in salivary secretion. Moreover, GLUT1 staining was observed mainly in basolateral membrane of ductal cells of salivary glands of non-diabetic and diabetic rats.

## Conclusions

We propose that in the salivary glands the GLUT1 participate of reabsorption of glucose in basolateral membrane, together with the SGLT1 in luminal membrane. In diabetes, reduced salivary flow was associated with increased SGLT1 protein in luminal membrane of ductal cells of salivary glands. Furthermore, insulin treatment reduced SGLT1 in luminal membrane of ductal cells, and increased salivary flow. These SGLT1 regulations, by modulating water reabsorption, might explain the alterations of salivary secretion observed in insulin-treated or untreated diabetic rats.

## P736

### The prevalence of diabetes mellitus in patients infected with human immunodeficiency virus on treatment with antiretroviral drugs

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## Background and aims

Acquired immunodeficiency syndrome (AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV). The therapy with antiretroviral drugs is associated with increasing reports of metabolic abnormalities such as body fat abnormalities, impaired glucose metabolism and insulin resistance, dyslipidemia. We investigated the prevalence of diabetes mellitus in patients with AIDS in treatment with antiretroviral drugs.

## Materials and methods

Forty-six patients (22 women and 24 men) with AIDS in treatment with antiretroviral drugs (2 nucleoside and nucleotide reverse transcriptase inhibitors + a protease inhibitor) after 5 years were recruited for this study. Fasting glycemia and 2 h OGTT (oral glucose tolerance test: ingestion of 75 g glucose

dissolved in 250–300 ml water) were assessed. Glycemia has been measured by glucose-oxidase method. Glycemia levels have been classified as recommended by World Health Organization definition. Results were compared with measurements in 38 patients with AIDS but without treatment with antiretroviral drugs.

## Results

The prevalence of impaired fasting glucose was 4.34% (2 patients), of impaired glucose tolerance was 8.69% (4 patients), and of diabetes mellitus was 10.86% (5 patients), in patients with AIDS treatment with antiretroviral drugs. The prevalence of impaired fasting glucose in patients with AIDS without treatment with antiretroviral drugs was 2.63% (1 patient) and of diabetes mellitus 2.63% (1 patient).

## Conclusion

This study showed that patients with AIDS in treatment with antiretroviral drugs have a greater prevalence of diabetes mellitus than patients with AIDS without treatment with antiretroviral drugs (level more than 3 times). Patients with AIDS in treatment with antiretroviral drugs should be routinely screened for diabetes mellitus.

## P737

### Linking CERN and TERM: testing the acceleration hypothesis

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## Objective

Catch-up growth might be associated with metabolic risk later in life. The  $\beta$ -cell has a higher energy requirement and poor antioxidant defense. The accelerator hypothesis predicts earlier onset in heavier people, without necessarily a change in risk, and views diabetes as the one disorder of insulin resistance, set against different genetic backgrounds. Insulin resistance is a function of fat mass, and increasing body weight is accompanied by earlier presentation (acceleration) of diabetes. We wanted to establish whether increasing body weight was associated with the earlier presentation of type 1 diabetes, as the hypothesis would predict. Research design and methods

The relationships between fatness and age at diagnosis were examined in context of birth weight, weight change since birth, weight and BMI at diagnosis in 130 children aged 1–16 years presenting for management of acute-onset type 1 diabetes.

## Results

BMI SDS at diagnosis and weight SDS change since birth were inversely related to age at presentation in mostly children. The girls were fatter than the boys and presented with diabetes at a younger age. The sex difference in age at diagnosis were no significant when corrected for BMI suggesting that something related to fatness was the responsible factor.

## Conclusions

The important question is how we can best fine-tune the nutritional intakes while maintaining neurodevelopment without cause long-term metabolic consequences. The implications for prevention of type 1 diabetes may be important.

Abbreviations: SDS, BMI, acceleration, diabetes mellitus, prevention.

## P738

### Metabolic syndrome in normotensive non diabetic population in Jeddah: a pilot study

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## Background and objectives

Identification of metabolic syndrome (MS) is important for the strategy to reduce the prevalence of NIDDM. The latest IDF consensus definition emphasizes waist circumference as the central criteria, while the earlier ATP III definition gives equal importance to different risk factors. In absence of local data on definition of central obesity, it might be inappropriate to use the IDF definition. Therefore, it is aimed to investigate the prevalence of metabolic syndrome in healthy individuals living in Jeddah using the two definitions to determine the relative importance of the different risk factors. It is also aimed to investigate the importance of family medical history and life style habits.

## Subjects and methods

Three hundred and fifty-seven healthy adults aged 18–50 years were recruited randomly from health centers in Jeddah, in a cross-sectional study design. Anthropometric and demographic information were taken. Insulin, glucose, and

lipids profile were measured in fasting blood samples. Individuals were identified using the two definitions of metabolic syndrome, and their anthropometric, demographic and biochemical characteristics were compared statistically to the rest of the population.

#### Results

Two hundred and thirty-three subjects satisfied the selection criteria. Using the IDF definition, 45.9% had abdominal obesity, 45.5% had high glucose, 21.7% had high triglycerides, 30.0% had low HDL-cholesterol, 12.0% had high BP, and 39 subjects (18.9%) were diagnosed with MS. These were significantly older, with higher BMI and fasting insulin. Using the ATP III definition more subjects (16.7%) were diagnosed. High glucose appeared in 45.5% of the population, low HDL cholesterol in 30%, high triglycerides in 21.7%, abdominal obesity in 25.3%, and higher BP in 12.0%.

#### Conclusion

Due to absence of local cutoff points for waist circumference, subjects might escape diagnosis if the IDF definition is used. More studies are needed to reach more accurate definition of MS in Saudis.

### P739

#### Cost of treating diabetes in a developing economy

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#### Objective

The study set out to determine the out-of-pocket and indirect costs of treating diabetes mellitus in Nigeria.

#### Method

The study was conducted at two tertiary health facilities that are 25 km apart and operate under the same management, the Wesley Guild Hospital (WGH) and the Ife Hospital Unit (IHU) both in southwestern Nigeria. An interview-structured questionnaire and case note records were used to determine demographic variables, how much patients had expended on diabetes care, sources of funds for care, ability to cope with paying, number of clinic attendance and number of days spent on admission all in the preceding 12 months.

#### Results

There were 94 patients in all (M: F = 1:1), the average age was 62 years, 29 were retirees and 83% of the patients were in the low socioeconomic class. The average clinic attendance was 8/12 months while the average duration of hospital stay was 38 days. The total cost of insulin, oral hypoglycaemics, other drugs and laboratory test was \$51,986.00. Only six patients had their own glucometer. With respect to the ability to cope with paying for care, 56% of the patients reported that they cope with difficulty or great difficulty while a third had to depend on relations for diabetes care payment.

#### Conclusion

Our study just as in previous studies recorded a higher prevalence of diabetes in the elderly population. Majority of our patients are from the lower socio economic strata with poor education further compromising their ability to make wealth and

fund their own care. This results inevitably to poor outcomes hence creating a vicious circle for perpetuating poverty.

The out-of-pocket and indirect cost of diabetes care appeared intolerably high to these mostly indigent patients. An effective health insurance scheme might ameliorate this presently unacceptable situation.

### P740

#### Comparison of the diagnostic criteria for PreDiabetes on a overweight population

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#### Introduction

'PreDiabetes' was considered as a categories of increased risk for diabetes on the last ADA recommendations in 2010. The proposed diagnostic criteria include an HbA1c value between 5.7 and 6.4%, impaired fasting glucose (IFG) (100–125 mg/dl) and impaired glucose tolerance (IGT) levels (2-h values in the oral glucose tolerance test (OGTT) of 140 to 199 mg/dl). The HbA1c as new diagnostic criteria may actually increase the number of patients diagnosed with PreDiabetes. Although this issue is controversial, originating a large discussion from different authors.

#### Objective

To compare the 3 mentions tests for the evaluation of dysglycemia on overweight population, without previous the diagnosis of PreDiabetes or Diabetes.

#### Methods

Observational study performed in our department. Clinical history was recalled and anthropometric measurements were performed in all participants: weight, height, waist circumference (WC) and % fat mass (FM). Glycaemia, lipids and blood pressure levels were measured in all patients. Three diagnostic criteria were considered: IFG, IGT and HbA1c value. Statistic tests (*T*-test,  $X^2$  test, Fisher test) were used to compare the differences between variables.

#### Results

The study included 53 individuals, with a mean age of 52 years, 66% being females. Between females and males, the mean weight was 81 vs 90 kg, BMI of 33 vs 31 kg/m<sup>2</sup>, WC 99.5 vs 104 cm and % FM of 40.5 vs 28.3%. Using only one diagnostic criteria, the PreDiabetes prevalence was 13.2% with FG, 9.4% with OGTT and 26.4% with HbA1c. Using the 3 criteria simultaneously, only 3.8% of the patients had PreDiabetes and none has diabetes.

#### Conclusions

Despite the high percentage of possible PreDiabetes based on HbA1c levels, this is not confirmed when all criteria are simultaneously used. Due to the low concordance between the 3 methods, other studies are required to evaluate the interests of HbA1c as new criteria for PreDiabetes diagnosis.

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# Targeting hyponatraemia secondary to SIADH



**Indication:** Treatment of adult patients with hyponatraemia secondary to syndrome of inappropriate antidiuretic hormone secretion (SIADH). **Please report Adverse Drug Reactions** to Otsuka Pharmaceutical Europe Ltd. Pharmacovigilance Department on telephone: +44 (0)1895 207100 (including out-of hours), fax: +44 (0)1895 207115. Prescribers are recommended to consult the summary of product characteristics before prescribing, particularly in relation to side effects, precautions and contraindications. Prescribing information is available on request from: [OtsukaMedicalEurope@otsuka-europe.com](mailto:OtsukaMedicalEurope@otsuka-europe.com). **Marketing Authorisation Holder:** Otsuka Pharmaceutical Europe Ltd., Hunton House, Highbridge Business Park, Oxford Road, Uxbridge, Middlesex, UB8 1HU, UK. **Legal Category:** POM.



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