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Abstract Book

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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 knowledge in the field of endocrinology through publication. This year's recipient is Professor Wiebke Arlt. The prize will be presented as part of the ECE 2009 opening ceremony where Professor Arlt will deliver her lecture. Professor Arlt will also write a review article based on this lecture to be published in the *European Journal of Endocrinology*. Further information can be found at http://www.euro-endo.org/about/about_prizes.htm

Wiebke Arlt, UK

Wiebke Arlt is 43 years of age and fell for Endocrinology as a medical student. She underwent clinical and scientific training in Germany and the USA. At present, she serves as Professor of Medicine and Head of the Centre for Endocrinology, Diabetes and Metabolism at the University of Birmingham, United Kingdom. She is a Consultant Endocrinologist at the University Hospital Birmingham, the Birmingham Women's Hospital and the Birmingham Children's Hospital. She is a committed clinician scientist and heads a research group working on basic and clinical aspects of adrenal and gonadal disorders, with a particular focus on steroid endocrinology. She is a steering committee member of the European Network for the Study of Adrenal Tumors, ENS@T, and the FP7 European Collaborative Network on Disordered Sex Development, EuroDSD. She is an editorial board member of the *European Journal of Endocrinology, Clinical Endocrinology, Journal of Endocrinology and Journal of Clinical Endocrinology & Metabolism.* She serves on several committees of the Society for Endocrinology, United Kingdom and the Programme Organising Committees for the ECE meetings 2005 Gothenburg and 2010 Prague. She represents the European Society of Endocrinology as a POC member for the ICE meeting 2010 Kyoto.

The *European Journal of Endocrinology* Prize Lecture

Sex, electrons and the adrenal – why endocrinology excites

Wiebke Arlt, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), School of Clinical & Experimental Medicine, University of Birmingham, Birmingham, United Kingdom

This lecture will cover clinical-experimental and molecular studies elucidating the role and regulation of the adrenal steroid dehydroepiandrosterone (DHEA), a crucial precursor of human sex steroid synthesis. DHEA and its sulphate ester DHEAS are the most abundant steroids in the human circulation and their intraadrenal synthesis represents a recent evolutionary development, only observed in higher mammals. Only humans and higher non-human primates show an age-specific pattern of DHEAS secretion with levels increasing from age 6–10 years ('adrenarche'), reaching its maximum during the third decade, followed by a gradual decline from middle age onwards ('adrenopause'). Adrenal DHEA serves as the major source for female androgen synthesis and several studies have demonstrated a beneficial role of DHEA replacement in patients with adrenal insufficiency.

Individuals with completely inactivating mutations in the *CYP17A1* gene encoding the enzyme responsible for DHEA biosynthesis produce no DHEA and thus no sex steroids at all. Consequently, affected boys present with a female phenotype, 46,XY disordered sex development (46,XY DSD). Recently, a novel form of congenital adrenal hyperplasia, combined CYP17A1 and CYP21A2 deficiency, has been shown to be caused by mutant P450 oxidoreductase (POR), which provides electrons to all microsomal CYP enzymes including CYP17A1 and CYP21A2. While the disruption of DHEA synthesis by mutant POR readily explains 46,XY DSD in some affected boys, the observation of severe virilisation in affected girls, 46,XX DSD, despite low circulating androgens appeared counterintuitive. However, recent studies have provided evidence for the existence of an alternative pathway to androgens in early human life, explaining the development of 46,XX DSD.

DHEA can be inactivated to DHEAS by DHEA sulphotransferase (SULT2A1), thereby preventing the conversion of DHEA to active androgens. SULT2A1 requires the sulphate donor PAPS for catalytic activity. Recent work has identified inactivating mutations in *PAPSS2* encoding human PAPS synthase 2 as the cause of androgen excess in a girl with premature pubarche, hyperandrogenic anovulation and undetectable DHEAS. These observations indicate PAPSS2 deficiency as a monogenic cause of androgen excess and highlight the crucial role of DHEA sulphation as a gatekeeper to human androgen synthesis.

Geoffrey Harris Prize Winner

This prestigious prize is intended for established workers in the field of basic or clinical neuroendocrinology, and is generously supported by Ipsen. This year's recipient is Professor Jan-Åke Gustafsson. The prize will be presented as part of the ECE 2009 opening ceremony where Professor Gustafsson will deliver his lecture. Professor Gustafsson will also deliver two other lectures at future ESE Scientific meetings. Further information can be found at http://www.euro-endo.org/about/about_prizes.htm

Jan-Åke Gustafsson, Sweden

Jan-Åke Gustafsson, MD, PhD, has played a pivotal role in discoveries of how nuclear receptors in the cell mediate actions of steroid hormones and other ligands to regulate gene expression. Dr Gustafsson is Professor of Medical Nutrition, Karolinska Institutet in Stockholm, Sweden, and is also, since January 2009, Professor and Director of the Center for Nuclear Receptors and Cell Signaling, University of Houston, Texas.

Dr Gustafsson and collaborators first described the three-domain structure of nuclear receptors, defined the function of these domains, ascertained how the nuclear receptor DNA-binding mechanism mediates effects on transcription of genes, and cloned the first (partial) cDNA sequence of a nuclear receptor. He also was the first to discover that fatty acids are natural activators of the peroxisome proliferator activated nuclear receptor (PPAR). Dr Gustafsson discovered the second type of estrogen receptor (estrogen receptor β) as well as a nuclear receptor that is important in cholesterol metabolism in many tissues, including the central nervous system (liver X receptor β).

Dr Gustafsson has received many rewards during his career: the Svedberg Prize in chemistry in 1982, the Fernström Prize of the Karolinska Institute in 1983, the Anders Jahre Prize in 1992, the Gregory Pincus Medal and Award of the Worcester Foundation in 1994, the Söderberg Prize in Medicine in 1998, the European Medal of the Society for Endocrinology, UK in 2000, and the Fred Conrad Koch Award from the Endocrine Society in the U.S. in 2002. Dr Gustafsson was elected to the Swedish Academy of Sciences in 1997, to the Swedish Academy of Engineering Sciences in 1998, became a foreign honorary member of the American Academy of Arts and Sciences in 2000, a foreign honorary member of the U.S. National Academy of Sciences in 2002 and a foreign honorary member of the American Philosophical Society 2008.

The Geoffrey Harris Prize Lecture

The new biology of estrogen signaling

J-Å Gustafsson, Department of Medical Nutrition, Karolinska Institutet, Stockholm, Sweden

Estrogen signaling is mediated by two isoforms of the soluble estrogen receptor (ER), ERα and ERβ. In general, ERα and ERβ appear to have distinct, specific actions, sometimes of antagonistic nature (yin/yang). ERβ is widely distributed and studies on mice with deleted ERβ show phenotypic alterations in many tissues, indicating that ERβ has essential roles in several physiological contexts. In the CNS, ERβ is essential for development of the brain and many aspects of estrogen signaling; in the ovary, ERβ is selectively expressed in the granulosa cells and is important for ovulation; in the lung, deletion of ERβ results in fibrosis and hypoxia; in the immune system ERβ deficiency leads to a syndrome reminiscent of chronic myeloid leukemia; in the bladder, female ERβ KO mice develop interstitial cystitis, probably secondary to disturbances in the immune system; in aging ERβ deleted mice, tumors develop in the prostate, ovaries and female pituitaries. The latter phenotypes reflect an antiproliferative action of ERβ, also seen in cultures of breast, colon and prostate cancer cell lines, where microarray studies have indicated that ERβ downregulates a multitude of genes involved in cell proliferation and, conversely, upregulates many genes with tumor suppressor function. ERβ also upregulates several adhesion proteins, notably E-cadherin, consistent with a role of ERβ in cellular differentiation. Proof of principle for the antiproliferative action ERβ has recently been obtained by the antiproliferative action in prostate gland and human prostatic cancer cell lines of synthetic ERβ specific drugs. Yet other ERβ specific drugs have been shown to ameliorate depression, as assessed in various mouse models. Pain is another possible indication for ERβ drugs; ERβ is involved in development of pain pathways in the spinal cord and ERβ drugs have been reported to increase the pain threshold in rodents. ERβ targeted, tissue specific drugs may soon prove useful against several diseases. This lecture will highlight some recent studies on ERβ in our lab with reference to a few of the themes described.

Plenary Lectures

Epigenetics and early enviromental exposure

PL1

Early influences on epigenetic regulation: relevance to chronic disease

Robert Waterland
USDA Children's Nutrition Research Center, Houston, Texas, USA.

Epigenetic mechanisms provide a potential explanation for how environmental influences in early life cause long-term changes in chronic disease susceptibility. Whereas epigenetic dysregulation is increasingly implicated in human developmental syndromes and cancer, the role of epigenetics in complex chronic diseases such as cardiovascular disease, type 2 diabetes and obesity remains largely uncharacterized. The inherent tissue-specificity of epigenetic regulation is the foremost impediment to an improved understanding of epigenetic dysregulation in human disease. Research in animal models is therefore crucial to enable the development of specific hypotheses that can be practicably tested in humans. We have developed a mouse model showing that methyl donor supplementation prevents transgenerational amplification of obesity, suggesting a role for DNA methylation in the developmental establishment of body weight regulation. Coupling such models with epigenomic technologies including DNA methylation-specific amplification and microarray hybridization should ultimately enable us to determine if epigenetics is an important link between early life events and adult disease.

Current and novel treatment targets for bone diseases

PL2

Current and novel treatment targets for bone diseases

Socrates Papapoulos
Leiden University Medical Center, Leiden, The Netherlands.

During the past few years there have been significant developments in the pharmacotherapy of bone diseases, especially of osteoporosis, and effective treatments have become available to physicians. These developments were paralleled by significant progress in our understanding of the local regulation of bone metabolism. Particularly, studies of human and animal genetics have led to identification of novel, more specific, signaling pathways in bone cells that can provide targets for new therapeutics.

Such novel targets in osteoclasts include, among others, RANKL and cathepsin-K. A fully human monoclonal antibody to RANKL (denosumab) was developed and a large phase three study in osteoporosis has just been completed while cathepsin-K inhibitors have been evaluated in phase two studies and one of them (odanacatib) is currently in phase three clinical development.

The PTH paradigm illustrated the possibility of stimulating bone formation in osteoporotic patients and opened the way for the development of bone forming agents and novel forms of PTH (e.g. PTH 1-31) or PTHrP. A particularly interesting approach has been the development of molecules that antagonize the calcium sensing receptor of the parathyroid cells and stimulate PTH secretion (calcilytics). The most exciting development of recent years has been, however, the recognition of the central role of the Wnt signaling pathway in bone formation which, in turn provided, a number of attractive targets for the development of pharmaceuticals. For example, inhibition of this pathway by blocking the action of sclerostin represents a very promising novel approach to stimulating bone formation in patients with osteoporosis.

The new developments may allow in the future tailoring pharmacotherapy to the specific needs and pathophysiological profile of the individual patient. However, apart from establishing the efficacy of these new molecules a critical issue for their introduction into clinical practice will be their tolerability and safety profile.

Stress and the brain: from adaptation to disease

PL3

Stress and the brain: from adaptation to disease

Florian Holsboer
Max-Planck-Institute of Psychiatry, Munch, Germany.

In response to stress, the brain activates several neuropeptide-secreting systems. This eventually leads to the release of adrenal corticosteroid hormones, which subsequently feed back on the brain and bind to two types of nuclear receptor that act as transcriptional regulators. By targeting many genes, corticosteroids function in a binary fashion, and serve as a master switch in the control of neuronal and network responses that underlie behavioural adaptation. In genetically predisposed individuals, an imbalance in this binary control mechanism can introduce a bias towards stress-related brain disease after adverse experiences. New candidate susceptibility genes that serve as markers for the prediction of vulnerable phenotypes are now being identified.

Genetics of diabetes and obesity

PL4

New insight in the genetics of type 2 diabetes and obesity from genome wide associations studies

Philippe Froguel[1,2]
[1]Genomic Medicine, Hammersmith Hospital, Imperial College, London, UK; [2]CNRS 8090-Institute of Biology, Pasteur Institute, Lille, France.

Recent large twin studies have definitively shown that more than 70% of the variance of the BMI and waist is genetically determined in both adults and children, suggesting that the epidemics of obesity and subsequent T2D are mainly due to the environmental pressure targeting individuals who are particularly vulnerable to metabolic diseases. The dissection of monogenic early onset severe obesity and T2D cases have identified a variety of causative genes that are involved in two fundamental pathways: pancreatic beta-cell function and the control of appetite.

Genome Wide Association approaches using high-density frequent Single Nucleotide Polymorphism micro-arrays have been developed to elucidate common forms of metabolic diseases. Since the report of the first GWA in T2D in early 2007 (Sladek et al, nature), several T2D case/control GWA studies have provided more than a dozen of loci that are consistently associated with increased risk for diabetes. Meta-analyses of GWA data from several populations should bring soon additional genes but their effect is likely to be modest. Most of the new T2D associated genes are expressed in the pancreas and are guessed to control insulin secretion. Their effect is additive which makes interesting their use to predict T2D incidence.

However, these loci only explain a small fraction of T2D heritability. Other form of polymorphisms, such as gene Copy Number Variations and rare variants may also greatly contribute to T2D risk. In addition other genes may also modulate phenotypes related to glucose control in the general (non diabetic) population, and in interaction with environmental factors might play an important role in the early development of glucose intolerance and in the mortality and morbidity associated with slightly elevated glucose levels. GWA analyses of general populations for quantitative traits related to glucose homeostasis identified glucose-6-phosphatase catalytic subunit-related protein 2 (*G6PC2)* and the melatonin receptor 2 gene (*MTNR1B*) has major regulators of fasting glucose. Both genes are expressed in the pancreatic beta-cells although the melatonin receptor is also acting in the retina as a mediator of the biological clock. Impairment of the circadian clock or of sleep quality is known to impair insulin secretion.

GWAs on BMI in general populations and in severe obesity cases and normal weight controls studies have identified the brain expressed *FTO* and Melanocortin 4 receptor as common obesity associated genes. Recent, GWA meta-analyses for BMI and in childhood obesity also found new genes contributing to obesity risk most of them expressed in the brain and potentially involved in the regulation of food behavior.

In conclusion, GWA conducted in both large well phenotyped general population and in well-defined cases and controls are equally useful to identify fundamental pathways involved in glucose and energy homeostasis. These studies should contribute to elucidate human metabolic physiology and to understand better the natural history of T2D and associated obesity.

New therapies for diabetes – genetically engineering tissues to maintain glucose homeostasis

PL5

New therapies for diabetes – genetically engineering tissues to maintain glucose homeostasis

Fatima Bosch
Department of Biochemistry and Molecular Biology, Center of Animal Biotechnology and Gene Therapy, School of Veterinary Medicine, Universitat Autònoma de Barcelona, Bellaterra, Spain.

Abstract unavailable.

Thyroid hormone transporters

PL6

Thyroid hormone transporters

Annette Gruters
Institute of Experimental Pediatric Endocrinology, Charité, Berlin, Germany.

Abstract unavailable.

Implications of adrenal hormone pulsatility

PL7

Implications of adrenal hormone pulsatility

Stafford Lightman
University of Bristol, Bristol, UK.

The HPA axis has a massive dynamic response rate. At nadir periods (at night in man and during the day in nocturnal rodents) there is a low level of activity which increases up until the circadian peak. This circadian rhythm is made up of an underlying ultradian rhythm of pulsatile glucocorticoid section, with pulse amplitude increasing from the circadian nadir to the circadian peak. Superimposed on this underlying rhythm is, of course, the stress response which can result in massive peaks of glucocorticoid secretion with high levels lasting much longer than the normal endogenous pulses. Why does the HPA axis have such a complex underlying rhythmicity and is it important for the ability of the organism to show both rapid and prolonged responses to homeostatic stress?

If the ultradian rhythmicity is important, rapid changes in endogenous glucocorticoids must have some rapid effects on cell signalling. We have shown that at the level of the whole animal, rapid changes in glucocorticoid can turn off HPA activity within about 20 min both in the rat and in man. Furthermore, using much lower concentrations of endogenous glucocorticoids we can show that each individual pulse of glucocorticoid results in a distinct translocation of GR from the cytoplasm to the nucleus, binding to promoter sequences of glucocorticoid responsive genes and transcription of pulses of hnRNA and mRNA. Interestingly in the brain there are distinctive time domains for the DNA binding of GR and MR. This provides scope for a digital signalling mechanism in which the frequency of pulses will determine the ratio of GR to MR binding to DNA – in effect an mechanism in which the response depends on the frequency of incoming signals acting on stochastic intranuclear events. Furthermore, it appears that translation of GR from the cytoplasm to the nucleus is not always necessary for these rapid intranuclear events and that there is an endogenous intranuclear cycle of GR activation, DNA binding and dissociation intimately related to chaperones and other accessory intranuclear proteins.

The HPA clearly uses rapid episodic changes to signal through both membrane associated and nuclear receptors. This allows an ability to respond to changes of great temporal and magnitude diversity. The next stage in our enquiries needs to be at the level of how this is reflected in the functional response to HPA signals.

11β HSDs-common lessons from rare mutations

PL8

11β-hydroxysteroid dehydrogenases: common lessons from rare mutations

Paul Stewart
University of Birmingham, Birmingham, UK.

In mammalian tissues, two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) catalyze the interconversion of hormonally active cortisol (F) and inactive cortisone (E). 11β-HSD2 is a high affinity dehydrogenase expressed in adult kidney that inactivates F to E protecting the mineralocorticoid receptor (MR) (which has equal affinity for F and aldosterone *in vitro*) from cortisol excess. 'Cushing's disease of the kidney' occurs in the hypertensive condition 'Apparent Mineralocorticoid Excess (AME)' because of mutations in the *HSD11B2* gene. Acquired inhibition of 11β-HSD2 explains the mineralocorticoid excess state that characterizes excessive liquorice ingestion. Heterozygous mutations in *HSD11B2* and polymorphic variation at this locus might be involved in the pathogenesis of salt-sensitive and 'essential' hypertension.

By contrast, 11β-HSD1 is a bi-directional enzyme but *in vivo* the predominant action in liver, adipose tissue and bone is E to F conversion. The putative 11β-HSD1 null state is the syndrome of Cortisone Reductase Deficiency (CRD) whereby patients are unable to convert cortisone to cortisol. Hyperandrogenism results because of increased ACTH drive to the drive secondary to increased cortisol clearance; as a result patients present with polycystic ovary syndrome and/or precocious puberty. Our clinical and laboratory studies indicate that the pivotal oxo-reductase activity of 11β-HSD1 is critically dependant upon the generation of NADPH within the endoplasmic reticulum from an accessory enzyme hexose-6-phosphate dehydrogenase (H6PDH). Mutations in the H6PDH gene explain the molecular basis for CRD – the HSD11B1 gene is normal. Recombinant mice lacking H6PDH have the predicted change in glucocorticoid metabolism (reduced oxo-hydroxyl ratios), and improved insulin sensitivity because of a failure to reactivate glucocorticoid locally within liver and fat. Lack of H6PDH specifically within muscle results in a type II fiber myopathy because of activation of ER stress pathways. Polymorphisms in HSD11B1/H6PDH genes may be implicated in explaining the variable phenotype of patients with PCOS.

Mutations in the HSD11B2 and H6PDH genes explain the monogenic diseases AME and CRD. In turn a greater understanding of the role of 11β-HSD1, 11β-HSD2 and H6PDH has increased our understanding of the role of corticosteroids in prevalent human diseases such as hypertension, metabolic syndrome and PCOS.

Symposia

PCOS

S1.1

Obesity, type 2 diabetes and PCOS: a common origin?

Bulent O Yildiz
Department of Internal Medicine, Endocrinology and Metabolism Unit, Hacettepe University School of Medicine, Ankara, Turkey.

Obesity and type 2 diabetes are common and complex traits that are closely related and the term 'diabesity' is being used for the twin global epidemic of these two disorders. PCOS is another common and complex disorder characterized by androgen excess, oligo-anovulation and polycystic ovaries on ultrasound. PCOS is linked with both obesity and type 2 diabetes. Although obesity and type 2 diabetes are not universally observed in PCOS, many women with PCOS are obese and the risk and prevalence of type 2 diabetes are significantly increased in PCOS. Alternatively, limited available data suggest an increased prevalence of PCOS in women with type 2 diabetes. Lastly, obesity is recognized as a major contributor to considerable variation in severity and expression of PCOS phenotype. Obesity, particularly the abdominal type, has significant impact on androgen excess and oligo-anovulation of PCOS through various mechanisms. Although underlying genetic and environmental factors are not fully understood for the linkage among obesity, type 2 diabetes and PCOS, insulin resistance appears to be a common denominator of these three disorders.

S1.2

Targeting insulin sensitivity in the treatment of PCOS

Renato Pasquali
Division of Endocrinology, Department of Clinical Medicine, St Orsola-Malpighi Hospital, University Alma Mater Studiorum, Bologna, Italy.

Lifestyle interventions and insulin sensitizers play a fundamental role in the treatment of PCOS, particularly in the presence of obesity. The rationale is represented by the reasoning that the decrease in insulin concentration, as a result of improved insulin resistance, may lead to metabolic alterations and have important effects on hyperandrogenism, and, in particular, on fertility. Insulin sensitizers can be added to lifestyle intervention, when obesity is present, although there is preliminary evidence that some behavioural modification in dietary habits may have a positive effect even in normal-weight insulin resistant PCOS women. Hopefully, weight reduction in the management of PCOS should be encouraged before any pharmacological treatment such as insulin sensitizers or antiobesity agents, although this does not represent a common rule worldwide.

Sustained weight loss can completely reverse the phenotype in a subset of obese women with PCOS, supporting the concept that a PCOS 'secondary' to obesity may exist. Interestingly, this is associated with a marked improvement of insulin resistance and a normalization of fasting and glucose-stimulated insulin levels. This adds new perspectives on the pathophysiological impact of obesity on PCOS. Future research should however investigate factors determining individual susceptibility to develop this disorder in the presence of obesity.

An important issue is represented by the individual responsiveness to insulin sensitizers, given alone or in combination. The effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action suggests that OCT1 genotype may be a determinant of metformin pharmacokinetics in PCOS. Preliminary clinical studies further support that clustering PCOS women by this genotype may partly explain individual responsiveness to metformin.

In addition, emerging data support the concept that metformin dose has an important impact in ameliorating insulin sensitivity and in decreasing circulation insulin levels.

Finally, insulin resistant PCOS women present an increasing list of monogenic disorders that represent a potential target for specific insulin sensitizers, such as tiazolidinediones.

S1.3

The role of HPA axis in metabolic derangements in PCOS

Djuro Macut
Institute of Endocrinology, Belgrade, Serbia.

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder present in 5–10% women of the reproductive period. The endocrine manifestations of PCOS include excess androgen production of ovarian and/or adrenal origin and arrested follicular development leading to chronic oligo- or anovulation. As a consequence, PCOS is associated with increased risk of infertility, and in long term to type 2 diabetes, and possibly cardiovascular disease. The ovary is generally considered the principal source of androgens, but many patients with PCOS also have increased adrenal androgen secretion. Previous works on this issue showed possible androgen hyperresponsivity to direct (ACTH) or indirect (CRH) stimulation of the adrenal cortex. Increased urinary free cortisol (UFC) has also been reported in PCOS patients. This alteration has been attributed to enhanced cortisol metabolism, followed by a compensatory overdrive of the hypothalamic–pituitary–adrenal (HPA) axis and hence increased androgen production. It was supposed an abnormal P450c17 function in PCOS that is principally responsible for the adrenal androgen excess, as well as increased peripheral metabolism of cortisol, either through enhanced 5α-reductase or impaired 11β-HSD1 activities. Known role of glucocorticoids (GCs) in the development of components of the metabolic syndrome (MS) led to the examination of possible hormonal dysregulation of HPA, by analyzing indices of glucocorticoid receptor (GR) binding in peripheral mononuclear leucocytes of women with PCOS. Although differences in some of the GR binding indices were not shown, it seems that number of the receptors and its affinity depends of the source of the androgens, namely adrenal DHEA-S, and its concentrations. Presence of several GR gene polymorphisms can modulate GCs effects. It seems that *BclI* polymorphism of the GR gene, could be related to the components of the metabolic syndrome in women with PCOS.

S1.4

Treatment of infertility in women with PCOS

Kursad Unluhizarci
Department of Endocrinology, Erciyes University Medical School, Kayseri, Turkey.

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies, affecting 5–10% of premenopausal women. Anovulatory infertility is an important feature of PCOS and the optimal treatment for infertile women with PCOS has not yet been defined. Weight loss is recommended as the first-line therapy in obese women with PCOS desiring pregnancy. It is clear that regular physical activity is an important component of weight loss programs since it is associated with better long-term weight loss maintenance. Many studies have shown that weight loss is associated with improved spontaneous ovulation rates in women with PCOS. Among the pharmacologic agents, clomiphene citrate (CC) remains the treatment of first choice for induction of ovulation in anovulatory women with PCOS. There are relatively few adverse effects and requires little ovarian response monitoring. There are no specific exclusion criteria for women with anovulatory PCOS who have normal baseline FSH and estradiole levels. However, older patients may show less response. Treatment generally should be limited to six ovulatory cycles and the starting dose is generally 50 mg/day, for 5 days. Common side effects are hot flushes, headaches and visual complaints. Although there are very limited experience, tamoxifen can be considered in women who are intolerable to hot flushes. There is considerable interest for insulin sensitizers (metformin, rosiglitazone and pioglitazone) in the treatment of women with PCOS. Metformin should be the choice if an insulin sensitizer is considered in the treatment of PCOS women. Although oligomenorrhea improves in some women with PCOS, the degree of improvement in ovulation frequency is similar to that obtained with weight reduction. Insulin sensitizers should not be used indiscriminately and should be restricted to those patients with glucose intolerance and/or metabolic syndrome. Another approach for the treatment of anovulatory infertility in women with PCOS is gonadotropin treatment. The use of exogeneous gonadotropins is associated with increased chances for multiple pregnancy and therefore, intense monitoring of ovarian response is required. Adherence to the chronic low-dose-regimen of FSH (37.5–50 IU/day) administration should markedly reduce the likelihood of excessive ovarian stimulation, namely ovarian hyperstimulation syndrome (OHSS). This issue should be discussed with the patient before ovulation induction. The duration of gonadotropin therapy generally should not exceed six ovulatory cycles. Laparoscopic ovarian surgery (LOS) may be used in CC resistant women with anovulatory PCOS. Mostly employed methods for LOS include diathermy and laser, known as 'ovarian drilling'. Between 4 and 10 punctures have been performed, and premature ovarian failure is a concern particularly in women who had a large number of punctures. Finally, after failure of weight reduction, anti-estrogen therapy or LOS and in women who have associated pathologies such as tubal damage, male factor infertility, *in vitro* fertilization is indicated.

β cell proliferation, survival and secretion

S2.1

Cell–cell communication and the regulation of insulin secretion

Peter Jones
King's College London, London, UK.

The mechanisms through which pancreatic beta cells recognise and respond to external signals Type 2 diabetes is becoming increasing well understood, but we have less understanding of how the responses of individual cells are integrated within the islet of Langerhans. Islets are heterogenous organs containing a variety of endocrine cell types including beta, alpha, delta and PP cells which synthesise and secrete insulin, glucagon, somatostatin and pancreatic polypeptide, respectively. Disaggregation of islets results in impaired insulin secretion from the beta cells, suggesting that interactions between cells within islets are important in regulating normal islet function. Islet cells express a variety of cell adhesion molecules which confer the capacity for the spontaneous re-aggregation of dispersed islet cells into anatomically-correct, three-dimensional islet-like structures, and this is associated with a return to more appropriate patterns of insulin secretion. There are numerous possible mechanisms through which intercellular communication within the islet may modulate beta cell function, including gap junctions, paracrine signalling and direct cell-cell interactions through cell surface molecules. *In vivo* studies using transgenic mice and *in vitro* studies using isolated islets or hormone-secreting cells lines suggest that several different mechanisms act simultaneously to maintain appropriate insulin secretion by co-ordinating beta cell responses to external stimuli. Thus, gene ablation studies have identified an important role for connexin-36 gap junctions in the synchronous behaviour of adjacent beta cells. Interactions between cell surface molecules such as E-Cadherin, Ephs and Ephrins have been implicated in communication between adjacent islet cells to regulate insulin secretion, beta cell proliferation and apoptosis. Finally, beta cells express numerous cell surface receptors to islet hormones and other potential auto/paracrine regulators, and there is considerable evidence of multiple levels of regulation of beta cell function by intra-islet diffusible signals. Understanding these complex interactions between islet cells may offer novel insights into the causes and treatments of Type 2 diabetes.

S2.2

Inflammation, cytokines and diabetes

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Beta-cell failure is central to the pathogenenis of both type 1 and type 2 diabetes mellitus. In type 1 diabetes immune-mediated beta-cell destruction takes place, whereas in type 2 diabetes metabolic factors are believed to induce progressively deteriation of beta-cell function and finally reduced beta-cell mass. The proinflammatory cytokine interleukin-1 could be a mediator of the beta-cell failure in both diseases: In type 1 diabetes by the secretion from actived macrophages inflitrating the islets, and in type 2 diabetes by glucose induced secretion from the beta-cell ifselves. Using the interleukin-1-receptor antagonist in patients with type 2 diabetes improves glycemic control and beta-cell function and reduces markes of systemic inflammation, indicating that type 2 diabetes could be an auto-inflammatory condition. Furthermore the involvement of interferon-gamma and tumor necrosis factor alpha in the pathogenesis of type 1 and type 2 diabetes is reveiwed.

S2.3

Endoplasma reticulum stress and beta-cell apoptosis

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Accumulated knowledge in the last five years suggests that components of the unfolded protein response (UPR) in the endoplasmic reticulum (ER) play a dual role in pancreatic beta cells, acting as regulators under physiological conditions or as triggers of beta cell dysfunction and death under situations of chronic and/or severe ER stress. These observations indicate that the large capacity of beta cells to synthesize, sort and secrete insulin may also make them vulnerable to chronic exposure to high glucose or free fatty acids, agents that contribute to beta cell dysfunction and apoptosis in type 2 diabetes. Beta cell ER stress is also present in the context of type 1 diabetes, but following different pathways. Thus, the cytokines IL-1β and IFN-γ trigger a severe ER stress by respectively inducing an NO-mediated depletion of ER calcium and inhibiting ER chaperones, thus hampering beta cell defenses. This results in amplification of the pro-apoptotic pathways and eventually beta cell death.
Some of the key issues that remain to be clarified in this novel field are: a. the transition from physiology to pathology, i.e. how the physiological UPR evolves to severe ER stress and, in some cases, beta cell death; b. the mechanism utilized by beta cells to recover from ER stress; c. the 'point of no return' for beta cell apoptosis, and the nature of the pro-apoptotic signals generated by ER stress.

S2.4

Incretin receptor signalling, β-cell proliferation and survival

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Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones secreted by gut endocrine cells that act on structurally related β-cell G protein coupled receptors to trigger glucose-dependent insulin secretion. Both peptide hormones augment glucose-stimulated insulin secretion although the actions of GIP are diminished in the setting of hyperglycemia. Moreover, the two incretin hormones, and their structurally related receptors, exert widely divergent biological actions on β-cell function following receptor agonist administration or genetic disruption of receptor signaling *in vivo*. Furthermore, there are significant differences in β-cell function and survival arising from pharmacological activation of incretin receptor signaling achieved using peptide agonists versus DPP-4 inhibition. The available data identify important differences in the endogenous physiological roles and pharmacological importance of murine GIP versus GLP-1 receptors versus DPP-4 inhibition for the preservation of β-cell mass and function *in vivo*.

Genetics in neuroendocrinology

S3.1

Familial hypopituitarism

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Growth is an inherent property of life. Normal somatic growth requires the integrated function of many of the hormonal, metabolic, and other growth factors involved in the hypothalamo-pituitary-growth axis. Discovery of transcription factors responsible for pituitary cell differentiation and organogenesis has had an immediate impact on understanding and diagnosis of pituitary hormone deficiencies. Importantly, combined pituitary hormone deficiencies (CPHD) have been associated with mutations in transcription factor coding genes that control organogenesis or multiple cell lineages, whereas isolated hormone deficiencies are often caused by transcription factors controlling late cell differentiation.
These transcription factors, mainly found and described primarily in transgenic and naturally occurring murine models, include factors such as HESX1, PROP1, POU1F1, LHX3, LHX4, TBX19, SOX2 and SOX3. Importantly, the expression of these various transcription factors dictates the phenotype that results when the gene encoding the relevant transcription factor is mutated. The highly variable phenotype may consist of isolated hypopituitarism, or more complex disorders such as septo-optic dysplasia and holoprosencephaly. As mutations in any of those transcription factors are rare, it is clear that many genes remain to be identified, and the characterization of these will further elucidate the pathogenesis of these complex conditions.

Furthermore, a normal development of a gland does not mean that the normal function will be maintained, as *GH-1* gene defects may end in CPHD as well. These findings are to be stressed and have an impact how these patients need to be followed in clinical practice.

S3.2

ACTH insensitivity syndromes

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ACTH insensitivity or familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder first described in 1959. We demonstrated in 1993 that about 25% of affected patients have nonsense or (more commonly) missense mutations in the ACTH receptor (melanocoprtin 2 receptor, MC2R). Functional analysis of these mutations had been especially difficult until our discovery in 2005 that the receptor requires an essential accessory factor – the melanocortin 2 receptor accessory protein (MRAP) for membrane trafficking and signal generation. Furthermore, mutations in MRAP account for about 20% of FGD patients. Availability of MRAP enables the development of efficient MC2R functional assays and it emerges that the majority of naturally occurring MC2R missense mutations result in failure of receptor trafficking and cell surface expression. A further group of patients have a form of FGD that was linked to a gene on chromosome 8 following a whole genome mapping strategy in 2002. Recently we have shown that this gene is that encoding StAR. The StAR protein is responsible for the transport of cholesterol across the mitochondrial membrane and is the first step in steroidogenesis. Typically mutations in StAR result in congenital lipoid adrenal hyperplasia. However certain mutations result in a StAR protein that retains some function, and consequently gonadal steroidogenesis is unaffected while adrenal glucocorticoid production is compromised, resulting in an FGD phenotype. In about 50% of FGD patients there is no defect in any of these genes, and further genetic loci remain to be identified.

S3.3

The GPR54 gene mutations as a cause for hypogonadotropic hypogonadism

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The identification of naturally occurring genetic mutations has provided unique insight into the current knowledge of the human hypothalamic–pituitary–gonadal axis. In the last 5 years, several loss-of-function mutations in the G-protein coupled receptor 54 (GPR54) gene have been shown to cause isolated hypogonadotropic hypogonadism. Although these mutations are not a common cause of hypogonadotropic hypogonadism, patients bearing mutations are critical to explore genotype-phenotype and gene function. The ligands for GPR54 are derived from the precursor protein, kisspeptin. The kisspeptins have been characterized as fundamental regulators of pubertal onset and powerful stimulants for GnRH-induced gonadotropin secretion. More recently, a GPR54 missense mutation (R386P) was reported in a girl with idiopathic gonadotropin-dependent precocious puberty. Functional studies *in vitro* demonstrated that this mutation leads to sustained activation of intracellular signaling pathways downstream of GPR54, suggesting that GPR54 defects can be also associated with central precocious puberty phenotype.

S3.4

Reversible hypogonadotropic hypogonadism

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Puberty is a complex biologic process inducing sexual development and fertility. Puberty is initiated by the secretion of pulsatile gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus. Severe anomalies in GnRH secretion or action may lead to idiopathic hypogonadotropic hypogonadism (IHH), a disorder where patients failed to go through puberty. While IHH was previously thought to require lifelong treatment, we hypothesized that activation of the hypothalamic–pituitary–gonadal (HPG) axis could occur in adulthood with the appropriate stimulus. Retrospective and prospective studies in a large cohort of male IHH (Kallmann syndrome (KS) and normosmic IHH) demonstrate reversal of HH in approximately 10% of cases. Those patients exhibited sustained adult serum T levels after hormonal treatment was discontinued. Mutations in *FGFR1, PROKR2, GNRHR* were identified in several cases of reversal.
In conclusion: 1) Sustained reversal of IHH occurs in about 10% of patients with KS or nIHH; 2) Genetic defects leading to IHH can be overcome, likely by environmental stimuli such as exposure to sex steroids; 3) Although the mechanism of reversal remains unclear, it may involve plasticity of the GnRH neurons in adulthood; and 4) IHH patients should undergo brief discontinuation of hormonal treatment to assess for reversal.

Gonadal steroid replacement

S4.1

Testosterone and the metabolic syndrome

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Abstract unavailable.

S4.2

Pharmacogenetics of androgen action

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Hypogonadism is still a poorly-defined clinical entity. Recently approved and published guidelines to diagnosis and treatment of hypogonadism claim the necessity of accompanying biochemical thresholds with clinical symptoms and monitoring under treatment a specific aspect of the 'wide spectrum' hypogonadism (libido, erection, bone mass, muscle strength and so on). This is due both to different levels of thresholds for different tissues and to individual variability.
Most part of interindividual variability relies on androgen receptor (AR) polymorphism linked to variations in the length of CAG repeats (CAGr) in exon 1. Many studies have shown that the longer the CAGr the weaker the androgen action and viceversa. This is true both for endogenous and exogenous androgens. In presence of similar testosterone (T) plasma levels, the final phenotypic androgenic effect or 'androgenicity' is mainly due to CAGr length. This is particularly relevant when exogenous T is administered. Data are emerging that androgen replacement treatment (ART) should be tailored on AR polymorphism to balance between clinical benefits and risks. Moreover, specific patient categories, such as obese men or patients with metabolic syndrome, represent clinical conditions that should deserve particular attention during ART, since shorter CAGr could amplify a clinical effect, such as polycitemia or sleep apnea.
It is rather strange to believe nowadays that strict diagnostic criteria and strict scheduled treatments could apply to all the spectrum of hypogonadal patients. Pharmacogenetically tailored diagnosis and treatment should be considered in this field of medicine.

S4.3

Androgen replacement in women

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Dehydroepiandrosterone (DHEA), the major product of the adrenal zona reticularis, is a crucial sex steroid precursor. Suppression of DHEA production in females, e.g. by exogenous glucocorticoids, results in significant overall

androgen deficiency. The ovaries contribute to some extent to circulating female androgens, mainly by converting the precursor steroid DHEA to androgens directly binding and activating the androgen receptor, testosterone and dihydrotestosterone. Loss of adrenal function, e.g. in adrenal insufficiency or due to chronic glucocorticoid treatment, results in pronounced androgen deficiency. Similarly, women undergoing bilateral oophorectomy often suffer a significant decrease in circulating androgens. Seminal studies in both these groups have provided the data that form the basis of our current recommendations for androgen therapy in women. However, it is important to stress that physiological menopause per se does not cause androgen deficiency, as androgen synthesis in the ovaries may persist postmenopausally despite declining estrogen production. The definition of female androgen deficiency in the 2002 Princeton consensus statement, androgen levels below or within the lower quartile of the normal range and concurrent sexual dysfunction, is not precise enough and may lead to over-diagnosis. On the other hand, the Endocrine Society USA guidelines published in 2006 and advising against all androgen replacement in women, is no better help for the concerned clinician. Androgen treatment should be considered in women with severe androgen deficiency due to an established cause such as adrenal insufficiency or bilateral oophorectomy and matching clinical symptoms. Replacement options include transdermal testosterone or oral DHEA, both of which have been shown to result in significant improvements in libido and mood and also of body composition and bone mineral density. It is important to keep in mind that the number of randomized controlled trials is still limited and we need to learn more about the respective benefit and risk ratios.

S4.4

Diagnosis and treatment of estrogen deficiency in men

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The discovery of naturally occurring, inactivating mutations of the aromatase gene and of the estrogen receptor (ER) gene in humans shed new light on the precise role of estrogen in several metabolic processes, both in male and female. To date, few clinical cases of males with well-documented congenital aromatase deficiency (11 females and 8 males) have been reported in medical literature. All mutations accounting for aromatase deficiency have been located in regions encoding essential functions in the aromatization process. Aromatase-deficient males show persistent linear growth with delayed skeletal maturation and epiphyseal closure, eunuchoid body proportions, osteoporosis, various degrees of fertility impairment and strong evidences of metabolic syndrome (impaired lipid, glucose and liver metabolism). Moreover preliminary data demonstrate an impaired GH response to GHRH plus Arginine and serum IGF-I levels at the lower end of the normal range in four patients with aromatase deficiency.
The estrogen treatment in some of these patients demonstrated the crucial role of estrogens also in men. Particularly, estradiol treatment induces the pubertal growth spurt, the achievement and maintenance of normal skeletal proportions and of peak bone mass, and the inhibition of bone resorption. Moreover, among gonadotropin feedback it was shown that estrogens are the most effective gonadotropin-secretion inhibitor at pituitary level, particularly with respect to FSH, and that they act also at hypothalamic level. Nevertheless estrogens are not able to improve the fertility and to restore GH-IGF-1 axis function. After all, the human model of aromatase deficiency shows that estradiol treatment could act on heart and cardiovascular system by possible cardio-protective effects and prevention of atherogenesis, and on dysmetabolic pattern by an improvement of lipid profile, insulin sensitivity, and of liver homeostasis.
By studying the naturally occurring aromatase-deficient patients we are expanding our understanding of the essential role of estrogens in human physiology.

IGF 1 survival, proliferation and cancer

S5.1

IGF1, proliferation and cancer

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The involvement of the insulin-like growth factors (IGF1, IGF2) in cancer biology has been the focus of extensive research. Ligand-dependent activation of the IGF1 receptor (IGF1-R) has been identified as a crucial step in cancer development. Epidemiological studies revealed that moderately elevated serum IGF1 is associated with increased occurrence of various tumours, including breast, prostate, and colorectal cancer. The IGF1-R is expressed in most transformed cells, where it displays potent antiapoptotic and cell-survival activities. The central role of the IGF1-R in cancer biology is illustrated by studies showing that IGF1-R blockade inhibits tumour growth and angiogenesis. Regulation of IGF1-R gene expression and activity is an important mechanism that allows the cell to 'decide' whether to go into arrest, to proliferate, or to apoptose. IGF1-R levels are controlled by secreted factors of endocrine or local (autocrine/paracrine) origin that can either stimulate or inhibit IGF1-R biosynthesis. In addition, a number of nuclear proteins with oncogenic or antioncogenic properties have been identified that regulate IGF1-R gene transcription. Transcription factors with tumour suppressor activity, such as p53, BRCA1, Von-Hippel Lindau (VHL), and Wilms' tumor-1 (WT1), negatively regulate IGF1-R expression. The etiology of neoplasias associated with loss-of-function mutation of tumour suppressors is, in many cases, linked to the inability of mutant forms to suppress their molecular targets, including the IGF1-R gene. Gain-of-function mutations of oncogenes are associated with increased transactivation of the IGF1-R promoter and/or augmented phosphorylation of its cytoplasmic domain and downstream signalling molecules. Interactions between stimulatory and inhibitory factors may ultimately determine the level of expression of the IGF1-R gene and, consequently, the proliferative status of the cell. Understanding the molecular basis of these interactions will be of significant value both in basic as well as in clinical terms.

S5.2

IGF, somatotropic plasticity and mammalian lifespan

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During recent years, insulin and insulin-like growth factors have been implicated in the control of lifespan in a variety of species. In mammals, substantial reduction of somatotropic signals generally extends lifespan. We showed recently in a conditional mouse mutant relevant for humans, that lifespan can be prolonged by inhibiting IGF-I signaling selectively in the central nervous system. This effect occurred through changes in specific neuroendocrine pathways. Investigating the pathophysiological mechanism, we found that IGF receptors in the brain steered the postnatal development of the somatotropic axis, which in turn altered the individual growth trajectory. This led to reduced adult body size, delayed mortality and longer mean lifespan. Our work suggested that chronically low IGF-I and low growth hormone levels favor long lifespan and may postpone age-related mortality. Together with other recent reports, these results challenge the idea that administrating GH can slow down or even prevent aging.
We then investigated whether early postnatal nutrition may participate in controlling the plasticity of the somatotropic axis. Using cross-fostering in newborn mice we manipulated early nutrition, and showed that underfeeding delayed growth, whereas overfeeding accelerated it. In both cases, final body size was permanently altered. We found significant alterations in pituitary GH, plasma IGF-I and ALS, and in gene expression of hypothalamic GHRH during postnatal development, that were consistent with the observed phenotypes and that persisted throughout adulthood. Although limited to the early postnatal period, both under- and overfeeding led to metabolic abnormalities, including diminished adult glucose tolerance, defective insulin secretion in previously restricted, and insulin resistance in overfed mice. Both restricted and overfed mice also showed increased arterial blood pressure, suggestive of vascular impairment. Collectively, these findings indicate a significant link between early diet, somatotropic development and specific pathology in mice, suggesting that, together with other hormones like leptin, IGF-I may play a role in modulating hypothalamic stimulation of the developing somatotropic function.
We propose that the underlying mechanism of the described phenotypes is an adaptive plasticity of the somatotropic function. This concept is particularly interesting from an evolutionary point of view, since it may allow individuals to decelerate growth and preserve resources, and thereby improve fitness in challenging environments.

S5.3

Nutrition, physical activity and cancer risks: the role of insulin and insulin-like growth factor-1

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Epidemiological observations increasingly imply nutritional energy balance as a key risk factor for cancer development. Excess body weight is associated with

increased risks of cancers of the endometrium, breast (postmenopausal women), kidney (renal cell tumours), colon, pancreas and oesophagus (adenocarcinomas). By contrast, regular physical activity reduces the risk of developing breast and colorectal cancers, and potentially other tumour types. Overall, excess weight and lack of physical activity may account for one quarter to half of the occurrence of the abovementioned tumour types.

The mechanisms that may underlie these relationships of nutritional energy balance with cancer development may depend on tumour type.

One major mechanism that is increasingly being implicated is alterations in the metabolism of insulin and/or insulin-like growth factors (IGFs) are as possible metabolic links between nutritional energy balance and cancer development. Prospective cohort studies have shown increased risks particularly of colon cancer and endometrial cancer among women and men with high fasting and non-fasting plasma insulin concentrations, and similar associations have been reported for pancreas cancer. Likewise, elevated plasma concentrations of IGF-I have been related to increased risks of cancers of the prostate, breast and colorectum. More independently of adiposity, higher plasma glucose levels (fasting and post-load) have also been associated with increased risks of cancers of the pancreas, liver and endometrium, in particular, as well as of the colon. Finally, there is increasing evidence to suggest that adiposity may also promote tumor development through the release of pro-inflammatory adipokines and cytokines, creating a state of chronic, low-grade inflammation.

In addition to insulin, IGF-I and glucose, endogenous sex hormones are strongly implicated in the development of cancers of the endometrium and breast, and especially among postmenopausal women that are overweight or obese. Among premenopausal women, development of ovarian hyperandrogenism (polycystic ovary syndrome) is a frequent phenomenon that is related to obesity and hyperinsulinaemia, which is associated with an increased risk of endometrial cancer because of reduced ovarian progesterone synthesis.

Besides the extracellular growth signals, there is increasing experimental evidence that intracellular energy sensing mechanisms are also central in controlling cell growth, proliferation and apoptosis. One mechanism of special interest, here, is the suppression of AMP-activated kinase (AMPK) activity, as a result of higher energy status of the cell.

Gaining a better understanding of the mechanisms relating excess weight and physical inactivity to cancer may lead to improved strategies for both cancer prevention and treatment.

S5.4

IGF-I and neuroprotection

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Neuroprotection defines a set of homeostatic, self-repair mechanisms that probably evolved for the specially demanding conditions imposed by brain function. Among these, intercellular messengers such as the insulin-like growth factors (IGFs), that appeared very early during phylogeny, apparently play a prominent role despite their peripheral origin. In contrast to the heterogeneity seen in primitive organisms where at least 30 different IGF-like peptides are described, in mammals only 3 of them, insulin and IGF-I/II are so far known. All exert neuroprotective actions, probably playing also a crucial role in a wide diversity of brain diseases. Although originally ascribed exclusively to the IGF-I receptor, the neuroprotective actions of these peptides in all probability also includes the insulin and IGF-II receptors. Several aspects of these peptides in relation to their brain actions warrant further investigation: 1) altered glucose metabolism, not only in brain but also in the periphery, is unusually common in brain pathologies. An as yet not well understood interaction between insulin and IGF-I signalling probably contributes to it. 2) Hetero-dimerization of insulin and IGF-I hemi-receptors and the unusual chaperone-like properties of the IGF-II receptor undoubtedly adds to the complexity of IGFs actions in the brain, and elsewhere. Their ultimate significance remains undetermined. 3) Insulin/IGF-I resistance triggered by neuro-inflammation, oxidative stress, excess excitatory neurotransmission, or endoplasmic reticulum stress (the four major disruptions linked to brain pathology) is a common process in pathological pathways in brain diseases. Their molecular underpinnings constitute an area for potential druggable targets. 4) Many neuroprotective mechanisms include activity-dependent processes and our recent evidence indicates that serum IGF-I enters into the brain in an activity-dependent fashion. These initial observations suggest an important role of IGF-I in cognitive reserve buildup. Thus, the concerted brain actions of IGFs likely contribute to environmental influence on brain health, brain aging and cognition.

Epigenetics and endocrine programing

S6.1

Glucocorticoids and developmental programming

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Epidemiological evidence suggests that an adverse fetal environment permanently programmes physiology leading to increased risks of cardiometabolic, neuroendocrine and psychiatric disorders in adulthood. We originally hypothesised that prenatal stress via fetal glucocorticoid excess might explain this link. Indeed, in rodents, prenatal stress, glucocorticoid exposure or inhibition/knockout of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), the feto-placental 'barrier' to maternal glucocorticoids, reduces birth weight and causes permanent hypertension, hyperglycaemia, increased hypothalamic–pituitary–adrenal (HPA) axis activity and anxiety-related behaviours in adult offspring. The phenotype persists into a second generation and transmits via male and female lines. This implies epigenetic mediation, a mechanism emerging for at least HPA axis programming. This also appears of potential clinical relevance. Thus, in a singleton-bearing, non-human primate model, exposure to glucocorticoids in the second half of gestation programmes cardiometabolic, HPA and behavioural parameters in 1-year old offspring. In humans, placental 11β-HSD2 activity correlates directly with birth weight and inversely with infant blood pressure. Moreover, low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming. Indeed, maternal glucocorticoid therapy alters offspring cognition and affect, and pregnant women exposed to the 9.11.2001 atrocity and who developed PTSD appear to transmit neuroendocrine changes to their one-year old offspring, but confined to third trimester exposure. Furthermore, exposure to the Nazi Holocaust exerted permanent effects upon glucocorticoid levels and steroid metabolism, effects dependent upon the age at exposure. Second generation effects also occur. Overall, the data suggest that developmental exposure to excess glucocorticoids/stress programmes peripheral and CNS functions in adult life, predisposing to affective and other pathology, and may be transmitted into at least one subsequent generation.

S6.2

Abstract unavailable.

S6.3

Programming of the stress system by the maternal care in animal models

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Life events occurring during the perinatal period have strong permanent long-term effects on the behavioural and neuroendocrine response to stressors. In rats, repeated restraint stress of the pregnant dam during the last week of pregnancy produces long lasting changes in the HPA axis function and behaviours in the offspring. These changes include a hyperactivity of HPA axis response associated with a reduction in the number of hippocampal corticosteroid receptors. The HPA dysfunctions have been reported in infant, young adult and aged animals, therefore suggesting a permanent effect of early stress. Interestingly, after the confrontation to an intense inescapable footshock, prenatal restraint stress (PRS) rats durably show a blunted corticosterone secretion after stress. PRS also induces a hyporeponse of the HPA axis when animals are exposed to an alcohol challenge. Rats exposed to a PRS also show behavioural disturbances known to be related to the HPA axis. Indeed, PRS produces high anxiety levels and depressive-like behaviour during adulthood including sleep disorders related to depression. With ageing, these animals exhibit memory impairments in hippocampo-dependent tasks. Despite the permanent imprinting induced by stress in utero, the dysfunctions observed after PRS can be reversed by environmental or pharmacological strategy. For example, early adoption or environmental enrichment during adolescence, as well as a chronic treatment with Insulin-like growth factor 1 in aged animals attenuated some HPA dysfunction's produced by PRS. Mechanisms underlying the PRS effects on the offspring remain largely unknown. However, previous works demonstrated that maternal glucocorticoids

during pregnancy may play an important role in the HPA disturbances reported. Thus, stressed mothers show high glucocorticoid levels during pregnancy. Furthermore, in the offspring of stressed mothers, the HPA response to stress is normalised by maternal adrenalectomy during pregnancy. Recently, our group has reported that repeated restraint stress during pregnancy leads to a decrease of the placental 11β-HSD2 activity. Finally, gestational stress has long lasting effects on HPA axis and behaviour in female dams. Thus, during lactating period, stressed mothers show an impairment of maternal care and low aggressive behaviour against a male intruder. Moreover, females stressed during pregnancy show an increase of anxiety-like behaviour several weeks after the end of the stress period. Given that, several evidences suggest that changes in maternal care may durably program offspring's HPA function and behaviours, it could be postulated that the alterations of the maternal behaviour during the early postnatal period may also strongly contribute to the long-term effect described after prenatal stress.

S6.4

Epigenetic programming and chronic physical aggression

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Chronic physical aggression has been linked to cortisol secretion and testosterone. Such links could be programmed by environmental effects on gene expression during pregnancy and early childhood. This paper will review research on the chronic aggression-cortisol-testosterone links and summarize a research program on pre and postnatal epigenetic programming.

Glucocorticoid action in the brain

S7.1

Glucocorticoid control of chromatin remodelling in stress-related learning and memory

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It is a well-known observation that glucocorticoid hormones facilitate the storage of stressful, emotional events into memory. How glucocorticoids act in these cognitive processes has still not been completely clarified. Since almost a decade we have been collecting data indicating that memory formation of stressful events involves epigenetic mechanisms coordinating transcriptional processes in dentate gyrus granule neurons. We found that such events evoke the phosphorylation of Serine-10 and the acetylation of Lysine-14 in the N-terminal tails of histone H3 molecules specifically in mature dentate neurons. Corresponding with *in vitro* findings, this epigenetic response is required for chromatin remodeling enabling the induction of immediate-early genes such as c-Fos specifically in these neurons. Subsequent studies employing a set of pharmacological and gene deletion approaches showed that the phospho-acetylation of histone H3, as well as associated gene expression and memory formation requires concurrent signaling via the glucocorticoid receptor and the NMDA/ERK/MSK (NMDA: *N*-methyl-D-aspartate; ERK: Extracellular signal-regulated kinase; MSK: mitogen- and stress-activated kinase) pathways. Thus, epigenetic processes regulating induction of gene transcription are involved in neuroplasticity processes in dentate neurons necessary for the formation of memories of the endured event. Glucocorticoid hormones secreted as a result of the stressful event play a critical role as signaling molecules in these epigenetic processes. Possible mechanisms of glucocorticoid action will be discussed.

Supported by the MRC and BBSRC

S7.2

Abstract withdrawn.

S7.3

Genomic versus nongenomic corticosteroid effects

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Neurons in the CA1 region of the rodent hippocampus express high levels of the nuclear mineralocorticoid (MR) and glucocorticoid receptors (GR). The MR affinity is such that low levels of corticosterone -as seen under rest- already substantially activate this receptor. Hence, for many years the GR was considered to be the main mediator of the stress response. This receptor causes (among other things) a delayed enhanced influx of calcium into CA1 neurons, an enhancement of serotonin responses and an impaired ability to induce long-term potentiation. Overall, these actions help to normalize hippocampal activity several hours after stress and to preserve information encoded shortly after stress. Recently, it has become evident that this delayed normalizing effect is complemented by a rapid nongenomic action of the same hormone. Thus, as soon as corticosteroid levels rise, hippocampal cells show an increased release probability of glutamate-containing synaptic vesicles. This is presumably due to MRs inserted into the presynaptic membrane, linked to the ERK1/2 signaling pathway. Interestingly, membrane-located MRs display a 10-fold lower affinity than their nuclear counterpart, allowing the former to be a prominent player in the stress response. MRs can also be inserted into the postsynaptic membrane and then (via G-proteins) mediate a rapid suppression of the K-conductance IA. Overall, these rapid and quickly reversible effects of corticosterone are expected to raise hippocampal excitability (in concert with other stress hormones) as long as the hormone levels are elevated, thus enabling the early stages of memory formation. Preliminary evidence suggests that both the nongenomic and the genomic effects of corticosterone show regional differentiation. This allows for region-specific facilitation or attenuation of neuronal activity, which is important for the role of the various areas in the neuroendocrine / cognitive processing of stressful information.

S7.4

Acute and chronic stress: central and peripheral actions of glucocorticoids and insulin

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Stressors engage a neural stress response network that is mediated in large part through the immediate actions of the stimuli on corticotropin-releasing factor (CRF) neurons in the amygdala (CeA) and in the long-term by the actions of glucocorticoids (GC) on increased synthesis of CRF in CeA and secretion of CRF on the monoaminergic cell groups as well as forebrain. The consequences of this bias behavioral, autonomic and endocrine outputs in the stressed organism. However, the GC also act both very rapidly and more slowly in hypothalamus and at the pituitary to damp further activity in the HPA axis. When elevations in GC are sustained, systemic insulin concentrations rise in parallel with GC. Together, elevated GC and insulin promote food intake, with a strong bias toward highly palatable calories in moderately stressed rats. This effect is also both acute and chronic. Systemically, the hormones promote increases in fat deposition. This combination over the long term increases fat depot weights. There is good evidence that a feedback signal denoting metabolic well-being acts on brain to reduce activity in the central stress response network, thus ameliorating the neural effects of stressors. Activity in common hepatic vagal afferents reduces intake of palatable calories, however, insulin overrides this action, and it is likely that the central action of insulin is responsible for fat and sucrose preferences shown in moderately stressed rats. Moreover, there is a strong, inverse relationship between mesenteric fat depot weights and hypothalamic CRF expression that supports the conclusion that metabolic well-being modulates the perception of stressors by the brain. Because the HPA axis appears to be in large part responsible for metabolic homeostasis and responds primarily to loss of metabolic stores, the dual feedback and feedforward actions of GC and insulin provide a highly appropriate means to re-establish metabolic equilibrium.

Pituitary cell biology

S8.1

Imaging pituitary cell networks and function

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The pituitary gland generates highly ordered hormone pulses to control basic body functions such as growth, fertility, and lactation. Using an approach combining transgenic mice models with cell-specific fluorescent tags (GH-GFP, PRL-DsRed, POMC-GFP, LH-Cerulean…) and functional optical imaging (pituitary-scale 2-photon excitation microscopy, cellular *in vivo* imaging), we recently unveiled that most, if not all pituitary cell types are much more organized than we first thought from earlier histological studies on thin tissue sections. During the presentation, I will present examples of how i) the sexual dimorphism of the GH axis implicates the GH cell network efficacy, ii) both the developmental pituitary program and external inputs are required for the optimized cell network organization, iii) structural and functional network motifs can differ from one cell network to another (GH cell network versus PRL cell network), and iv) the organisational relationship between parenchymal cell networks and the blood flow circuitry is important for generating hormone pulses.

S8.2

New regulatory mechanisms controlling pituitary hormone secretion

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Hormone release by pituitary cell types is primarily regulated by stimulatory and inhibitory factors secreted by the hypothalamus. In turn, factors produced by the target organs controlled by the pituitary exert a feedback regulation on the corresponding pituitary cells. However, recent data indicate that, besides these factors, other mechanisms operate to finely tune pituitary function. These include both novel peripheral regulatory factors as well as intrinsic cellular elements. Regarding the first group, we will discuss our recent results on the interaction of adipocyte-derived adipokines involved in the regulation of metabolism and energy balance, with the pituitary. Thus, we have shown that adiponectin inhibits both basal GH and LH release as well as ghrelin-induced GH release and GnRH-stimulated LH secretion in rat pituitary cell cultures, wherein the adipokine also increases GHRH-R and ghrelin/GHS-R mRNA content while decreasing that of GnRH-R. We will also discuss the role played by the KISS1/KISS1R neuroendocrine system, which controls puberty and other reproductive functions, at the pituitary level. Specifically, our *in vitro* data using kisspeptin-10 (kp10) show that this peptide acts directly on pituitary somatotropes and gonadotropes to increase both free cytosolic Ca^{2+} and to stimulate modestly but significantly the release of GH and LH. Finally, we will consider the involvement of the different somatostatin receptors (sst1–sst5) in the differential regulation of somatotropes by their classic inhibitor somatostatin and the somatostatin-related peptide cortistatin. We recently cloned two novel human and porcine truncated isoforms of sst5 (sst5B and sst5C) which are selectively activated by somatostatin (psst5B) or cortistatin (psst5C) and can interact with and functionally modulate full length sst5 and sst2. When viewed together, these data suggest that far from representing simple lineal models of regulation, pituitary cell types are controlled by complex multifactorial systems, comprising both intrinsic and peripheral factors, which will have to be uncovered in order to fully understand the precise regulation of the distinct pituitary cell types and, accordingly, their physiological role and pathological implications.

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S8.3

New regulators of pituitary cell proliferation

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Sporadic and familial pituitary adenomas are being recognised and diagnosed with increasing frequency due to better diagnostic techniques and improving awareness.

Recently, a number of important steps have been taken to clarify the molecular pathological events leading to familial pituitary tumorigenesis, with the recognition of the tumour suppressor genes *p27* and *AIP* as causes of familial pituitary tumours in addition to previously-established genes such as *MEN1* and *PRKAR1A*. The abnormal expression of p27 (CDKN1B, which is under-expressed) and AIP (which is over-expressed) in sporadic pituitary tumours has been reported, but no somatic mutations have been recognised in these genes. Furthermore, germline mutations in apparently sporadic cases are extremely rare. The mechanism of p27 haploinsufficiency leading to tumorigenesis is supported by previous data showing p27 as an important cell cycle inhibitor. However, the mechanism whereby AIP causes tumorigenesis is unclear as this molecular co-chaperone has many potentially important partners. The most logical candidates are the phosphodiesterases due to their involvement in the cAMP pathway, which has in turn been previously implicated in somatotroph cell tumorigenesis via the gsp-mutation (GNAS1) and that of the PKA regulatory subunit (PRKAR1A). Nevertheless, there are increasing data that AIP acts as a classic tumour suppressor gene, regardless of its precise mode of action.

A number of outstanding questions remain regarding familial pituitary adenomas including (1) what are the causative genes in cases of AIP mutation-negative familial isolated pituitary adenomas, (2) what genes are responsible for the MEN1 and CDKN1B mutation-negative sporadic and familial MEN1-syndrome patients, and (3) what gene is behind ~40% of Carney complex cases without PRKAR1A but which segregate to the 2p16 area?

There are also exciting and novel developments in studies of the genesis of sporadic pituitary tumours. In addition to previously identified important players such as PTTG and HMGA2 (high mobility group A2 protein), recent studies have implicated the involvement of Akt and ERK pathways, MEG3, a non-coding RNA in non-functioning pituitary adenoma tumorigenesis, E-cadherin and the Wnt pathway, Pi-class glutathione-S-transferase (GSTP1), p21, p16, CXCL12 and its receptor CXCR4, DNA methyltransferase-3 (DNMT-3), Rab18 a protein involved in secretory granules, the folate receptor, pituitary microRNAs, and even the cooking spice curcumin.

Tumorigenesis seems to be a multifaceted process in the different type of pituitary adenomas, the increasing amount of information may lead to novel pathways and possibly novel treatments in the future, but the prime causative mechanisms remain elusive.

S8.4

Dual function of dopamine/somatostatin hybrid agonists

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Somatostatin acts as an inhibitor of hormonal secretion and cell proliferation by acting through 5 somatostatin receptors subtypes (sst1–5). Coupling with Gi/o proteins is associated with effects on various transduction pathways, as adenylate cyclase inhibition or phosphatases activation. Dopamine receptor subtype 2 (D2DR) acts through coupling with similar G-proteins and transduction pathways. Sst, mostly sst2 and sst5, are coexpressed with D2DR in many neuroendocrine normal or tumoral cells. Both sst2 and sst5 are able to form heterodimers with D2DR, modifying ligands binding and signal transduction in a positive cooperation manner. Coactivation (of sst2 and D2DR) with clinically available sst2 (octreotide, lanreotide) and D2DR agonists (cabergoline) in different tumors or cellular models is rarely associated with additive effects in the suppression of cell secretion and proliferation. Availability of hybrid dopamine and somatostatin agonists (dopastatins), combining in the same molecule structural parts of somatostatin and dopamine, opened new possibilities for sst-D2DR cooperation. In the first and most studied cellular model, GH tumoral cells *in vitro*, dopastatins showed clearly a synergic effect on cell secretion and proliferation by acting through both sst and D2DR receptors. In others pituitary tumors as lactotroph and gonadotroph and in most other neuroendocrine and non-neuroendocrine cellular models, coexpressing various levels of sst and D2DR, dopastatins showed an effect closer to that of D2DR agonists. Transduction pathways involved in sst – D2DR cooperation is currently under investigation in various cell models, while a dopastatins, BIM-23A760, is starting clinical studies in acromegaly.

Addison's disease from genetics to clinical outcome

S9.1

Addison's disease: natural history and long-term outcome

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Primary adrenal insufficiency was first described in 1855 by Thomas Addison, demonstrating that the adrenal cortex is essential for life. Life-saving

glucocorticoid replacement became widely available only with the clinical introduction of cortisone in 1949. Chronic primary adrenal insufficiency (PAI) has a prevalence of 93–140 per million and its incidence is rising due to an increase in autoimmune adrenalitis. In developing countries tuberculosis is still a leading cause of PAI. Despite significant improvements in therapeutic regimens for PAI there is mounting evidence that well-being is not fully restored by current replacement strategies. Impaired well-being has been repeatedly and consistently demonstrated in PAI irrespective of the glucocorticoid used or the distribution of hydrocortisone doses. This may be related to the missing early morning rise in glucocorticoid availability with current replacement regimens. Furthermore, there is now growing evidence that not only quality of life but also life expectancy may be affected by PAI including increased cardiovascular mortality and an increased cancer risk. However, these data have been challenged by a recent investigation from Norway suggesting that increased mortality is restricted to younger age ($<$40 years) and male sex. In particular, patients with PAI are at risk of life-threatening adrenal crisis. Retrospective analysis in 444 patients with adrenal insufficiency revealed an incidence 5.1 crises per 100 patient years. Major precipitating causes were gastrointestinal infections and fever of any cause. In 8% no specific cause was identified. Patients with PAI and significant non-endocrine comorbidities had a moderately higher risk of crisis (RR 1.24, $P=0.057$). In secondary adrenal failure female sex and concomitant diabetes insipidus were risk factors for adrenal crisis (RR 1.26 and 1.25, respectively, $P<0.05$). We currently collect data on adrenal crisis prospectively in a large cohort of patients with adrenal insufficiency to better define the risk factors for this emergency which will be presented. In summary, impaired well-being and altered morbidity and mortality in PAI indicate the need to improve current replacement and surveillance strategies in Addison's disease.

S9.2

Genetics of autoimmune Addison's disease

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Autoimmune Addison's disease (AAD) can be divided into two distinct genetic aetiologies. The childhood onset type 1 polyendocrinopathy (aka APECED) syndrome is a monogenic autosomal recessive trait. Whereas the common-or-garden AAD that may be found as an isolated endocrinopathy, or in association with thyroid disease or type 1 diabetes as part of the autoimmune polyendocrinopathy type 2 syndrome (APS2). Isolated AAD (IAD) and APS2 can be considered as very similar from the genetic standpoint, and are inherited as complex multigenic conditions. Importantly, although families with more than one case of IAD/APS2 in them are rare, they have been found and allow an estimate for the heritability (lambda-sib) of IAD/APS2 of about 150. This means IAD/APS2 has a very strong genetic load, but that the disease alleles are likely to be rather rare in the population. Thus far, the major susceptibility loci for IAD/APS2 have been uncovered by studying candidate genes for the commoner complex autoimmune endocrinopathies such as type 1 diabetes or autoimmune thyroid disease. For instance, there are IAD/APS2 susceptibility alleles within the MHC region of chromosome 6p21, CTLA4 (2q33) and PTPN22 (1p13). Additional loci that have come to light in recent years include CYP27B1, FCRL3 and CIITA. However, none of these loci have strong effects and so none are helpful for diagnostic or predictive use in clinical practice. This is a marked contrast to the situation with APS1, where the mutational spectrum is well-defined in several populations and analysis has particular clinical utility in many circumstances.

S9.3

Early subclinical Addison's disease

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The adrenal autoimmune process that causes primary adrenal insufficiency is made evident by the appearance of circulating adrenal autoantibodies directed against the enzyme steroid 21-hydroxylase (21OHAb), in genetically predisposed individuals. Adrenal autoantibodies appear months to years before the appearance of clinical signs of adrenal insufficiency and a pre-clinical phase of the disease can be recognised. Subjects positive for 21OHAb present with a variable degree of pre-clinical adrenal insufficiency as revealed by the low-dose ACTH stimulation test (LDT) and by aldosterone concentration and plasmatic renin activity. The progression of the destructive process against the adrenal cortex is accompanied by a progressive increase in 21OHAb levels, more evident in subjects with an impaired response to the LDT. A spontaneous remission of early subclinical adrenal insufficiency is observed in the majority of subjects with normal response to the LDT. On the contrary, a pathologic LDT is invariably followed by progression of the adrenal dysfunction that ultimately leads to clinical Addison's disease (AAD). Factors increasing significantly the risk of progression towards clinical adrenal insufficiency include: male gender, presence of other concomitant autoimmune diseases, impaired LDT and a high 21OHAb titre. Among genetic factors, HLA-DR3-DQ2, DR4-DQ8, MICA5.1 and CTLA gene polymorphism are significantly associated with appearance of 21OHAb, but do not influence the natural history of the disease and do not predict future clinical adrenal insufficiency. On the contrary, the presence of the DRB1*0403 allele in 21OHAb-positive subjects is significantly and negatively associated with progression to clinical Addison's disease (AAD), and represents the major protective gene marker. The combined use of biochemical and genetic tests in 21OHAb-positive subjects enables the accurate estimate of the risk for future development of clinical AAD and paves the way to clinical studies aimed at preserving the residual adrenal function in subjects with early subclinical AAD.

S9.4

New ways of delivering glucocorticoids

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Replication of physiology is a basic tenet of endocrinology but this is rarely achieved. We developed a modified-release hydrocortisone to provide circadian cortisol. The adrenal glucocorticoid, cortisol, is an essential stress hormone and its secretion follows a distinct rhythm regulated by the central circadian oscillator in the suprachiasmatic nucleus. Circulating cortisol levels are low at sleep onset, rise between 0200 and 0400 h, peak within an hour of waking and then decline through the day. Loss of this rhythm, as occurs in adrenal insufficiency, is associated with metabolic abnormalities, fatigue and poor quality of life, despite replacement with immediate release hydrocortisone. Our aim was to investigate whether an oral formulation of modified release hydrocortisone (Chronocort) could replicate the physiological cortisol rhythm in normal healthy volunteers. Using reference subjects ($n=33$) we defined the normal cortisol rhythm. We then tested Chronocort against immediate-release (IR-HC) in dexamethasone suppressed healthy volunteers ($n=28$). Chronocort 15mg demonstrated delayed and sustained release: mean (S.E.M.) C_{max} 457 (38·4) nmol/l at 7·41 (0·57) hrs after drug. Bioavailability of Chronocort 5, 10, 15 & 30 mg was 100, 79, 86, & 69% that of IR-HC. In patients with CAH, Chronocort 30 mg, showed a similar pharmacokinetic profile to that seen in healthy volunteers and controlled early morning (0800 h) ACTH and 17OH-progesterone. Modelling demonstrated that Chronocort 15 to 20 mg at 2300 h and 10 mg at 0700 h could reproduce physiological cortisol levels. In conclusion, using modern formulation technology it is possible to generate physiological cortisol profiles. This approach provides a new paradigm for glucocorticoid replacement therapy with important clinical implications for the current management of congenital adrenal hyperplasia and adrenal insufficiency.

Secondary osteoporosis

S10.1

Growth hormone deficiency: bone matters

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Growth hormone (GH) plays a critical role for longitudinal bone growth in children, the achievement of a normal peak bone mass in young adults, and it also affects bone mass and bone remodelling in adults. Among the most reported features of severe growth hormone deficiency (GHD) are abnormal body composition, in particular, increased fat mass and reduced lean body mass, osteopenia and increased risk of fracture. Low bone mass has been reported using dual energy X-ray absorptiometry (DEXA) and other quantitative methodologies. Bone quality in GHD adults is not studied. Reduced serum concentrations of the markers of bone turnover and the scarce histomorphometry data suggest that GHD is, probably, a state of low bone turnover.

Clinical studies have shown that GH replacement therapy accelerates bone turnover within a few weeks, whereas changes in bone mineral density (BMD) and bone mineral content (BMC) were observed much later, 1–2 years after initiating of GH therapy because of initially negative bone remodelling balance. There are few studies determining the effects of prolonged GH replacement. Seven years of GH replacement therapy in 20 adults resulted in increased lumbar

spine and forearm BMD between 1 and 6 years. In our study, 10 years of GH therapy in 87 GHD adults produced sustained increases in bone mass and density with the maximum effect after 7–10 years.
T-scores were almost normalized. As *t*-score in lumbar spine and femur neck is strongly related to the risk of fractures in these regions, the 10-year replacement is likely to reduce the risk of fractures in GHD adults.
The differences in the treatment responses between genders, age groups and groups of adults with different onset of GHD will be discussed.

S10.2

Glucocorticoid effects on bone

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Glucocorticoid-induced osteoporosis (GIO) is the most relevant form of secondary osteoporosis and fractures occur in 30–50% of individuals. Moreover, glucocorticoids (GC) may cause osteonecrosis in as many as 25% of patients on high-dose or long-term therapy. Bone loss occurs fast and may be as high as 10–15% within the first 3–6 months and preferentially affects bone sites rich in cancellous bone such as the ribs, vertebral bodies and the femoral neck. It appears that there is no 'safe' dose of GC and even inhaled GC in higher doses suppress bone formation and accelerate bone loss.
The effects of GC on bone are primarily direct and here the major effect is on osteoblasts and osteocytes. GC lead to premature apoptosis of these two cell systems and inhibit osteoblastogenesis at the same time. Newer insights into the pathophysiology of GIO has led to the discovery of the importance of the local activity of the 11β-hydroxysteroid dehydrogenase (11β-HSD) system which consists of two isoenzymes determining the local concentration of active cortisol. Various activation of this system is thought to be responsible for the clinical observation whereby patients are more or less prone to the effects of GC. Other important mechanisms i.e. impaired production of IGF-1 and testosterone support the decrease in osteoblastic activity. Although absolute osteoclastic activity does not appear to be increased in GIO it is nevertheless too high when compared to the simultaneous marked decrease in bone formation. Here, the primary driving factor seems to be a decrease in local osteoprotegerin production that allows RANKL to increase osteoclastogenesis and promote osteoclast life span.
The cornerstones of prevention and treatment of GIO include a calcium (1200–1500 mg) and vitamin D3 supplementation (800–1200 IE) as well as a bisphosphonate treatment or, in high-risk patients teriparatide injections.

S10.3

Defining the target level for vitamin D

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Vitamin D is an essential hormone for skeletal metabolism across the lifecycle. Rickets and osteomalacia, uncommon manifestations of vitamin D deficiency in western countries, are still common in the Middle East and Asia (1, 2). Furthermore, low bone mass and fractures, latent manifestations of vitamin D insufficiency, are common conditions worldwide (3, 4). Serum 25-hydroxy-vitamin D (25-OHD) level is the best index of vitamin D nutritional status, and whereas it is generally accepted that a level below 5–10 ng/ml (multiply by 2.5 to convert to nmol/l) represents vitamin D deficiency, what constitutes a desirable level is now emerging based on the evidence detailed below.
Vitamin D sufficiency in adults and elderly can be defined by evaluating discrete biochemical or physiological outcomes that this hormone modulates. These include intestinal calcium absorption, serum parathyroid hormone levels, bone mass, muscle function, and fractures. Intestinal calcium transport increases linearly from 15% to 35% when serum 25-OHD level rises from 10 to 32 ng/ml (5); whereas the vitamin D level at which PTH levels tends to decrease and follow a shallower curve varies from study to study, with a range of 20–40 ng/ml (6). In the NHANES III study, higher serum levels of 25-OHD were associated with higher bone mass of the hip in older (and younger) men and women; the curves being steepest for 25-OHD levels between 10 and 40 ng/ml (7). As for musculoskeletal outcomes, the elderly need a 25-OHD level of around 26 ng/ml to improve muscle function and reduce the risk of falls, and a level above 30 ng/ml to reduce the risk of hip and non-vertebral fractures (8). Therefore, based on the above body of evidence, a desirable target level for 25-OHD would be above 30 ng/ml. Using this cut-off, it is estimated that 1 billion individuals would suffer from hypovitaminosis D worldwide, regions at higher risk are the Middle East and Southern Asia (2, 3). Each 100 IU of vitamin D taken orally would raise 25-OHD level by 1 ng/ml, consequently the current recommendations for an adequate intake for vitamin D, of 400 IU in adults and 600 IU in elderly, would be sub-optimal to reach a target level above 30 ng/ml. Daily doses of 800–1600 IU have been suggested (9) and would vary depending on the nutritional status of the individual at the start of supplementation.
Hypovitaminosis D is a major public health problem across all life stages, with deleterious immediate and latent manifestations (1–4). Strategies to address this often silent disease should include public education, national health policies for screening and prevention through food fortification, and treatment of high risk patients through vitamin D supplementation. In addition to the above, further research is needed to standardize vitamin D assays, conclusively define optimal vitamin D levels, and determine the doses and regimens of vitamin D supplementation for pregnant and lactating women, infants and adolescents.

S10.4

Sex steroids in the regulation of bone metabolism in men

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Osteoporosis-related fractures constitute a major health concern not only in women but also in men. The relative contribution of estrogens and androgens for the male skeleton remains unclear. Most epidemiological studies demonstrate that serum estradiol is a stronger predictor of bone mineral density than serum testosterone. However, conflicting results have been presented regarding the impact of serum sex steroids for fracture risk in men, probably because previous studies have been underpowered and have analyzed the serum sex steroid levels using immunoassay-based techniques with a questionable specificity at lower concentrations. We recently showed that elderly men with low serum estradiol have an increased risk of fractures in the large population-based MrOS Sweden study, with serum sex steroids analyzed by the specific gas chromatography–mass spectrometry technique. In contrast, serum testosterone was not an independent predictor of fracture risk.
There are two main sources of sex steroids in elderly men, the testicles and the adrenals. Interestingly, we found that low serum DHEA was related to fracture risk independently of serum sex steroids in the MrOS Sweden study, indicating that adrenal-derived DHEA, which is locally converted to estradiol and/or testosterone, has an impact on fracture risk.
Experiments using mice with inactivated sex steroid receptors demonstrated that both activation of the estrogen receptor (ER)α and activation of the androgen receptor (AR) result in a stimulatory effect on the cancellous bone mass in males. ERβ was of no importance for the skeleton in male mice while it modulated the ERα-action on cancellous bone in females. *In vitro* studies demonstrated that the G-protein coupled receptor GPR30 is a functional ER. Our recent *in vivo* analyses of GPR30-inactivated mice revealed no function of GPR30 for cancellous bone mass but it is involved in the regulation of longitudinal bone growth.

New aproaches to epigenetics and hormone/gene regulation

S11.1

Abstract unavailable.

S11.2

Existence of a 'dormant' androgen receptor-regulated gene program revealed by ChIP-seq and its influence in prostate cancer progression

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Prostate cancer (PCa) patients are typically treated by androgen ablation therapies that ultimately fail when PCa enters in androgen-depletion independence (ADI),

leading to metastasis and death. To develop better clinical treatments it is crucial to understand the mechanisms underlying progression towards ADI and metastasis. Using ChIP-seq technology, I will show how the forkhead factor FoxA1 precisely restricts the extension of the androgen receptor (AR) binding program maintaining as 'dormant' 75% of the potentially AR-regulated program. Once 'awaked' -when FoxA1 is absent-, this previously unknown AR-regulated gene program would promote the progression to aggressive PCa by potentially conferring metastatic properties to the cell, including cell motility and cell migration. Together, this cascade of events provides a potential direct link between FoxA1-dependent regulation of AR, progression to ADI, and metastatic behaviour. These studies show how fine modulation of transcription factor levels can drastically modify the complete set of directly-regulated gene programs, deriving in dramatic consequences for tumour progression.

S11.3

MicroRNA and glucocorticoid signaling

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The absolute protein level of hormone receptors is a crucial determinant for cellular responsiveness towards hormone exposure. In that respect, it is important to note that, by recent progress in gene expression profiling techniques, a novel class of small non-coding RNAs has been discovered. These non-coding transcripts of approximately 21 nucleotides are called microRNAs (miRNAs) that downregulate protein levels in a cell by translational repression. Given the increasing and varied number of functions attributed to miRNAs, it is plausible to hypothesize that miRNAs regulate levels of proteins involved in glucocorticoid feedback mechanisms, in particular because miRNAs preferentially target transcription factors. In the lecture, I will highlight the role of miRNAs in endocrine signalling and present experimental evidence that protein levels of the glucocorticoid receptor are under control of cell-type specific microRNAs.

Growth factors and signaling networks in pituitary tumours

S12.1

New mechanisms involved in pathogenesis of prolactinomas: critical role of the *HMGA* genes

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HMGA2 gene amplification and overexpression in human prolactinomas and development of pituitary adenomas in HMGA-transgenic mice showed that HMGA proteins play a crucial role in pituitary tumorigenesis. Recently, we have explored the pRB/E2F1 pathway to investigate the mechanism by which HMGA proteins act, showing that HMGA2 interacts with pRB and induces E2F1 activity in mouse pituitary adenomas by displacing HDAC1 from the pRB/E2F1 complex – a process that results in E2F1 acetylation. We found that loss of E2F1 function (obtained by mating HMGA2 and E2F1$^{-/-}$ mice) suppressed pituitary tumorigenesis in HMGA2 mice, thus demonstrating that HMGA2-mediated E2F1 activation is a crucial event in the onset of these tumors.
To identify other genes involved in the process of pituitary tumorigenesis induced by the HMGA proteins, more recently we have analysed the gene expression profile of pituitary adenomas developed by HMGA2- and HMGA1-transgenic mice in comparison with normal pituitary glands from control mice. We have identified 82 transcripts increased and 72 transcripts decreased of at least 4-fold in all the mice pituitary adenomas analyzed compared with normal pituitary glands. Among these genes, we focused our attention on two genes, *Mia/Cd-rap* and *Ccnb2*, the first down- and the latter up-regulated in the adenomas compared to normal tissue. We demonstrated that the HMGA proteins directly bind to the promoter of both these genes and are able to regulate their expression.
For *Ccnb2*, the gene coding for cyclin B2, we also analysed the expression of its human homologue in a panel of human pituitary adenomas of different histotype and correlated the results with the expression of the *HMGA* genes. We found a statistical direct correlation between *CCNB2* expression and each of the *HMGA* genes, thus indicating that HMGA-induced cyclin B2 overexpression gives an important contribution to human pituitary tumorigenesis.

S12.2

MAPK and PI3K/AKT pathways in pituitary tumorigenesis

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Mitogen-activated protein kinases (MAPK) are a family of serine/threonine kinases and are key signaling pathways involved in the regulation of normal cell proliferation, survival and differentiation. In mammals, there are more than a dozen MAPK genes. The best known are the extracellular signal-regulated protein kinases (ERK1 and 2); c-Jun N-terminal kinases (JNK1-3); p38s (α, β, γ, δ) and ERK5. Aberrant regulation of MAPK cascades contribute to cancer and other human diseases. In particular, ERK 1/2 which is a downstream component of a signaling module that is activated by the Raf serine/threonine kinases has been the subject of intense research. Raf activates the MAPK kinase (MEK1/2) dual-specificity protein kinases, which then activate ERK1/2. The mutational activation of Raf in human cancers supports the importtant role of this pathway in human oncogenesis. Additionally, the Raf-MEK-ERK pathway is a key downstream effector of the Ras small GTPase, the most frequently mutated oncogene in human cancers.
The serine/threonine protein kinase PKB/Akt is a crucial regulator of cell growth, proliferation, differentiation and apoptosis. Mitogenic signaling by receptor tyrosine kinases that increase phosphatidylinositol 3-kinase (PI3K) activity lead to activation of PKB/Akt which in turn triggers a number of responses like cell growth, survival and increased motility. Interactions between MAPK and PI3K/Akt pathways have also been reported (1).
There are a number of studies investigating the role of these signaling cascades in pituitary tumorigenesis. In somatolactotroph GH4C1 cell lines, both gsp oncogene and over-expression of wild-type $G_s\alpha$ protein was found to initiate a sustained MAPK ERK 1/2 activation (2). In a study performed in GH-secreting adenomas and non-functioning pituitary adenomas, the activation of G-protein-coupled receptors by neurohormones caused an increase in ERK 1/2 activity, while increasing cAMP by forskolin increased ERK 1/2 activity only in GH-omas (3). In a study by Muşat *et al*. Akt mRNA was found to be over-expressed and immunohistochemical expression of phosho-Akt was found to be higher in pituitary adenomas compared to normal pituitaries (4). In a knock-in mutant mouse model carrying a mutation in thyroid hormone receptor-β gene, spontaneous development of TSH-omas with accompanying activation of Akt and its downstream effectors were noted (5). In a recent study, mutations and amplifications in PI3K3CA gene of the PI3K/Akt pathway have been found in invasive pituitary tumors compared to noninvasive ones (6).
In conclusion, there are reports showing both MAPK and PI3K/Akt over-activation in pituitary tumors, however whether activation of these pathways are primary events or their activation results from upstream regulators of these pathways needs to be revealed.

S12.3

Molecular and histological prognostic markers in pituitary tumors

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Although most pituitary tumors are benign, some are invasive or aggressive. In the absence of specific markers of malignancy, only tumors with metastases are considered malignant. Recently we identified a marker set of invasion, proliferation and aggressiveness in prolactin (PRL) tumors1. We will present the prognostic value of histological and molecular markers in PRL tumors and their usefulness in other types of pituitary tumors.
Forty-five patients, 23 men and 22 women, with a PRL tumor were operated by transsphenoidal surgery, with a long post- surgical outcome (mean follow-up: 124

months; 36–300 months). The tumor size and the invasion were studied by radiology (MRI). Histological (mitoses and labeling for Ki-67, P53, PTTG) and transcriptomic (microarrays and q-RTPCR) methods were performed. Thirty-nine tumors were classified into 3 groups: non-invasive ($n=17$), invasive ($n=15$), and aggressive-invasive with histological signs of proliferation ($n=7$) tumors. Two tumors are considered as malignant, on the presence of metastasis, 5 and 16 years after primary surgery. Two statistical analyses, taking into account the histological groups and the post-surgical out come were performed in 39 patients (15 patients with remission and 24 patients with persistence and progression in 9 of them). The expression of each gene was compared to the histological classification using a non-parametric test and to the follow-up using a survival model.
By radiology, these 45 PRL tumors consisted of 8 microadenomas and 37 macroadenomas (volume >0.5 cm^3). By histology, the detection of 4 markers of proliferation (mitosis, Ki-67, PTTG and P53) confirmed that no marker per se could distinguish between invasive and non-invasive tumors. However, mitoses and Ki-67, PTTG and p53 labelings were significantly different in 7 invasive tumors, which were classified as aggressive-invasive tumors. These results are consistent with the existence of 3 groups of PRL tumors. By q-RTPCR analysis, we found that one gene implicated in invasion (ADAMTS6) and 6 genes of proliferation (CRMP1, PTTG, ASK, CCNB1, AURKB, CENPE) were differentially expressed between the 3 groups of tumors ($P=0.038$ to $P<0.0001$). The survival analysis revealed that 6 genes (ADAMTS6, PTTG, ASK, CCNB1, AURKB, CENPE) were differentially up- or down regulated with high degree of significance ($P=0.0028$ to $P<0.0004$) in those patients either in remission or with persistent or progressive tumors. By the same methodology applied to other types of tumors other genes were found.
In conclusion, molecular and histological markers are useful in classifying the PRL tumors into three groups. Differential expression of some genes may predict the aggressiveness and recurrence potential specifically in PRL tumors, but other genes are found in other types of pituitary tumors.

S12.4

Predictive markers of pituitary adenoma behaviour

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Markers to predict tumor biology represent an important tool for the optimal management of pituitary adenomas. Specific morphologic features may serve as predictive markers of tumor behavior. These include a) tumor cell-specific markers, b) stromal-related elements involving vasculature and angiogenetic factors, and additional stromal substances and c) miscellaneous tumor type-associated features.
Macroscopic invasion of the perisellar tissues, defined as radiographic or gross operative finding, is considered a more consistent prognostic indicator. Regarding morphology, cytologic atypia is not a reliable feature. In contrast, the number of mitoses is very important for prognosis. Given that only scarce mitoses can be identified, particularly in some aggressive cases, the Ki-67 represents an alternative key feature to assess tumor proliferation. In the recent WHO classification, the Ki-67 labeling index (LI) represents a major prognostic indicator for pituitary adenomas. In addition, expression of the p53 gene product is very important marker to access tumor biology. Adenomas with more than 3% Ki-67 LI and extensive p53 immunoreactivity are classified as 'atypical adenomas'. Some investigators have proposed to designate adenomas as 'atypical' when Ki-67 LI is more than 10% irrespectively of p53 status
Apoptosis and mitoses represent two adverse and asynchronous events, which under physical conditions maintain the optimal cell numbers. Apoptoses can be recognized by histology alone. Using DNA labeling techniques we can identify apoptotic cells, higher apoptotic labeling index was found in functioning compared to nonfunctioning adenomas, in microadenomas, particularly in corticotroph adenomas, and in untreated adenomas, particularly prolactinomas.
Cytogenetic analysis of chromosomes may provide important information regarding tumor development and progression. Increased chromosome 11 copies are more frequent in functioning, aneuploid pituitary adenomas. Monosomy or partial loss of chromosome 11 in adenomas with normal or increased DNA labeling index indicates complex genomic abnormalities of chromosomes, other than chromosome 11.
Immunohistochemical detection of somatostatin receptors is important, as their density in the cytoplasmic membrane is directly related to the effectiveness of somatostatin analogues. Therefore, morphologic assessment of the somatostatin receptor profile can predict the responsiveness and also validate the effectiveness of treatment with somatostatin analogues.
New drugs such as temozolomide can be used to treat aggressive pituitary tumors. 0–6 ethylguanine DNA methyltransferase (MGMT) serves as a predictive marker for this new option of treatment. It is important to know that only patients with adenomas showing low MGMT levels respond, whereas those with high levels are resistant to temozolomide therapy.
We can conclude that several predictive factors are currently available that may be applied using morphologic methods.

Pro & con – Surgery for 'asymptomatic' hyperparathyroidism

S13.1

Surgery for asymptomatic primary hyperparathyroidism

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Primary hyperparathyroidism (PHPT) is not an uncommon endocrine disease in regions where there are liberal indications for serum calcium measurements. There is a general consensus that virtually all patients with symptomatic PHPT should undergo an operation. The proportion of patients with mild or 'asymptomatic' PHPT varies between 40 and 80% in published reports, but a frequent estimate is that about 50% of all PHPT patients belong to this category. The controversy regarding the role of surgery in asymptomatic PHPT involves a number of medical, social, economical as well as patient preference issues, i.e.,
Will mild neurocognitive symptoms improve after surgery?
What is the effect on bone density and future fracture risk?
Long-term effects on cardio-vascular disease and survival?
Apart from the short- and long-term effects of a cure from the disease, several aspects of the surgical procedure itself need to be taken into account:
The role of pre-operative localisation methods.
The extent of the operation (local-, regional-, or full surgical exploration).
The expertise of the surgeon performing the operation (operative volume for PHPT; success- and operation-specific complication rates).
The patient's co-morbidity, age and expected life expectancy.
Cost-effectiveness of the operation vs. continued medical observation.
Patient preferences.
The overall analysis of the role of surgery in asymptomatic PHPT patients involves an estimate of the balance between benefits and risks. A surgeon with a special interest and experience with PHPT is probably the best individual to explain the risks, benefits and alternatives to operative intervention.

S13.2

Management of mild primary hyperparathyroidism

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The clinical presentation of primary hyperparathyroidism 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. Several consensus development conferences have discussed management of these patients with mild, borderline pHPT during the last twenty years.
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 (QoL) and cognitive function would improve following operative treatment. Systematic randomized studies on these patients with up to two years observation time have so far not indicated benefit of surgery. It has to be taken into account that the studies were based on generic not disease specific questionnaires. At present, it is not recommended to regard impaired QoL and psychiatric symptoms as indications for operation. Another central question is the cardiovascular (CV) aspects of pHPT. Case control studies have indicated increased CV-risk and benefit of operation. Mild pHPT has also been related to central obesity and insulin resistance. However, recent data do not indicate benefit of operation on CV-profile including markers of insulin resistance, despite normalisation of PTH and bone turn-over markers following surgery.
Long term prospective studies in mild pHPT have indicated significant bone loss in the ten to fifteen year perspective, however based on very few observations. On the other hand, there is growing evidence from recent prospective studies based on consensus guidelines that patients safely can be followed conservatively for some years. In years to come these studies may reveal whether this also holds for the longer perspective. However, at present no prospective studies are powered to give final answers on hard endpoints such as cardiovascular events and fractures.

Thyroid

S14.1

TSH receptor and thyroid diseases

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The thyrotropin receptor (TSHr) is a glycoprotein hormone receptor controlling the thyroid growth and the thyroid hormones production, upon stimulation by its natural ligand, the thyrotropin hormone (TSH). In some pathological situations, this receptor is activated in the absence of TSH, either by autoantibodies from patients with Graves' disease (an autoimmune thyroid disease where the TSHr is targeted and activated by autoantibodies), or by activating mutations (as observed in autonomous thyroid adenomas), or by abnormally high levels of another glycoprotein hormone, the chorionic gonadotropin (hCG), produced by the placenta during the pregnancy (which results in gestational hyperthyroidism).
So far the mechanism of activation of this receptor by the TSH, autoantibodies, hCG or natural mutations remains poorly understood.
We propose here a model of activation for the TSHr which takes into account all these physiological and pathological observations.

S14.2

DUOX2 gene and thyroid disease

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Generation of hydrogen peroxide (H_2O_2) in thyroid cells is essential for the synthesis of thyroid hormone. H_2O_2 is produced by the Dual Oxidase 2 (DUOX2) at the apical membrane of the thyrocyte, where it is required by thyroperoxidase for the iodination of thyroglobulin. A dual oxidase maturation factor 2 (DUOXA2) was recently identified as an endoplasmic reticulum-resident protein required for expression of DUOX2 activity. DUOX2 and DUOXA2 genes are located in immediate vicinity at human chromosome 15q, and constitute a functional unit evolved from ancient prokaryotic operons.
Deficiency of DUOX2 leads to congenital hypothyroidism (CH) in humans and mice. We described biallelic inactivating mutations in DUOX2 in patients with severe and permanent CH, while monoallelic defects cause milder and transient CH. These findings represented the first proof of a direct involvement of DUOX2 in human thyroid hormonogenesis, and showed that a (permanent) genetic defect could cause a *transient* CH phenotype. Missense DUOX2 defects in compound heterozygosity with nonsense mutations were later shown to contribute to permanent but milder forms of CH, adding to the hypothesis that biallelic defects are necessary for permanency of the disease. However, transient CH was recently reported in children with biallelic mutations in the gene, albeit not functionally tested.
We performed a large-scale screening in 102 patients with thyroid dyshormonogenesis and identified DUOX2 mutations in 33% of the cohort, and a much lower prevalence of DUOXA2 defects of only 2%. Even when DUOX2-DUOXA2 genes are also expressed in other tissues as the lung or the gastrointestinal tract, we did not identify any extra-thyroidal symptoms in patients with DUOX2 or DUOXA2 genetic defects. This probably reflects the tight and complex tissue-specific regulation of H_2O_2/superoxide generation systems, including overlapping functions of the DUOX1/DUOXA1 paralog pair in human mucosal host defence.

S14.3

Molecular basis of non thyroidal illness syndrome

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The term nonthyroidal illness syndrome (NTIS) refers to characteristic changes in thyroid hormone (TH) levels during illness and starvation. These changes are low T3 and high rT3 in serum and tissues, normal or low serum T4 and inappropriately normal or low serum TSH. It remains unclear whether NTIS is a beneficial adaptive response to reduce energy consumption, or a form of secondary hypothyroidism that requires TH treatment, and there is no clinical evidence that TH treatment is advantageous, or indeed disadvantageous.
NTIS patients have an impairment of hypothalamic–pituitary function. The increase in proinflammatory cytokines and endogenous glucocorticoids typically seen in critically ill patients, together with administration of glucocorticoids and dopaminergic drugs, could directly suppress TRH secretion, the pituitary response to TRH and TSH secretion.
About 80% of T3 is produced by extrathyroidal deiodination of T4. In NTIS low T3 and high rT3 are related to a decrease in liver type 1 iodothyronine deiodinase (D1) and skeletal muscle (SM)-D2 activities and to the increase of D3 activity in liver and SM.
NTIS patients show decreased T4 and T3 in most tissues caused in part by reduced uptake, although this is not limiting if appropriate replacement therapy is given.
TH action depends on the tissue expression of TH receptors (TRs), retinoid X receptor (RXR) and corepressors and coactivators. In patients with NTIS *THRB1* and *RXRG* expression decreased in SM and adipose tissue and *THRA1* expression and MCT8 transporter decreased in adipose tissue, indicating a decreased potential for TH. Septic NTIS patients showed a decrease in *D2* expression and an increase in D3 activity in SM, but no changes in deiodinase activities were observed in adipose tissue. Septic shock NTIS patients tend to decrease production and increase degradation (muscle) or decrease uptake (adipose tissue) of T3 that probably decrease TH actions.

S14.4

Type 3 deiodinase and cancer

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Thyroid hormones (TH) are endocrine molecules necessary for multiple biological processes that are crucial to tumor growth and differentiation. Several reports pointed out a pivotal role of thyroid status in the tumoral behavior.
TH action is regulated by the action of the deiodinases. Type 2 deiodinase (D2) activates thyroxine (T4) by converting it to T3, whereas D3, by inactivating T3, terminates thyroid hormone action.Thus, the deiodinase family of selenoprotein constitutes a potent mechanism to control thyroid hormone signaling, allowing cells to customize their own T3 intracellular concentration in a spatial- and temporal-dependent/specific fashion.
D3 is an oncofetal protein frequently expressed in proliferating and neoplastic cells, but its role in this context is unknown. D3 mRNA and/or activity has been reported in several tumoral cell lines (breast and colon carcinoma, hepatocarcinoma and neuroblastoma) and human tumors, including astrocytomas, gliomas, TSH-secreting pituitary tumors. High levels of D3 expression in vascular tumors, a condition that can cause consumptive hypothyroidism.
At the same time, tissues expressing D3 have lower T3 concentrations than what would be expected from plasma contribution; thus, D3- expressing tissues have a gene expression profile typical of hypothyroid cells (28, 175). This is explained by the inactivation of T3 and T4 that takes place at the plasma membrane level immediately after these hormones enter the cell.
We have recently demonstrated that the Shh pathway, through Gli2, directly induces D3 in proliferating keratinocytes and in mouse and human basal cell carcinoma, the most frequent human cancer. We further demonstrate that Gli-induced D3 over-expression reduces intracellular active thyroid hormone (T3), thus resulting in increased cyclin D1 and keratinocyte proliferation.
Whether and how the control of local TH homeostasis contributes to the neoplastic growth will be discussed and is the object of active investigations.

Progress in understanding and management of diabetes

S15.1

The metabolic syndrome is getting nervous

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The classical diagnostic strategy of internal medicine, including medical history, physical examination and additional diagnostic test, is unable to assess the activity of the autonomous nervous system in detail and, consequently, has resulted in negligence of the involvement of the autonomic nervous system. Nonetheless, evidence is emerging that the autonomous nervous system is

involved in the pathophysiology of complex diseases. These conditions include insulin resistance, type 2 diabetes mellitus and dyslipidemia, which have traditionally been interpreted in brainless concepts of disease. Several lines of evidence obtained in rodent studies have provided support for this involvement of the autonomous nervous system. These include experiments emploing retrograde neuronal tracers, which have documented for instance tissue specific neuroanatomical representation of different fat compartments in hypothalamic nuclei (Kreier *et al*., Endocrinology 2007). Conversely, experiments using tissue specific denervation in combination with neuroendocrine interventions have documented that hypothalamic nuclei and the two branches of the autonomic nervous system are powerful modulators of tissue specific hormone sensitivity. For instrance, NPY induces hepatic insulin resistance of VLDL production through neuronal activity of hepatic sympathetic nerves (van den Hoek *et al*. *Diabetes* 2008). In humans the involvement the hypothalamus in the pathophysiology of type 2 diabetes mellitus was proven by functional MRI (Vidarsdottir *et al*. *Diabetes* 2007). These observations support the notion that the metabolic syndrome is getting nervous, even though we lack the tools to assess this involvement in all details in humans *in vivo*.

S15.2

The metabolic memory

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Large randomized studies have established that early intensive glycemic control reduces the risk of diabetic complications, both micro and macrovascular. However, epidemiological and prospective data support a long-term influence of early metabolic control on clinical outcomes. This phenomenon has recently been defined as 'Metabolic Memory.' It was first hypothesized by Brownlee (Nature 2000), and since shown by many researchers that overproduction of free radicals, superoxide anion ($O_2^{-\bullet}$) in particular, forms the unifying link between hyperglycemia and the complications of diabetes. It has also been shown that antioxidant molecules can at least partially reverse these complications both in the laboratory bench and clinically.
In this study we have confirmed in three different models (human endothelial cells, retinal cells and retina from diabetic animals) that, even normalizing glycemia, a persitent activation of many pathways involved in the pathogenesis of diabetic complications is still present. However, the major finding has been the demonstration that even normalizing glycemia an overproduction of free radicals is still evident and, overall, that inhibiting their production, particularly at the mitochondrial level, can switch off the memory' effect of hyperglycemia.
These findings clearly open a new field of research, aiming to obtain specific compounds able of blocking the 'Metabolic Memory'.

S15.3

Role of the fatty liver (NAFLD) in the pathogenesis and treatment of Type II Diabetes

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Several prospective studies have shown that fat accumulation in the liver due to non-alcoholic causes (NAFLD) preceeds and predicts type 2 diabetes and cardiovascular disease independent of obesity and even fat distribution, although individuals with a fatty liver tend to be more abdominally obese than those without. All components of the metabolic syndrome also correlate with liver fat independent of obesity. Both genetic and acquired factors regulate liver fat content. The heritability of liver fat based on twin studies is ~60%. The rs738409[G] allele in the *PNPLA3* (adiponutrin) gene strongly associates with increased liver fat content in 3 different ethnic groups, also in Finns. Of acquired factors, changes in body weight markedly and rapidly change liver fat. Of dietary factors, diets rich in saturated fat and those stimulating *de novo* lipogenesis appear harmful. Regarding the mechanisms of fat accumulation in the liver, peripheral lipolysis is increased independent of obesity in subjects with NAFLD. In such subjects, adipose tissue in inflamed and insulin resistant and characterized by an excess of ceramides which could be mediators of high fat induced insulin resistance. In the human liver, there is an excess of triglycerides which contain saturated fatty acids, consistent with tracer studies that both increased adipose tissue lipolysis and *de novo* lipogenesis (which produces saturated fatty acids) contribute to excess fat accumulation in the liver. Liver fat content and inflammation can be reduced by PPARγ agonists. This effect is unlikely to be direct s PPARγ2 expression is increased and likely to involve adiponectin from adipose tissue. The main target of adiponectin is the liver and its circulating concentrations are markedly increased by PPARγ agonist therapy. Indeed patients who are very resistant to insulin because of a fatty liver or who have inflammatory changes in addition to increased fat content (NASH) might form a subgroup which benefit from PPARγ treatment. Liver fat is also the best preodctor of insulin requirements in type 2 diabetes. Simple equations to predict liver fat based on routinely available clinical data have recently been developed.

S15.4

Insulin analogues in type 2 diabetes: how far have we come?

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The introduction of chemical and genetically modified short and long acting insulin molecules, as well as mixed insulin preparations, has provided novel tools to improve glycaemic regulation in patients with diabetes. Reaching the glycaemic target of an HbA1c below 7% represent a major challenge in many patients with type 2 diabetes. Different insulin preparations can be used in various different combinations with a variable number of daily injections as well as in different combinations with oral antidiabetic agents. In this lecture, some of the most important questions regarding insulin treatment with different insulin analogues in patients with type 2 diabetes will be addressed including: 1. When initiating bed time insulin treatment in patients with type 2 diabetes, what can be achieved using modern basal insulin analogues as compared with human NPH-insulin?, 2. Which treatment regimen is best when initiating insulin treatment in patients with type 2 diabetes using human insulin or insulin analogues: Bed time basal, biphasic or prandial only insulins?, 3. Do multiple injections with human insulin or insulin analogues improve control in patients with type 2 diabetes on biphasic insulins who have not reached target?, and finally 4. Should insulin treatment in obese and non-obese patients with Type 2 diabetes be combined with metformin, insulin secretagogues or both? In addition, the lecture will address the future of insulin analogues in the treatment of type 2 diabetes.

Neuroendocrine tumors

S16.1

Novel approaches in the treatment of NET

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Neuroendocrine tumors (NETs) constitute a rather heterogenous group of malignancies that are considered to be rare. However, resent data is indicating a significant increase in both incidence and prevalence over the last decades, with an overall incidence of 5/100 000/year and prevalence of 25/100 000/year.
The treatment of NETs is based on the tumor biology (proliferation capacity and differentiation), tumor localisation and spread (TNM-staging). Surgery is important in most patients with NETs with or without a curative intent combined with radiofrequency ablation and embolization of liver metastases. PRRT, peptide radio receptor treatment, with radioactive somatostatin analogs has increased in importance over the last years with objective response rates (PR + SD) of 30–45%. The medical treatment consists of cytotoxic agents and biologicals such as somatostatin analogs, alpha interferon, VEGF- and mTOR-inhibitors. The cytotoxic therapy consist of various agents such as streptozotocin, cisplatinum, etoposide, temozolomide, capecitabine and doxorubicin in different combinations for high proliferating tumors (Ki-67 < 10%). Somatostatin analogs (octreotide, lanreotide) are standard of care for functioning NETs with low proliferation capacity and Ki-67 less than 5%. The biochemical and subjective improvement are ranging from 35 to 50% with significant tumor reduction in about 5%.Treatment with non-functioning tumors with somatostatin analogs is still controversial but most resent data are indicating a benefit. Alpha interferon has been registered for mainly classical midgut carcinoids with carcinoid syndrome with biochemical and symptomatic improvement in 35–60% of the patients and tumors shrinkage in 10–15%. Both somatostatin analogs and alpha interferon can be combined. VEGF-inhibitors have been applied during the last years for treatment both as single drug but also in combinations with cytotoxic giving response rates of 10–20% and most recently mTOR-inhibitors have been applied in different subtypes of NETs with response rates between 5 and 15%.
The future treatment of NETs will be based on molecular genetics and tumor biology for personalized treatment.

S16.2

Moleculargenetics of neuroendocrine tumours

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Neuroendocrine tumor (ENET) is a distinct term and includes specific selected tumors of the foregut, midgut and hindgut. The term separates other neurocrest-derived tumors likewise pheochromocytomas, paragangliomas as well as medullary thyroid carcinoma and parathyroid adenoma. There are sporadic and hereditary ENETs. The prominent hereditary group of ENETs forms the syndrome of multiple endocrine neoplasia type 1 (MEN1). Other ENETS are regarded as sporadic.
The Preventive Care Unit of the Freiburg University Medical Center has focused over years on pheochromocytoma-associated syndromes including von Hippel-Lindau disease (VHL). Among the major manifestations of VHL are pancreatic islet cell tumors (ICT), thus forming a third group of ENET patients. Both genes, MEN1 and VHL have been identified. We have subjected patients with pancreatic islet cell tumors to genetic screening of the genes MEN1 and VHL.
The NET registry included 68 patients with pancreatic neuroendocrine tumors. These 68 subjects comprised 28 women and 40 men diagnosed with ICT at age 12–82 (mean 59 years). Twenty three functional tumors are registered, 13 insulinomas, 3 VIPomas, 2 glucagonomas, 1 gastrinoma and 4 tumors without specification. Moleculargenetic analysis revealed 4 patients with MEN1 germline mutations. In 36 patients no MEN1 and no VHL mutation was found. These patients and tumors were classified as sporadic.
The VHL registry included 23 subjects had ICT. Gender was female in 15 and male in 8 subjects. All these 30 patients were primarily symptomatic for other VHL-associated tumors but not because of ICT. Single ICT was present in 19 subjects, whereas in 4 subjects multiple tumors were present. Tumor diameter varied from 0.5 to 15 cm. Insulin, C peptide and gastrin were measured in 10 cases, but elevated C peptide and gastrin was only found in one subject. This patient had malignant ICT. Transformation from benign to malignant ICT was observed in 2 subjects at age 24 and 61 years respectively. Malignant tumors were found to be statistically larger than benign tumors. malignant. Moleculargenetic analyses revealed germline VHL mutations in all patients with VHL-associated ICT. There are 20 different mutations, 13 missense, 6 truncating mutations and one large deletion comprising exon 1. Of note, there was a clustering of mutations in nucleotides 691–713 (14/30 subjects) or nucleotides 666–761 (21/30 subjects).

S16.3

Peptide receptor radiotherapy for NET

Dik Kwekkeboom
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Abstract unavailable.

S16.4

PET based somatostatin receptor imaging of NET

Irene Virgolini
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Abstract unavailable.

Stem cells niches in the endocrine system

S17.1

Stem cell biology: lessons to learn from the fly

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Many adult organs harbor stem cells that are used to sustain homeostasis and to replenish damaged tissue following injury or disease. While the therapeutic potential of stem cells has been much discussed its practical use is still lacking. One major obstacle is the difficulty of re-introducing stem cells into their organs. Adult stem cells reside within a special environment (niche), which participates in every aspect of stem cell behavior. Many of the biological principles that govern stem cell behavior within a living body were worked out in fruit flies. These principles suggest that stem cells cannot be considered separately from their niches. They form one unit.
Oogenesis in the fly depends on germ line stem cells (GSCs). GSCs divide continually to produce one daughter cell that remains a stem cell, while the other differentiates to form an egg. Niche cells secrete factors that maintain GSCs and control their rate of division. They also tether GSCs and induce asymmetric localization of proteins and organelles within GSCs.
Our data suggests that the stem cell unit (GSCs and their niche) develops in unison. Most importantly, while niche cells affect GSCs, the opposite is also true. Stem cells affect their own support cells. Germ cells and their somatic support cells communicate via a feedback loop. GSCs produce a factor required for somatic cell survival. When GSCs are missing, support cells die. The support cells, on the other hand, produce a factor repressing germ cell proliferation. This feedback loop controls tissue homeostasis and allows the ovary to control the number of stem cells it possesses.
The conservation of biological principles and molecular players between flies and mammals suggest that many of the lessons learned in flies are applicable to humans. The implications for the interdependence of stem cells and niche cells will be discussed.

S17.2

A population of progenitor/stem cells in the adult pituitary

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Tissue-specific progenitors play essential roles for organ development and homeostasis but they are not present in all tissues. Throughout life, the pituitary gland adapts the proportion of its endocrine cell types to meet hormonal demands. This plasticity may rely on adult progenitor cells and we have recently described such a population. These cells express SOX2, an HMG box transcription factor, marker of several embryonic progenitors and stem cells, and form 'pituispheres' in culture, which can grow, self renew, and differentiate to all pituitary endocrine cells. Differentiation is associated with expression of SOX9, a related HMG box factor. $SOX2^{+ve}$ cells are found throughout Rathke's pouch in embryos and persist in the adult gland. However most of these adult $SOX2^{+ve}$ cells also express SOX9. This $SOX2^{+ve}/SOX9^{+ve}$ population may represent transit amplifying cells, whereas the $SOX2^{+ve}/SOX9^{-ve}$ cells could be progenitor/stem cells. In order to prove this hypothesis and also better characterize the newly developed pituisphere cultures we are currently following different approaches. We have first undertaken lineage marker analysis in order to prove that $SOX2^{+ve}$ cells give rise to endocrine cells *in vivo*. We are also developing genetic tools to specifically inactivate *Sox2* and *Sox9* to understand their function in the embryonic and adult pituitary and to learn more about the cells in which they are expressed. Finally, to establish the pituisphere culture as an *in vitro* system to understand endocrine cell differentiation we are currently developing assays to manipulate/control differentiation of specific endocrine lineages.

S17.3

Thyroid: stem cells: normal development and tumorigenesis

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There is some evidence from different studies that (1) stem cells reside in thyroid tissue (as in all other tissues) for life-time of the organism, (2) stem cells and their progeny are under the control of niches that limit proliferation of these undifferentiated cells, (3) induction of apoptosis and (excessive) growth stimulation can overcome strict niche control, (4) under these conditions actively cycling, more or less differentiated progenitor may grow faster than the surrounding differentiated thyrocytes. Based on this evidence, epidemiological data, and the general concept of stem cells as a source of benign and malignant tumours a role of stem cells and their progeny in the pathogenesis of benign thyroid nodules (A) and thyroid cancer (B) is hypothesised: (A) Population studies have demonstrated that nodular transformation is increasing with age whereas the goitre size is decreasing. Throughout the aging thyroid gland, adult stem cells are detectable that maintain the capacity of proliferation and differentiation. Experimental studies revealed that growth factors, their related

receptors and growth-related signalling peptides are highly expressed or even overexpressed in thyroid nodules and nodular goitres. Some of the growth factors are potent stimulators of thyroid stem cell growth. The proliferation of quiescent stem cells is controlled by signals from putative niche cells. *In vitro*, malnutrition can limit or even overcome the control which results in an outgrowth of stem cells as thyro-spheres. Histological and immunohistochemical studies demonstrated hypofunction, destruction and necrosis of normal thyroid tissue in goitre tissues, conditions that may be equivalent to *in vivo* focal malnutrition thereby affecting the control of niches on thyroid cell growth *in vivo*. In addition, there is some experimental evidence that apoptosis of thyrocytes is a main factor of cell loss during goitre formation. Apoptosis of thyrocytes is, however, a prerequisite for thyro-sphere formation and therefore the proliferation of stem and progenitor cells *in vitro*. Thus, the short but intense stimulation of stem cells by growth factors *in vitro* may correspond to processes of nodular transformation *in vivo* that last for months, years or even decades. During this time, some cells may additionally accomplish molecular aberrations that provide a second growth advantage, for example ras mutations in few non-functioning thyroid nodules. (B) Malignant transformation and in turn thyroid carcinogenesis may occur by mutagenesis of more or less differentiated (early) stem/progenitor cells with a high proliferative capacity. Thus, the stem cell hypothesis fits well in with the known genetic aberrations in thyroid cancers but is contradictory to the concept of rarely dividing normal thyrocytes as the origin of thyroid carcinomas.

Should adrenal venous sampling should be performed before adrenalectomy in primary aldosteronism?

S18.1

Adrenal venous sampling should be performed before adrenalectomy in primary aldosteronism?

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Primary aldosteronism (PA) is the most frequent form of endocrine hypertension. The identification of the subtype of PA is fundamental to distinguish between subtypes that benefit from surgery and subtypes that should be treated pharmacologically with mineralocorticoid receptor antagonists. CT scanning is the most sensitive imaging technique for the identification of adrenal nodules but lacks sensitivity and specificity. Also posture test and other clinical criteria of 'high probability' of APA have shown to be unreliable in distinguishing between PA subtypes. Adrenal vein sampling (AVS) is considered the only reliable technique that allows the clinician to define the patients that should undergo unilateral adrenalectomy. During AVS, blood is collected from the inferior vena cava, from a peripheral vein and from both adrenal veins and aldosterone and cortisol are measured in each sample. The cortisol concentration in the samples are a measure of the adequacy of the adrenal vein cannulation. There is no agreement on which criteria should be used for considering a successful cannulation of the adrenal veins and for considering the aldosterone secretion 'lateralized' and therefore, to suggest adrenalectomy. We suggest to use restrictive criteria, especially for demonstrating the correct cannulation of the adrenal veins. Monitoring of cortisol during the catheterization procedure allows any improperly collected adrenal samples to be immediately re-collected. We recently reported a quick and reliable cortisol assay performed in the operating room during the AVS, allowing the radiologist further attempts at cannulation until cortisol measurements demonstrate the success of the sampling. The recently published Guidelines of the Endocrine Society suggest that AVS should be performed in all patients in whom the adrenalectomy is considered. In fact adrenal vein sampling is the only reliable technique that allows the differentiation between PA subtypes and therefore, it should always be performed in patients who are potential candidates for surgery.

S18.2

Should adrenal venous sampling be performed before adrenalectomy in primary aldosteronism? Con

Laurence Amar
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There is no doubt that AVS plays a central role in differentiating PA subtypes and that adrenalectomy is only indicated in patients with unilateral aldosterone hypersecretion.

However, AVS is not widely available. It is invasive and not devoid of morbidity (pain, adrenal hematoma). Cannulating the two adrenal veins is technically demanding and may fail in up to one patient in four. The procedure is not standardized (sequential or simultaneous bilateral AVS? with or without cosyntropin stimulation?). There is no consensus regarding the threshold for the cortisol-corrected aldosterone ratio that indicates a lateralized AVS. Even the use of cortisol-corrected aldosterone ratios can be questioned as it relies on the unproven assumption that cortisol secretion in symmetrical in all cases of surgically-curable PA.

The presence of a unilateral adenoma at computed tomography is a surrogate marker of unilateral aldosterone hypersecretion. Whether a lateralized AVS improves the prediction of surgery outcome in younger patients (e.g. aged 40 or less) with a unilateral adenoma is not documented. The prevalence of non-functioning adrenal masses increases with age. Therefore younger patients have a very low probability for the combination of idiopathic PA with a non-functioning adrenal adenoma.

Consequently, AVS cannot be considered a *sine qua non* condition for adrenalectomy in PA, specifically in younger patients, i.e. in patients who are the best candidates for surgery.

Should AVS be performed in a many patients with PA before surgery? YES

Should all young patients with PA and a typical adenoma at computed tomography undergo AVS before surgery? NO.

Bone endocrinology

S19.1

Endocrine regulation of energy metabolism by the skeleton

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That obesity protects mammals from osteoporosis led us to show in the past that bone remodeling, and thereby bone mass, is regulated by the adipocyte-derived hormone leptin via a central relay and the sympathetic nervous system. To test whether in response to this regulation bone could exert a feedback control of energy homeostasis we performed a functional genetic screen in mice designed to identify genes expressed specifically in osteoblasts, encoding signaling molecules and affecting energy metabolism. Through this effort we identified two genes, Osteocalcin and Esp, whose products are both involved in the regulation of glucose metabolism and of pancreas biology. Indeed, mice lacking the product of *Esp* are hypoglycemic and protected from obesity and glucose intolerance because of an increase in β-cell proliferation, insulin secretion and insulin sensitivity. In contrast, mice lacking the osteoblast-secreted molecule osteocalcin display decreased β-cell proliferation, glucose intolerance and insulin resistance. Genetic and biochemical analyses revealed that *Esp* and *Osteocalcin* lie in the same molecular cascade and that the *Esp*−/− mice are a model of osteocalcin's gain-of-activity. Highlighting the importance of this novel regulation of energy metablism *Esp*−/− mice are protected from diet-induced obesity and diabetes. These studies establish that the skeleton, via its secretion of osteocalcin, exerts an endocrine regulation of sugar homeostasis thus expanding the biological importance of this organ as well as our understanding of the regulation of energy metabolism.

In a new set of studies, we have tested the effect of recombinant osteocalcin on energy metabolism in WT mice as well as in a model of diet-induced obesity. Results of these experiments will be presented at the meeting.

S19.2

Neuronal regulation of bone remodeling

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The process of bone remodelling enables the conservation of an optimal bone mass and properties during adult life. Recent evidences, based on genetic and pharmacological data, revealed that this process is under the control of hypothalamic and neuronal signals released by sympathetic nerves in the bone microenvironment. The implication of these findings is that bone remodelling can be considered as a classical homeostatic process, coupled and fully integrated with other endocrine systems of the body. These studies also revealed that beta2 adrenergic receptors in osteoblasts mediate the anti-osteogenic effect of autonomic nerves, and that pharmacological blockade of b2 adrenergic receptor signalling by beta-blockers can increase bone mass in mice and rats. These findings as well as their clinical implications will be discussed.

S19.3

Role of oxidative stress in skeletal involution

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In contrast to traditional ideas that loss of estrogens at menopause is the seminal mechanism of osteoporosis, bone loss begins as early as the early part of the third decade in both women and men; substantial trabecular bone loss occurs in sex steroid sufficient young adult women and men; and after the first few years of accelerated bone loss in postmenopausal women, bone mass and strength decline in both sexes at the same rate. Consistent with these clinical observations, mechanistic studies in mice show that aging, and specifically increased oxidative stress, rather than age-associated failure of other organs, is a fundamental pathogenetic mechanism of age-related bone loss and strength, leading to, among other changes, a decrease in osteoblast lifespan and bone formation. Loss of estrogens or androgens accelerates the effects of aging on bone by decreasing defense against oxidative stress. Oxygen radical-induced activation of the FoxO family of transcription factors defends against such an increase by up regulating free radical scavenging and DNA repair enzymes, thereby representing an indispensable homeostatic mechanism for skeletal health. Consistent with this, loss or gain of function of FoxOs decreases and increases bone mass respectively. Albeit, excessive or protracted FoxO activation diverts ß-catenin away from Wnt signaling, leading to a decreased osteoblastogenesis. Excessive FoxO activation may also lead to a decrease in bone strength, independently of bone mass, by compromising the bone vasculature and the hydration of the aging skeleton. Fascinatingly, attenuation of Wnt-mediated transcription, resulting from an autosomal dominant missense mutation in LRP6 or LRP5 -co-receptors for the Wnt-signaling pathway has been linked recently genetically not only to premature osteoporosis, but also to coronary artery disease as well as several features of the metabolic syndrome including hyperlipidemia, hypertension, and diabetes, but not obesity. Hence, antagonism of Wnt-signaling by oxidative stress—induced activation of FoxOs with increasing age may be a common molecular mechanism contributing to the development not only of involutional osteoporosis, but several pathologies like atherosclerosis, insulin resistance, and hyperlipidemia – all of which become more prevalent with advancing age (Manolagas & Almeida *Mol. Endocrinol.* 2007 **21** 2605–14).

S19.4

Lipoprotein involvement in bone metabolism

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Osteoporosis is a common, age-related disease with a strong genetic component. Mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) gene are known to cause rare bone-related syndromes including loss-of-function mutations with decreased bone mineral density (BMD) and gain-of-function mutations with high bone mass. The initial discovery of lipoprotein involvement in bone metabolism was surprising, but revealed a strong regulatory pathway by the Wnt-signalling cascade. In addition, several attempts to investigate the genetic background of osteoporosis by whole genome investigations resulted in the affirmation of the lipoprotein receptor gene loci in association with bone fractures and BMD.

Recent studies demonstrate the LRP6 coreceptor as a key element of the PTH signalling that regulates osteoblast activity. Furthermore, lipoproteins and the respective genes regulate atherogenesis and bone properties – more investigations are on the way to elucidate their involvement in bone metabolism using new insights in the calcification of bone and vasculature.

Defining targets for investigating new therapies and possible individual pharmacogenomics in osteoporosis patients by lipoprotein research will expand our repertoire in diagnosis and treatment of this widespread disease.

Thyroid cancer

S20.1

C Cell neoplasia

Rossella Elisei
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Parafollicular C-Cells represent 1% of thyroid cells and differ from follicular cells for their origin from the neural crest. At variance with follicular cells, their growth and function are independent from thyrotropin stimulating hormone (TSH), they do not take up iodine and they produce and secrete calcitonin (CT) but not thyroglobulin. C-Cell Hyperplasia (CCH) is defined as an increased number of normal C-cells (i.e. 50 or more C-cells in at least one low-power field (100×)), more commonly with a diffuse pattern. Although rare, CCH has been described in normal thyroids and more than 10% of lymphocytic thyroiditis are accompanied by CCH as well as some micropapillary thyroid cancer. The pathological role of this CCH is still unclear.

Malignant transformation of C cells leads to the development of medullary thyroid carcinoma (MTC). It is a well-differentiated thyroid tumor maintaining the biochemical and pathological features of C cells and elevated concentration of serum CT are strongly suggestive of the presence of either primary MTC, before thyroidectomy, or metastatic MTC, after thyroidectomy. Only rare cases of de-differentiated MTC are associated to low or absent levels of serum CT.

The prevalence of MTC varies from 5 to 10% among all thyroid tumors and from 0.4 to 1.4% among thyroid nodules. Females and males are almost equally affected. The mean age at diagnosis is around 40 years, but a wide range of age at onset is reported. In about 25% of cases MTC is one of the components of the Multiple Endocrine Neoplasia type II syndromes, which are autosomal dominant inherited syndromes involving other endocrine glands. The pathogenic mechanism of these syndromes has been recognized in the activation of the RET protoncogene. Several germline RET mutations, mainly concentrated in exons 10–16 of the RET gene have been discovered to be associated with the hereditary MTC. Somatic RET mutations are found in about 45% of sporadic MTC and have been reported to have a poor prognostic role both for the outcome and the survival.

The biological behaviour of MTC is quite aggressive and only 50% of MTC patients are still alive after 10 years. The only possibility to improve the cure and survival is the early diagnosis and the early surgical treatment when the MTC is still intrathyroid. This can be obtained by routine measurement of serum CT in patients with thyroid nodular disease and with RET genetic screening in hereditary forms. Metastatic MTC patients have only few therapeutic options and conventional therapies have been demonstrated to be ineffective. The recent development of new targeted drugs such as tyrosine kinase inhibitors able to act against both RET and other tyrosine kinase receptors (i.e VEGFR, PDGFR ecc) are very promising and several clinical trials are already on going.

S20.2

Ultrasound diagnosis and follow-up of thyroid cancer

Murat Erdogan
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Utlrasonography is now a crucial part of diagnosis and the follow-up of thyroid cancers as well as other thyroid disorders. If you are an endocrinologist of the age 40 or below and if you still do not perform ultrasonography by yourself, we believe you have an important skill to learn, which will guide you through out your thyroid practice and totally change your vision to thyroid disorders.

We hereby discuss the importance of thyroid ultrasonography for the diagnosis and follow up of thyroid malignancy under three headings.

(1) Picking up the high risk cases among a large group of patients with nodular thyroid disorders.

(2) Preoperatively, to define the operation strategy for the FNAC(+) patients.

(3) Postoperative management of differentiated and medullary thyroid cancer patients.

If one can use ultrasonography for these three headings in a given thyroid cancer patients, we believe that the outcome of the patient will be improved to a great extend.

S20.3

Advances in management of thyroid cancer with novel chemotherapy agents

Christopher Nutting
Royal Marsden Hospital, London, UK.

Recently there has been resurgence in interest in the treatment of iodine negative differentiated thyroid cancer and medullary thyroid cancer with novel agents.

This presentation will review the recent literature on treatment of thyroid cancers with multi-targeted tyrosine kinase inhibitors. Data on the experience of the Royal Marsden Hospital with Sorafenib will be presented. Vascular endothelial growth factor inhibition appears to be a particularly important target for these agents, and future directions of research will be discussed.

S20.4

Pregnancy after exposure to radioiodine

Steve Hyer
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The administration of ^{131}I should strictly be avoided in pregnancy. Iodide concentrating capacity can be detected in the thyroid of the 10–11 week fetus. If administration is within the first 8 weeks, the major problem is fetal whole body dose due to gamma emission from ^{131}I in the maternal bladder (about 50–100 μGy/ MBq of administered activity). This dose is reduced if the mother is well hydrated and voids frequently. Generally the lifetime risk of fatal cancer is considered to be 10–15% per Sv (stochastic).
If the fetus is more than 8 weeks post conception, the developing fetal thyroid may accumulate iodine. Fetal thyroid doses are very high which can cause significant fetal thyroid damage leading to permanent hypothyroidism. The whole body fetal dose is likely to be less than 100 mSv and would not necessarily indicate termination of the pregnancy on the basis of the exposure alone. The mother should, however, be given usual doses of replacement thyroid hormone. If discovered within 12 hours of administration, 60–130 mg of stable KI is recommended. Since ^{131}I is concentrated in maternal milk, breast feeding is contra-indicated after radioiodine administration to the mother. A radiation dose of approx 550 cGy would be delivered after the administration of 1mCi to the mother.
This presentation will consider the risk assessment after exposure to ^{131}I with case studies taken from clinical practice. Dose-dependent effects of ^{131}I on gonadal function will also be discussed.

Current problems in the management of pituitary tumours

S21.1

Cardiovascular morbidity and mortality in patients treated for craniopharyngioma

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The treatment of craniopharyngiomas is associated with long-term morbidity. Although histological benign, intrinsic aggressive biological properties of craniopharyngioma, such as invasion of surrounding tissues, apparently preclude an indolent course. Cardio- and cerebrovascular mortality risk in craniopharyngioma patients is approximately three fold increased. This risk seems to be even greater in estrogen-deficient premenopausal women. In addition, there is a high prevalence of features of the metabolic syndrome, such as type 2 diabetes mellitus, obesity and dyslipidaemia, when compared to normative data. Long-term follow-up studies all involve retrospective historical cohorts. The potential role of treatment modalities and hypopituitarism to the enhanced cardiovascular morbidity, is discussed. Then, the question arises as to the mechanism(s) involved in the explanation for the increased prevalence of cardiometabolic complications in these patients. One of these explanations is irreversible hypothalamic dysfunction. Evolution has provided us with powerful tools to keep our internal environment stable by synchronizing activity and rest to the day/night cycle by means of biological clock mechanisms. The nuclei within the hypothalamus are crucial in integrating and conveying the different signals, informing the brain of the internal and external environment. Considering the large proportion of patients with damage to the optic nerves, it is hypothesized that craniopharyngiomas and/or the effects of treatment may result in damage to the suprachiasmatic hypothalamic nucleus. This may result in altered regulation of central clock mechanisms, which predisposes to alterations in metabolism. In addition, the possibility of damage to other hypothalamic nuclei involved in the integrative physiology of metabolism cannot be excluded. Hypothalamic damage thus may have contributed to the observed morbidity but direct clinical evidence is not yet available. Patients treated for Craniopharyngioma have excessive long-term multisystem morbidity and mortality. Besides appropriate endocrine replacement, especially estrogen replacement in premenopausal females, intensive control of glucose, lipids, blood pressure and weight should be achieved, as in any other patient with increased risk for cardiovascular disease.

S21.2

Factors associated with hypothalamic morbidity in patients with craniopharyngiomas

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Background
Adult craniopharyngioma (CP) patients without GH therapy exhibit high risk for cardiovascular disease (CVD) and mortality with a higher risk in women than in men. Approximately 50% of children treated for CP are obese at follow-up and hypothalamic damage seems to be a major cause. In GHD CP children GH treatment improves linear growth but does not ameliorate weight gain, but the impact of long-term GH therapy into adulthood is presently unknown.
The results from a cross-sectional study of 42 operated childhood onset (CO) CP patients on complete replacement therapy including GH will be presented. They were compared with population controls matched for age-, gender-, smoking, and residence for CVD risk, bone mineral density (BMD), basal metabolic rate (BMR), energy intake (EI), dietary intake and attitudes towards eating. The impact of disease related factors were also studied. Duration of GH therapy was 10 years in women and 12 years in men.
Results
Significant increases in BMI, fat mass, insulin and leptin were recorded in both male and female CP patients. CP women had increased CVD risk and the high BMI was the primary cause but long-term GH therapy was unsuccessful to affect this risk. Hypothalamic involvement by the tumour was superior to female gender risk in CVD risk prediction.
Despite increased fat mass, adequate muscle mass, physical activity and calcium intake when compared to controls, 45% of CO CP women, but not men, had *z*-scores below −2.0 SDS. Hypothalamic dysfunction with leptin resistance may have contributed.
The ratio EI/BMR was low in the patients with tumour growth into the hypothalamus. CP men had significantly higher percentage of energy from sugar and sweets compared to controls. CP women had significantly more percentage of energy from light meals and higher values on cognitive restraint and disinhibition compared to controls.
Conclusions
Women with CO CP have increased CVD risk and lower BMD in spite of long-term GH therapy. CO CP patients with hypothalamic involvement have low EI. Hypothalamic involvement predicts CVD risk and may contribute to low BMD and EI/BMR.

S21.3

Treatment and prognosis of non-functioning pituitary adenomas

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Surgery is the first-line treatment of patients with clinically non-functioning pituitary adenomas (NFA). Because of lack of clinical syndrome these tumours are diagnosed with a variable delay when patients suffer from compression symptoms (hypopituitarism, headache, visual field defects) due to the extension of the tumour outside the pituitary fossa. Surgery is followed by residual tumour tissue in most patients. In these cases, radiotherapy is generally used to prevent tumour re-growth. However, NFA cell membranes, in analogy with GH- and PRL-secreting adenoma, express somatostatin and dopamine receptors. Treatment with somatostatin analogues and dopamine-agonists induced some beneficial effects on visual field defects and was also followed by tumour shrinkage in a minority of cases. Dopamine-agonists seem to be more effective on tumour shrinkage than somatostatin analogues. More recently, a combination treatment with both somatostatin analogues and dopamine-agonists have been tested in a few patients with interesting results.
Lack of randomized, placebo-controlled trials prevents any conclusion on the efficacy of these drugs. In contrast, use of gonatotropin-releasing hormone analogues has been abandoned.

S21.4

Treatment options for aggressive pituitary tumors

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Pituitary tumours have recently been shown to have a prevalence of around one in a 1000, but the overwhelming majority of these are benign and readily treated. Nevertheless, while the initial therapy of the majority of non-secreting macroadenomas is transsphenoidal surgery, these have a tendency to recur even when apparently totally removed. Recurrence seems not to depend on dural invasion, and it cannot at present be predicted by any histopathological markers. Indeed, some 50% of such adenomas will recur over 10 years, although this recurrence rate is

reduced to <5% with standard 3-portal conformal external beam radiotherapy. Such radiotherapy, while highly effective, does carry the risk of progressive hypopituitarism, but the risk of second tumour development or of visual path abnormalities is very low. Nevertheless, there may be an increased risk of cerebrovascular disease, and it would be extremely helpful to be able to predict which patients would benefit from this treatment. Radiosurgery appears to be a useful alternative where the recurrence is limited in size and is >5 mm from the optic chiasm ad nerves, although claims of an increased rate of effectiveness have not been easy to substantiate.
Only around 0.2% of pituitary tumours are carcinomas, as defined by the presence of intra- or extra-cranial metatsases, but these offer an exceptional challenge. The majority of these tumours are either prolactin- or ACTH-secreting tumours. Such patients may occasionally respond to dopamine- or somatostatin-recepor agonists, but in general require repeated transsphenoidal or even transcranial surgery. Conventional chemotherapy has not been shown to be particularly effective, but recent case reports with the alkylating agent temozolomide have shown scattered cases of impressive tumour control, at least in the short and medium term. The enzyme MGMT reverses the effect of temozolomide by removing the methyl adduct from DNA, and recent reports suggest that tumours lacking MGMT are especially sensitive to temozolomide. In such tumours there is evidence for methylation of the MGMT gene promoter. However, an extensive study has shown that only some 15% of pituitary adenomas lack MGMT, although this proportion may be higher in prolactinomas.

Tumorigenesis in pheochromocytoma/paraganliomas

S22.1

The Warburg effect in pheochromocytoma: a link between genetic disorders and cell metabolism

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Tumorigenesis and intermediary metabolism have a long common history. Eighty years ago, the biochemist Otto Warburg pioneered a large field of researches devoted to the metabolism of tumour cells. He reported a spectacular shift from a normal aerobic metabolism to a highly glycolytic metabolism, associated with a low respiration rate, despite aerobic conditions. After being forgotten for decades, a renewed interest in the Warburg effect has resulted from the report that, mitochondrial enzyme involved in the tricarboxylic acid cycle, also known as the Krebs Cycle, can act as tumor suppressor genes in hereditary tumor syndrome.
Three of the four subunits of succinate dehydrogenase, or mitochondrial complex II, namely, SDHB, SDHC, and SDHD, have been involved in the tumorigenesis resulting in paragangliomas and pheochromocytomas. Primary mutations in the fumarase hydratase (FH)-encoding gene unexpectedly result in uterine fibroids, skin leiomyomata, and papillary renal cell cancer. These inactivation seem to have a common pathway with the Von Hippel Lindau (VHL) tumor suppressor gene inactivation, as they all lead to the abnormal activation of hypoxia-inducible factors (HIF) in normoxic conditions, a phenomenon know as pseudo-hypoxia.
We used the heterogeneous genetic determinism of pheochromocytomas as a tool to decipher the molecular basis for the Warburg effect and its link with pseudohypoxia in tumors. We studied oxidative phosphorylation (OXPHOS), angiogenesis and glycolysis in pheochromocytomas induced by germ-line mutations in *VHL* (Von Hippel Lindau disease type 2A, 2B and 2C) and compared them to those associated with mutations in *RET, NF1* and *SDH* genes. *SDH*, VHL-2A and VHL-2B tumors, but not *RET*, *NF1* and VHL-2C pheochromocytomas, have been suggested to be related to a pseudo-hypoxic drive. Our findings suggest an unexpected association between pseudohypoxia and loss of p53, which leads to a distinctive Warburg effect, specifically in VHL-2A- and -2B-related pheochromocytomas. This study illustrates how the molecular consequences of genetic disorders can strongly modulate metabolic functions in tumor tissues.

S22.2

Hypoxia-induced angiogenesis in pheochromocytoma

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Predisposition to phaeochromocytoma is a feature of germline succinate dehydrogenase subunit mutations (*SDHB*, *SDHC* and *SDHD*), von Hippel-Lindau (VHL) disease, multiple endocrine neoplasia (MEN) types 2A and 2B and neurofibromatosis type 1. However, although each of these disorders is associated with phaeochromocytoma they differ with respect to susceptibility to other associated tumours, However despite this clinical heterogeneity, there is evidence for shared mechanisms of tumourigenesis. Thus *VHL*, *SDHB* and *SDHD* inactivation have been linked to both dysregulation of the HIF-1 and HIF-2 transcription factors and a non-HIF dependent pathway involving JunB, cJun and EglN3/PHD3. The VHL tumour suppressor protein (pVHL) has multiple functions, but regulation of proteosomal degradation of HIF-1 and HIF-2 is the best studied. Most VHL mutations associated with phaeochromocytoma susceptibility result lead to loss of HIF regulation and SDH inactivation (by inhibiting the prolyl hydoxylases that modify HIF allowing pVHL to bind) also results in a pseudohypoxic gene response with overexpression of a wide range of HIF target genes including angiogenic growth factors. Although somatic VHL and SDH subunit mutations are rare in sporadic phaeochromocytoma, similar changes in gene expression to those observed in VHL-related phaeochromocytoma have also been reported in some sporadic phaeochromocytoma. However the relationship between hypoxic gene response pathways and phaeochromocytoma is complex as some germline VHL mutations associated with predisposition to phaeochromocytoma only retain the ability to regulate HIF. Thus although dysregulation of HIF pathways may not be essential for the development of phaeochromocytoma, HIF dysregulation is frequent and closely associated with phaeochromocytoma tumourigenesis.

S22.3

Antiangiogenic medication in malignant pheochromocytoma

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Inhibiting tumor angiogenesis by targeting the activity of angiogenic growth factors, especially the vascular endothelial growth factor (VEGF)-A signalling pathway, is a new promising therapeutic strategy for patients with different cancer types. Several VEGF inhibitors have been developed including a humanized anti-VEGF-A monoclonal antibody (bevacizumab), an anti-VEGFR-2 antibody, a VEGF receptor chimeric protein and various small molecules inhibiting VEGFR-2 signal transduction, such as sunitinib or sorafenib. Several of them are now approved for the treatment of cancer, especially for advanced renal and colonic cell carcinomas. Complete/partial response to sunitinib has been reported in very few patients with advanced malignant paraganglioma/pheochromocytoma. The use of these drugs has been associated with several side effects, including early hypertension necessitating close blood pressure monitoring, renal toxicity (proteinuria), cardiovascular toxicity (decrease in left ventricular ejection fraction, congestive heart failure), hypothyroidism, bleeding, gastrointestinal perforation, wound-healing complications, and venous thrombosis. Initial and follow-up work-up should include: home or 24-h ambulatory blood pressure monitoring; urine dipstick for proteinuria detection and estimated glomerular filtration rate determination (MDRD); TSH measurements; ECG; echocardiography if necessary. Optimal care is best achieved within a network of professionals including general practitioners, oncologists, cardiologists and nephrologists.

S22.4

Clinical, biochemical and genetic aspects of malignant pheochromocytoma

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Pheochromocytoma (Pheo) is a chromaffin tumor of the adrenal gland. When extra-adrenal, the tumor is called paraganglioma (PGL). At present, the only objective criterion for malignancy is the presence of metastases (i.e. spreading of the tumor in bones, liver, lungs or lymph-nodes where chromaffin tissue is normally absent). Extensive invasion of adjacent tissues can be considered only an indicator of malignant potential as well as high cellularity, necrosis, vascular/capsular invasion and high Ki-67 immunoractivity at histology. Malignancy is more frequently associated with extra-adrenal localizations and with tumor of large size and of irregular shape. Metastases can be found at diagnosis or develop after primary surgery, sometimes also after many years. The biochemical profile of malignant Pheo/PGL is generally represented by high levels of norepinephrine and/or dopamine. Rarely malignant tumors can be non-secreting. Genetic analysis has demonstrated that malignant Pheo/PGLs are frequently associated with mutations of the gene encoding the B subunit of the succinate-dehydrogenase (SDHB). In fact, at variance with carriers of mutations in the other susceptibility genes who present malignancy in about 5%, in patients with a germline SDHB mutation

malignancy ranges from 30 to 60%. Unfortunately, at present the therapy of malignant Pheos/PGLs is palliative. Radiometabolic therapy using I-131-MIBG is the first option when metastases result positive at scintigraphy. Chemotherapy has been used alone or in association with radionuclide treatment but always with limited results. Therapy with anti-angiogenetic drugs is a putative option that might be tested in the next future.

Adrenocortical tumours – pathogenesis and management

S23.1

Molecular pathogenesis of adrenocortical tumors

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Tumors of the adrenal cortex can present as incidental findings during abdominal imaging, as the cause of steroid excess and/or as malignancy. The most common adrenal disorder encountered today is the adrenal incidentaloma, which is mostly benign but can be associated with (subclinical) Cushing's syndrome or primary aldosteronism. In contrast, adrenocortical carcinoma (ACC) represents a rare but highly malignant tumor entity. Over the last years studies including expression profiling of tissue samples, *in vitro* examination of tumor related pathways and genetic examination of patient cohorts with specific adrenal disorders have uncovered a number of mechanisms relevant for the molecular pathogenesis of adrenocortical tumors. Furthermore, hereditary diseases including Li-Fraumeni and Beckwith-Wiedeman syndrome have allowed important insights in mechanisms of adrenal tumorigenesis. Finally, during the last decade a number of mouse systems have been developed with distinct features of adrenal tumorigenesis that have aided as *in vivo* models. IGF-2 is considered one of the most potent growth factor for the adrenal. Accordingly, genetic studies and expression profiling on adrenocortical carcinomas have demonstrated a variety of genetic alterations resulting in IGF-2 overexpression. Conversely, loss of peptide hormone expression such as the Bone morphogenetic proteins (BMPs) also have been demonstrated to impact growth and function of ACCs. Furthermore, the Wnt/β-catenin pathway has been recently suggested to be activated in both benign and malignant adrenocortical tumors. Activating mutations of the β-catenin gene were found with similar frequencies in adrenal adenomas and carcinomas whereas abnormal localization of β-catenin was observed at a higher rate in adenomas than in carcinomas. In addition, somatic mutations in the regulatory R1A subunit of protein kinase A (PRKAR1A), which is a key component of the cAMP signaling pathway that has been implicated in endocrine tumorigenesis have been demonstrated in sporadic secreting adrenocortical adenomas. Taken together, a number of molecular mechanisms have been recently identified that contribute to adrenocortical growth and function. Future challenges will include translation of these molecular advances into clinical practice to improve diagnosis and treatment of patients with adrenocortical tumors.

S23.2

Aberrant receptors (AR) in adrenal Cushing's syndrome

Rossella Libe[1,2], Lionel Groussin[1,2], Guillaume Assie[1,2], Xavier Bertagna[1,2], Olivier Chabre[3], Hervé Lefebvre[4] & Jérôme Bertherat[1,2]
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Some elegant clinical observations of dysregulated cortisol-secretion in cases of ACTH-independent macronodular hyperplasia (AIMAH) and more rarely adrenocortical adenomas (ACA) have led to the concept of 'aberrant' or 'illegitimate' membrane receptors (AR) in adrenal Cushing syndrome. It this situation cortisol secretion is regulated by an extra-cellular ligand that usually does not stimulate cortisol secretion in normal adrenals. This abnormal response might be due to an ectopic expression or overexpression of a receptor. It is also possible that post-receptors signalling alterations play a role in this phenomenon. The most investigated example of AR is the case of food-dependent Cushing's syndrome due to illegitimate expression of the GIP receptor. This receptor stimulates intracellular levels of cAMP as does ACTH. Others G-protein-coupled receptors (i.e. receptors for LH/HCG, ß-adrenergic ligands, vasopressine, serotonin …) have been described. *In vitro* studies have shown that stimulation of these receptors often stimulate cAMP signaling. Ligands of these receptors might circulate from the general circulation, but also, as demonstrated for vasopressine or serotonin, be produced by the adrenal tumor itself. Clinical screening of AIMAH patients have shown that an abnormal response suggestive of the presence of one or several AR is very frequent. In some cases this has been used for targeted therapy of cortisol oversecretion. The list of receptors that could be 'aberrant' might not be yet complete. *In vitro* studies of cortisol secretion regulation by various ligands as well as gene profiling studies are interesting tools for a systematic extensive screening. The concept of AR raised two interesting issues: 1) its role in the development of cortisol-secreting tumors 2) its pathogenesis. The expression of a functional AR clearly takes part in cortisol dysregulation. However animals studies have shown that ectopic expression of LH/HCG or GIP receptors also stimulates tumor growth. Concerning the mechanisms leading to AR in adrenal Cushing's syndrome no genetic alteration of the gene encoding these receptors have been reported so far. Transcriptome studies have shown that the gene expression profile of AIMAH varies according to the type of AR. The genetic study of familial cases of AIMAH with AR might in the future give new insights in the pathogenesis of this disorder.

S23.3

Prognostic factors and adjuvant mitotane therapy for ACC

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Adrenocortical carcinoma (ACC) is a rare tumour characterized by a dismal prognosis. The most important predictor of outcome is the possibility to attain a complete resection and prognosis is extremely poor when complete surgical removal of ACC is not feasible. Most patients have resectable disease at presentation; however, fully half of the patients who have undergone complete removal of the tumour are destined to relapse. The high recurrence rate of ACC has prompted many investigators to consider the use of adjuvant therapy following radical resection of the tumour. Very recently, the results of a retrospective analysis, performed by our group, involving a large cohort of patients with ACC, who were followed for up to 10 years at different institutions in Italy and Germany, demonstrated that recurrence-free survival was significantly prolonged in the mitotane group, as compared with untreated patients, who had a significantly higher risk of recurrence than those receiving mitotane. Multivariate analysis indicated that shorter disease-free survival was associated with older age and more advanced stage.
In contrast to our experience, Bertherat *et al.* did not observe a significant advantage with adjuvant mitotane after complete removal of ACC, even if survival of mitotane-treated patients was better in secreting ACC. They found that steroid secretion had a prognostic value: the poorer prognosis of cortisol-secreting tumours could be related to co-morbidity of Cushing's syndrome. Alternatively, the pathophysiology of cortisol-secreting ACC may lead to the development of more aggressive tumours. It is also possible that the adrenolytic action of mitotane requires CYP11B activity within the tumour: this enzyme is probably expressed in cortisol-secreting tumours, accounting for the more potent effect of mitotane in such tumours.
To conclude, ACC is a rare disease with a high risk of relapse after radical surgery. In our centers, institution of adjuvant mitotane therapy following complete removal of ACC has become the standard of care, at least for patients at high risk of recurrence, as defined by advanced stage and/or high proliferation of ACC, as defined by elevated mitotic count or high Ki-67% index. A prospective randomised trial comparing the effect of mitotane versus observation in low-risk patients is currently ongoing.

S23.4

Chemotherapy and radiotherapy for adrenocortical carcinoma (ACC)

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ACC is a rare, heterogeneous malignancy with poor prognosis. Data from the German ACC Registry ($n=478$) indicate a 5-year survival rate of 47%. In addition to mitotane, cytotoxic drugs are standard of care in advanced ACC. The best results have been reported by Berruti *et al.* for the combination of mitotane with etoposide, doxorubicin and cisplatin with an objective tumor response rate of 49% in 72 patients. A response rate of 36% was published for the combination of mitotane and streptozotocin in 22 patients. Currently these two most promising regimens are compared in the first ever phase III trial (www.firm-act.org). Up to Dec 2008, 238

patients have been randomized and results will be available in 2011. The first experience using 'target therapies' for ACC was disappointing. The EGFR inhibitor gefitinib, erlotinib (EGFR inhibitor) + gemcitabine or bevacizumab (VEGF antibody) + capecitabine exhibited only limited efficacy. However, trials testing IGF-1 receptor inhibitors or multityrosine kinase inhibitors like sunitinib or sorafinib are ongoing and will hopefully hold more promise.
The role of radiotherapy is not well defined and in the past some authors judged ACC as radio-resistant. By reviewing the literature we could identify ten articles covering radiotherapy in a total of 129 patients (64 postoperative and 65 palliative irradiations). In addition, we analyzed 26 patients receiving palliative radiotherapy from the German ACC Registry. In an adjuvant setting radiotherapy was able to prevent local recurrence in most patients. In advanced disease, response to radiotherapy was seen in 57% of patients. Therefore, ACC is not resistant to radiotherapy, but prospective investigations are needed to fully define its therapeutic potential.
Therefore, further cooperative efforts including well designed clinical trials are needed to improve outcome in patients with ACC.

Environmental pollutants as endocrine disruptors

S24.1

Environmental chemical and thyroid signalling

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Despite the recognized importance of thyroid hormones (TH) for normal brain development, little is known about the critical molecular events underlying this role. We investigated the molecular basis of TH action on the developing brain by: (1) comparing genome-wide gene expression patterns in the cerebellum of euthyroid, hypothyroid and hyperthyroid juvenile mice treated with 6-propyl thiouracil or mercapto-methylimidazole /perchlorate using DNA microarrays; and (2) investigating genes associated with TH receptor-binding sites in post-natal day 15 mouse cerebellum using chromatin immunoprecipitation combined with customized microarrays (ChIP-on-chip). The vast majority of genes that were altered in animals rendered hyper- or hypothyroid were involved in metabolism, apoptosis and REST (RE1-silencing transcription factor). The effect of TH on gene expression differed between males and females; hypothyroidism had a greater effect in male relative to female pups. Sex-specific effects were confirmed in rats treated with PTU. ChIP-on-chip identified 91 genes associated with TH receptor (TR) binding sites in their promoter regions or other genomic locations; 10 of the 13 binding fragments were confirmed with ChIP-PCR. A TRβ binding site upstream of the coding region of myelin associated glycoprotein was demonstrated to be TH responsive using a luciferase expression system. Motif searches did not identify any classical binding elements, indicating that not all TR binding sites conform to variations of the classical form. Genes that are directly-regulated by TH-TR may provide useful biomarkers of exposure to chemicals that operate through the TH-TR pathway (e.g., polychlorinated biphenyls or benzo(a)pyrene). These findings provide mechanistic insight into impaired neurodevelopment resulting from TH deficiency and a rich bioinformatics resource for developing a better understanding of TH function.

S24.2

Molecular mechanisms of AhR and NR crosstalk

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The AhR is a ligand dependent transcription factor which belongs to the bHLH-PAS family of transcription factors. Together with it partner factor ARNT the AhR mediates the biological of numerous ligands includings dietary factors, tryptophane derivatives and environmental pollutants like polyaromatic hydrocarbons and poly-chlorinated dioxins.
Exposure to these environmental pollutants leads to, among other effects, disruption of hormonal signaling pathways. Ample scientific evidence has demonstrated that exposure to dioxin inhibits for example estrogen receptor signaling pathways.
One of the mechanisms behind the disruptive effects of dioxin on E_2 signaling is due to recruitment of ARNT to the AhR, an event that lowers the intracellular pool of ARNT available for the estrogen receptors ERα and ERβ.
We have continued to study the mechanisms by which dioxin and other AhR ligands modulate estrogen receptor signaling. Using a combination of bioinformatics and molecular biology methods we have shown that AhR ligands can either activate or repress ERα or ERβ signaling depending on cell-context. This cell and ligand specific effects are depending on P450 enzymes and their ability to generate metabolites that activate ERα and/or ERβ transcription. Interestingly, our observations show that exposure to different AhR ligand activate different gene expression profiles and thus different cellular outcomes.

S24.3

Endocrine disruptors or goitrogens? Effects of UV screens, synthetic and nutritive compounds on thyroid function

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Thyroid hormone (TH) biosynthesis, storage and secretion is organized in a complex series of biochemical reactions round an evolutionary conserved functional unit, the thyroid follicle, a highly vascularised epithelial structure enclosing the colloidal lumen mainly composed of thyroglobulin (Tg). The luminal apical surface of this tight epithelial monolayer is the site of H_2O_2 dependent TH biosynthesis and is involved in mobilization of Tg, the colloidal matrix and storage protein. The initial step of biosynthesis, basolateral iodide uptake by the sodium-iodide symporter NIS, is blocked by voluminous anions (e.g. ClO_4^-), a rocket fuel increasingly contaminating the global surface. Nutritive components have been identified as goitrogens, such as C-and *O*-glycosidic glucosinolates (*cruciferacea*), cyanates, isocyanates and thiocyanates (e.g. cassava), which (irreversibly) inhibit thyroperoxidase (TPO). Several flavonoids and isoflavonoids, widely used as 'green' 'natural plant steroids' in postmenopausal hormone replacement therapy such as genistein and UV screens (e.g. benzophenone 2) regularly applied as sun protectants to prevent erythema, sun burns and skin cancer are potent TPO inhibitors and lead to goiter formation if iodine supply is inadequate as in many parts of the world. No specific inhibitors of thyrooxidase (Duox) or cathepsins involved in T4 and T3 liberation from Tg or of plasma membrane transporters (MCT8, OATP14, LATs) involved in thyroid secretion or cellular uptake of T4 and T3 have been identified among endocrine disrupters. Several endocrine disrupters have been reported to displace T4 and T3 from its binding to the serum distributor protein transthyretin (TTR), resulting in altered free TH levels, increased cellular uptake or renal excretion. Among these agents are flavonoids (F21388), flame retardants (TBBPA) and other phenolic or aromatic compounds with structural similarities to T4. However, several endocrine disrupters are also potent inhibitors of intracellular deiodinase enzymes, (de-)conjugating enzymes (sulfotransferase, glucuronidase, sulfatase) and T3 receptors, thus interfering with intracellular availability and action of the ligand T3, which modulates gene expression by T3 receptors TRα and TRβ. Therefore, several of the identified endocrine disruptors exhibit multiple modes of interference in the TH axis and raise major concern especially under conditions of inadequate iodine supply and during life phases sensitive to altered TH availability such as fetal, neonatal, pubertal development, pregnancy, aging and euthyroid sick syndrome. More data on human exposure and risk assessment need to be collected in the REACH project of the EU.

S24.4

Amphibians as sensors of endocrine disruptors

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Environmental compounds can interfere with endocrine systems of wildlife and humans. The main sink of such substances, called endocrine disruptors (ED), are surface waters; and thus aquatic vertebrates such as fishes and amphibians are most endangered. Despite numerous reports on ED in fishes exist, information about ED in amphibians is relatively scarce but emerging. Amphibians can be affected sensitively by ED via adverse effects on reproductive biology and development e.g. metamorphosis triggered by the thyroid system. In amphibians, ED can affect reproductive biology by (anti)estrogenic and (anti)androgenic modes of action resulting in severe endocrine effects including abnormal sexual differentiation. These effects are mainly driven by direct interferences of ED with sex steroid receptors or indirectly by impacting synthesis and bioavailability of sex steroids. Recent development of flow-through exposure systems indicate that larval exposure of amphibians results in a similar sensitivity concerning impacts on sexual differentiation compared to established fish models. ED actions on thyroid system cause acceleration or retardation of metamorphosis mainly via changes in bioavailability of thyroid hormones rather than by direct interferences with thyroid hormone receptors. Our broad knowledge of amphibian biology and

endocrinology indicates that amphibians are very suitable sensors for the study of ED. In particular, the effects of ED on the thyroid system triggering metamorphosis can be determined easily and most sensitively in amphibians compared to other vertebrates including mammals. Methods and strategies are proposed for tracking and risk assessment of ED using amphibians as model. Recently, the emerging ecotoxicological issue of pharmaceuticals present in the environment indicates a high potential of further modes of action disrupting endocrine system of amphibians for instance by glucocorticoids and progestogins.

Pathophysiology and treatment of Type 2 Diabetes

S25.1

Abstract unavailable.

S25.2

Monogenic obesity

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Whilst the recent rise in the prevalence of obesity has been driven in part by environmental factors, there is considerable evidence from twin and adoption studies that body weight and fat mass are highly heritable traits and differences in susceptibility to obesity have strong genetic determinants. The identification of patients with mutations in the gene encoding the adipocyte-derived hormone leptin, and their successful treatment with recombinant human leptin, have provided insights into the role of leptin responsive pathways in the regulation of eating behaviour, intermediary metabolism, the onset of puberty and T-cell mediated immunity. Leptin acts by regulating a complex network of brain responses that can be studied using functional imaging, to co-ordinate changes in nutritional state with changes in food intake and the 'liking' of food. A downstream target of leptin action, the melanocortin 4 receptor (MC4R), plays a key role in modulating sympathetic nervous system mediated changes in blood pressure. Recently, genome wide approaches are proving to be an increasingly important tool in understanding the genetic heterogeneity associated with common obesity. The recognition that copy number variants contribute to a significant proportion of genetic variation at an individual and at a population level opens up new routes to gene discovery which will drive further understanding of the molecular pathways involved in energy homeostasis.

S25.3

FTO and obesity

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Three independent studies identified SNPs in the first intron of the gene FTO as being strongly and consistently associated with BMI and/or obesity at significance levels ($P=10^{-7}$ to $P=10^{-30}$) unobserved so far in genetics of obesity. The three studies estimated the putative effect around 1% of the total variance of BMI. So far, this represents the strongest effect for a common variant in obesity.
This result was replicated in at least 5 studies, either case–controls or general population of European and, less strongly Asian, descent. Interestingly, so far, the association has not been replicated in a 1100 African Americans cohort. However, both different allele frequencies and, for individual of African descent, lower linkage disequilibrium could explain this lack of replication.
Associated SNPs are located within a high LD block spanning 47 kb which includes exon 2 where both initial studies failed to find any mutation and thus the functional variant is not yet determined.
The *FTO* gene is ubiquitously expressed with a maximum in hypothalamus, which plays a major role in control of energy homeostasis. Contradictory results as to correlation of mRNA levels within hypothalamus in response to fasting rodent or correlation of *FTO* mRNA and obesity were found.
This gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase that could be involved in demethylation or DNA repair but its *in vivo* function is so far unknown.
Noticeably, another gene, *RPGRIP1L* (*FTM*) is close to associated SNPs and could be involved in obesity together with, or instead of *FTO* although initial KO study does not support this hypothesis.
Thus, at the time being, both the functional variation and the physiopathology of FTO action are partially unknown. I will provide an update of the ongoing research on this subject.

S25.4

Incretin-based therapies

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone with potent glucose-dependent insulinotropic and glucagonostatic actions. The glucose dependency of its antihyperglycaemic effects minimize any risk of hypoglycaemia. In higher concentrations GLP-1 slows gastric emptying and reduces appetite and food intake, resulting in weight loss. Taken together these actions give GLP-1 an unigue profile as an antidiabetic agents. Since GLP-1 is highly susceptible to enzymatic degradation by dipeptidyl peptidase-4 (DPP-4) strategies for developing GLP-1 as therapeutic agent include DPP-4 resistant GLP-1 analogues and selective DPP-4 enzyme inhibitors to prevent degradation of the peptide.
Treatment with a DPP-4 inhibitor (sitagliptin and vildagliptin) increases intact GLP-1 with a factor 2–4 and improved HbA1c with 0.8–1.0%, without risk for hypoglycaemia. The DPP-4 inhibitor is without gastrointestinal side effects and weight neutral. The GLP-1 analogue exenatide has a half-life of 3–4 h and needed to be administered twice daily before breakfast and dinner. Exenatide improves glycaemic control with an average reduction in HbA1c of about 1.0% and caused a weight loss of 2–3 kg after 30 weeks of treatment. The side effect is primarily nausea during the first days of treatment. Liraglutide, a once daily GLP-1 analogue with a half life of 12 h, reduces HbA1c with 1.0 to 2.0% and weight with 2–4 kg. Liraglutide has been compared with sulfonylurea, rosiglitazon and insulin glargine and in all studies reduction in HbA1c was greater or similar with liraglutide, and weight loss was in favour of liraglutide. At present several GLP-1 analogues for once-weekly administration are in phase 3 development. The final place of incretin-based therapies in the diabetic treatment algorithm will be clarified when we have long-term trials with cardiovascular end-points and data illustrating the effects on beta-cell function and the progression of type 2 diabetes.

Thyroid, Pregnancy and Fertility

S26.1

Thyroid disorders, infertility and miscarriages

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Italy.

Before and during pregnancy the thyroid gland and gonadal axes interact continuously. During reproductive life, normal levels of thyroid hormones are required for the maturation of oocytes. Hypothyroidism influences ovarian function by decreasing levels of sex-hormone-binding globulin and increasing the secretion of prolactin. In women of reproductive age, L-thyroxine therapy reverses hypothyroidism improving fertility and avoiding the need for use of assisted reproduction techniques. Infertile women undergoing medically assisted reproduction technologies are treated with a controlled ovarian hyperstimulation to increase circulating estrogen concentrations, which can, on the other hand, severely impair thyroid function. These changes are transient in healthy women, but in women affected by autoimmune thyroid diseases, estrogen stimulation might lead to an altered thyroid function during pregnancy. The frequency of thyroid autoimmunity is raised in infertile women with ovulatory dysfunction and endometriosis whereas hypothyroidism associated with infertility seems to be increased only in women with ovulatory dysfunction. Presence of thyroid autoimmunity does not interfere with normal embryo implantation, but is associated with a significantly raised frequency of miscarriages, even when thyroid function is apparently normal. Subclinical and overt hypothyroidism is associated with an increased risk of pregnancy-related morbidity, for which L-thyroxine therapy is required. Systematic screening for thyroid disorders in pregnant women is still controversial but can be considered an adjunctive tool in women at high risk, particularly infertile women.

S26.2

Thyroid dysfunction during pregnancy

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Thyroid physiology demonstrates certain specificity during pregnancy. Due to the increased thyroid hormone formation, observed in the course of gestation, the requirements for dietary iodine increase substantially – according to the current recommendations – to 250 μg/day (but <500 μg/day). Therefore, additional iodine supplementation is advised at the level of 150 μg/day to be administered to every pregnant (and lactating) woman. Thyroid hyperstimulation, caused by human chorionic gonadotrophin (hCG) in the first trimester, is another physiological change during pregnancy, assuming relatively frequently the form of gestational transient thyrotoxicosis which, however, usually needs no treatment. Concerning thyroid pathologies in pregnant women, thyroid dysfunctions, i.e. hyper- and hypothyroidism, occur most frequently in developed countries, both being predominantly of autoimmune etiology. Thus, hyperthyroidism in pregnancy is usually associated with Graves' disease, whereas hypothyroidism – with Hashimoto's thyroiditis. The diagnosis is based on abnormal values of thyroid hormones and thyrotropin concentrations, with some difficulties in the interpretation of results occurring in the first trimester, while thyroid antibodies should always be measured. Medical treatment in hyperthyroid pregnant women is the management of choice, with propylthiouracil being the preferred antithyroid drug, although thiamazole is also recommended by some authors as a safe and even more effective agent. Careful control of maternal thyroid function is required during antithyroid drug treatment to avoid fetal hypothyroidism. Replacement therapy with levothyroxine is the treatment of choice in hypothyroidism. Patients with pre-existing hypothyroidism generally require increased thyroxine doses during pregnancy. Subclinical hypothyroidism during pregnancy definitively requires thyroxine treatment. Summing up, appropriate management of thyroid dysfunction in pregnancy ensures excellent maternal and fetal outcomes.

S26.3

Postpartum thyroiditis

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Postpartum thyroiditis (PPT) is an autoimmune disorder characterized by lymphocytic infiltration of the thyroid gland and by the occurrence, in the postpartum period, of transient hyperthyroidism and/or transient hypothyroidism. Most women return to the euthyroid state by 1 year postpartum. The prevalence pf PPT is about 7.5–8.5%, then it represents a quite common disease, even if it may be asymptomatic in most cases. Women positive for thyroid peroxidase antibodies (TPOAb(+)) are prone to develop hypothyroidism during pregnancy and thyroid dysfunction after delivery. About 50% of TPOAb(+) pregnant women have PPT, and among these, more than 40% are affected by permanent hypothyroidism that develops in subsequent years. Factors predictive of thyroid dysfunction include a hypothyroid form of postpartum thyroid disease, high TSH values, and high TPOAb titers.

The clinical presentation of thyroid dysfunction occurring after delivery, may be characterized by hypothyroidism, hyperthyroidism, or hyperthyroidism followed by hypothyroidism (biphasic form).

Two studies, in 1990 and 2000, have investigated the chance of preventing PPT. Results showed that the administration of levothyroxine or iodine during or after pregnancy is not able to reduce the incidence of thyroid dysfunction after delivery.

Selenium (Se) exerts multiple actions on endocrine systems by modifying the expression of at least 30 selenoproteins, many of which have clearly defined functions. Well-characterized selenoenzyme families include the glutathione peroxidases (GPx), thioredoxin reductases, and iodothyronine deiodinases. These selenoenzymes influence cell function by acting as antioxidants, modifying redox status and thyroid hormone metabolism. Se supplementation may decrease inflammatory activity in patients with autoimmune thyroiditis, especially in those with high activity, reduces TPOAb titers, and ameliorates the thyroid echogenicity pattern.

To test the hypothesis that Se supplementation may be beneficial on thyroid autoimmunity and post-partum thyroid dysfunction (PPTD), 169 euthyroid, TPOAb(+) pregnant women were randomly divided into two groups: group S1 (85 women), designed to receive selenomethionine 200 μg/d after 12 week gestation; and group S0 (84 women), designed to receive placebo. In addition, 85 TPOAb(−) age-matched women were recruited as the control group (group C). Results showed that Se supplementation reduced the number of thyroid dysfunction during the postpartum period (28.6 vs 48.6%) and the number of permanent hypothyroid patients at 12 months after delivery (11.7 vs 20.3%); furthermore, lower TPOAb titers were observed in the Se-treated patients during and after gestation, and the same patients 12 months after delivery displayed a significantly better ultrasound pattern.

This study shown for the first time that Se supplementation during and after pregnancy inhibits the progression of autoimmune chronic thyroiditis. Se administration in the dosage of 200 μg/d during pregnancy and the postpartum period exerted an anti-inflammatory action, reduced TPOAb titers, and ameliorated the ultrasound echogenicity pattern with respect to controls. Se supplementation improved the course of the destructive thyroid gland process that occurs after parturition, reducing the incidence of PPTD and hypothyroidism.

S26.4

Consensus guidelines for the management of thyroid disorders associated with the pregnancy: an overview

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The wealth of new information, published over the past two decades, has led an international committee (under the auspices of the American Endocrine Society) to propose clinical guidelines for the management of thyroid diseases associated with the pregnant state. These guidelines were reviewed and endorsed by the four world Thyroid Associations (ETA, ATA, LATS, AOTA) and the Endocrine Society. They have been published in August 2007 as a supplement to the Journal of Clinical Endocrinology and Metabolism (Vol: 92; pp S1–S47).

The topics under scrutiny were maternal & foetal aspects related to both maternal hypothyroidism & hyperthyroidism (including Graves' disease & GTT 'gestational transient non-autoimmune thyrotoxicosis'), infertility and miscarriage, postpartum thyroid disorders, iodine nutritional status, thyroid nodules & cancer diagnosed during pregnancy, and finally the heated subject of universal screening. Altogether, 35 recommendations for 'good clinical practice' were edited, based on a systematic review of all publications related to each of these topics, published over the past two decades. For each recommendation, grading of the evidence was achieved based on the USPSTF system and, when possible, on the GRADE system.

Management of thyroid diseases during pregnancy involves many different care providers (family doctors, obstetricians, endocrinologists, nurse midwives, etc.) thus making the development of such guidelines all the more needed. Also, thyroid diseases during pregnancy require special consideration because pregnancy *per se* induces significant changes in thyroid function and, conversely, maternal thyroid disease can have adverse effects on the pregnancy and foetal development.

Since it is not possible, in the time allotted for this lecture, to review systematically the entirety of the 35 recommendations, we will focus our attention on the following topics: a) the difficulties encountered in grading the available scientific evidence and lack of sufficient randomized clinical trials; b) the need for continuing and better education of the multiple care providers concerning thyroid disease in pregnancy; c) the difficult issue of targeted *versus* universal screening of pregnant women to search for thyroid autoimmunity features and mild thyroid dysfunction, and finally d) an attempt to draw perspectives on 'where to go to' in the near future.

In summary, pregnancy has profound effects on the regulation of thyroid function in healthy women and in patients with thyroid disorders. These effects need to be recognized, precisely assessed, clearly interpreted, and correctly managed in order to drastically reduce the detrimental effects of thyroid dysfunction on pregnancy outcome.

Impact of SNPs on Hormone Function

S27.1

Pitfalls and chances of association studies

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Genetic association studies have been very successful and very reliable in many fields of medicine. Cross replication between studies now is the rule rather than the exception, which is very different from the situation a few years ago.

Nonetheless, there are still possible problems with association studies which need close attention. These problems include population stratification even in the days of whole genome data sets, inappropriate methods of analysis and finally over-interpretation of the data.

I will try to give an overview of theses chances and problems, highlighted by examples and will attempt a guide to the implementation and interpretation of association studies nowadays.

S27.2

Impact of SNP on hormone function: FSH receptor

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The *FSHR* is characterised by a large number of SNPs (1636 listed in the NCBI SNP database), mostly located in intronic regions and of unknown heterozygosity rate. Some SNPs, especially those which are nonsynonymous and located in exons have been studied in association with gonadal function.
The SNPs at nt position 919 and 2039 in exon 10 are very common (heterozygosity: 0.469) and result in the aminoacid transition Thr/Ala at codon 307 and Asn/Ser at codon 680, respectively. In the Caucasian population they are in linkage disequilibrium with the Thr307-Asn680 variant covering 55% and the Ala307-Ser680 variant 45% of the alleles. The other two possible combinations represent $<1\%$ of all alleles in Caucasians, while they are more frequent in the East. A G/A SNP is located in the promoter region (-29), with the G allele covering 75% and the A allele 25% of the alleles in Caucasians, while the distribution is equal (50%) in Indonesians. These SNPs do not have any apparent functional effect *in vitro*, but influence the receptor activity *in vivo*, at least in women. We could show that the Ala307-Ser680 variant is associated with higher basal serum FSH levels and lower sensitivity to FSH stimulation in women with normal ovarian function undergoing ovarian hyper stimulation for assisted reproduction and during normal menstrual cycle. However, these two SNP apparently do not influence serum FSH levels and semen parameters in men with normal or reduced spermatogenesis. However, when the haplotypes resulting from the SNPs in exon 10 and from the SNP at position -29 in the promoter region are considered together the two allelic variants A-Ala-Ser and G-Thr-Asn showed a statistically significant different distribution between controls and men with non-obstructive azoospermia, suggesting that the FSHR genotype might constitute a risk factor for spermatogenetic failure.

S27.3

Estrogen and androgen receptor variants

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Androgens and oestrogens, are acting through specific receptors, belonging to the nuclear receptor family and both androgen receptor (AR) and the two forms of oestrogen receptor, α (ER1) and β (ER2) are richly expressed in different parts of the male reproductive system.
There are several examples indicating that polymorphisms in these sex hormone receptors can be associated with some types of disease or milder dysfunction of the sex hormone regulated organs.
In the AR gene not only single nucleotide polymorphisms (SNPs) but also variation in the length of two repetitive sequences, CAG (glutamine encoding) and GGN (glycine encoding) repeats, can have an impact on the receptor function. Thus, extremely long (>40) CAG repeats are associated with Kennedy's disease, a late onset disturbance in the neuromuscular function. Even variations in the normal range (10–30) of CAG numbers have suggested as implicated in pathogenesis of conditions as male infertility, prostate cancer, testicular cancer and metabolic syndrome.
Less information is available as considers the impact of GGN repeat length variation but polymorhisms in this part of the AR gene have been linked to the risk of e.g. cryptorchidism and hypospadias.
In vitro studies have confirmed that AR activity varies with different CAG and GGN length and these variations may not only have influence on the effect of the natural ligand of the receptor but even modify the endocrine disrupting effect of environmental pollutants.
Less information is available as considers SNPs in the AR gene and risk of disease; however, we have recently identified a SNP which may be associated to the risk of testicular cancer.
SNPs in the ER1 and ER2 have also been reported as risk factors of pathological conditions in the male reproductive system, e.g. infertility and cryptorchidism. However, these findings need to be confirmed in more studies.

S27.4

LH receptor variance

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The LH receptor is a member of the large receptor family of GTP-binding protein coupled receptors. Mutations in the LH receptor gene cause severe sex hormone-related disease, ranging from very early precocious puberty in boys (activating mutation) to slight undervirilization, severe hypospadias or complete 46XY pseudohermaphroditism (inactivating mutation). Thus, LH receptor gene mutations have profound effects on sex hormone production and on physiology of patients. In addition, the strength of the phenotype follows the *in vitro* activity of the various LH receptor mutant proteins. Thus the question has arisen whether slight changes in LH receptor activity, such as caused by polymorphic gene variants, may have subtle effects on susceptibility, disease progression or response to treatment of sex hormone-dependent disease. The most frequent LH receptor polymorphisms that involve an amino acid change are the absence or presence of a two amino acid (LQ) insertion at position 18 in exon 1 (rs4539842) and two variable amino acids at position 291 and 312 respectively: 291Asn/Ser (rs12470652) and 312Ser/Asn (rs2293275). The latter are of interest because of their location in exon 10, which is involved in the differential sensitivity of the LH receptor to LH and hCG. The 291Asn/Ser and 312Ser/Asn SNPs are located at or near glycosylation sites, respectively. The polymorphic LQ insertion is located in the signal peptide, which enables translocation of the LH receptor into the endoplasmic reticulum where protein maturation processes are carried out. The LQ insertion had a clear effect on *in vitro* LH receptor expression and showed an association with worse disease free survival in breast cancer patients. Although we could not detect a functional effect of the 312SN polymorphism, this allele appears to be a weak risk allele for breast cancer. The 291S LH receptor variant is more sensitive to LH *in vitro*, but its low frequency did not allow association studies in our breast cancer cohort.

Receptor Modulators

S28.1

Diverse actions of the nuclear receptor corepressor RIP140 in metabolic regulation

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PGC1 and RIP140 are key regulators of nuclear receptor signalling that control metabolic gene expression in adipose tissue, liver and muscle. PGC1 promotes whereas RIP140 represses the expression of a network of catabolic genes in adipose and muscle. Thus mice devoid of RIP140 accumulate less fat in adipose tissue and liver while mitochondrial biogenesis and respiration is increased in type 2 muscle fibres; as a consequence, the mice maintain their insulin sensitivity as they age or are fed a high fat diet. Expression profiling indicates RIP140 suppresses the expression of genes in adipose and muscle that are involved in energy expenditure, including fatty acid oxidation, oxidative phosphorylation and mitochondrial biogenesis but is necessary for the expression of genes involved in lipogenesis and triglyceride storage. Analysis of cultured adipocytes and myofibres after *in vitro* differentiation demonstrates that RIP140 functions as a corepressor for PPARs and ERRs. Analysis of the UCP1 gene, which is de-repressed in white adipose tissue, indicates that the repression of metabolic genes is achieved by the recruitment of a combination of histone modifying enzymes and DNA methyl transferases which leads to methylation of specific histone residues and DNA. Interestingly, many of the metabolic genes repressed by RIP140 are activated by the coactivator PGC1. It appears that these two cofactors function to remodel chromatin in the vicinity of metabolic genes and thereby regulate their transcription through mutually antagonistic mechanisms including post translational modifications.

S28.2

Glucocorticoid receptor ligands, dissociating between transrepression and transactivation

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Glucocorticoid hormones (GCs) remain the mainstay for the treatment of various inflammatory disorders, because of their great efficacy. The long-term usage of GCs is, however, overshadowed by the occurrence of debilitating side-effects, like osteoporosis, skin and muscle atrophy, diabetes and neurological disorders. GCs exert their functions through binding to the glucocorticoid receptor (GR), a transcription factor that regulates gene transcription in a positive or negative way. Direct binding of activated GR in the promoter of target genes is believed to be the main pathway leading to metabolic gene expression (mainly hold responsible for the unwanted side-effects), whereas the interference of GR with the activity of other transcription factors, such as NF-κB or AP-1, greatly contributes to its desired anti-inflammatory capacities. 'Dissociated ligands' thus aim to separate GR-mediated transcriptional activation from transcriptional repression in order to achieve better side-effect profiles.

In this respect, a newly characterized, plant-derived, non-steroidal GR modulator, i.e. Compound A (CpdA) was tested both *in vitro* and *in vivo* for its dissociative effects.

Methodology

We have used CpdA in several cellular *in vitro* assays as well as in *in vivo* disease models to test its dissociated properties, as compared to glucocorticoids.

Results

CpdA behaves as a potent (although weaker) anti-inflammatory agent, both *in vitro* as *in vivo*, as compared to the synthetic glucocorticoid Dexamethasone. However, as opposed to steroidal ligands, CpdA does not give rise to the gene-activating effects in cells, nor to increased blood glucose levels or hyperinsulinemia in the tested animals. Furthermore, as opposed to glucocorticoids, CpdA does not lead to GR desensitization.

Conclusions

It is possible to fully dissociate the gene-activating effects from the inhibitory actions of GR by imposing a monomeric structure to the receptor by so-called 'specific GR modulators' (SGRMs), like CpdA. Moreover, GR desensitization can be avoided which adds to the beneficial effects for long-term treatments.

S28.3

Selective androgen receptor modulators: mechanisms and therapeutic potential

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Testosterone supplementation increases whole body and appendicular skeletal muscle mass, maximal voluntary muscle strength, and leg power. However, concerns about the long term risks of prostate and cardiovascular disorders in older men treated with testosterone have encouraged efforts to develop selective androgen receptor modulators (SARM) that increase skeletal muscle mass and improve physical function without the adverse effects on prostate and cardiovascular outcomes. These nonsteroidal SARMs do not serve as substrates for CYP19 aromatase or 5α-reductase, act as full agonists in muscle and bone and as partial agonists in prostate and seminal vesicles. The differing interactions of steroidal and nonsteroidal compounds with the AR may at least partially contribute to their unique pharmacologic actions. Bicalutamide adapts a greatly bent conformation in the AR. Although A-ring and amide bond of the bicalutamide molecule overlaps the steroidal plane, the B-ring of the molecule folds away from the plane, pointing to the top of the ligand binding pocket (LBP), which forms a unique structural feature of this class of ligands. These H bonding interactions are believed to be critical for high binding affinity. Structural modifications of aryl propionamide analogs bicalutamide and hydroxyflutamide led to the discovery of the first generation of SARMs. The first generation SARM pharmacophores can be classified into four categories: aryl-propionamide, bicyclic hydantoin, quinoline, and tetrahydroquinoline analogs.

The mechanistic basis of the tissue selective actions of SARMs is poorly understood, although several mechanisms have been proposed. Ligand binding induces specific conformational changes in the ligand binding domain, which could modulate surface topology and subsequent protein–protein interactions between AR and other coregulators involved in genomic transcriptional activation or cytosolic proteins involved in nongenomic signaling. Differences in ligand-specific receptor conformation and protein–protein interactions could result in tissue-specific gene regulation, due to potential changes in interactions with ARE, coregulators or transcription factors.

It is generally believed that the downstream signaling mechanisms that mediate the anabolic effects of SARMs on the skeletal muscle are similar to those of testosterone. Testosterone induces hypertrophy of both type I and type II fibers and an increase in the number of satellite cells. Testosterone promotes the differentiation of mesenchymal, multipotent cells into myogenic lineage and inhibits their differentiation into adipogenic lineage. Testosterone and DHT regulate mesenchymal multipotent cell differentiation by promoting the association of AR with β-catenin and translocation of the AR-β-catenin complex into the nucleus, resulting in activation of TCF-4. The activation of TCF-4 modulates a number of Wnt-regulated genes that promote myogenic differentiation and inhibit adipogenic differentiation. The effects of testosterone on myogenic differentiation are mediated through an AR pathway. Testosterone increases fractional muscle protein synthesis and improves the reutilization of amino acids by the muscle. We do not know whether conversion of testosterone to DHT is required for mediating androgen effects on the muscle.

Preclinical studies have demonstrated the ability of SARMs to increase levator ani muscle mass in the castrated rat and to increase bone mass and strength. Efficacy trials of several SARMs in humans are in early stages and have generally shown modest increments in fat-free mass. The first generation SARMs do not undergo aromatization or 5-alpha reduction; it is unknown whether this may pose long term risks. The efficacy and the safety of SARMs as function promoting therapy is just beginning to be evaluated.

S28.4

Thyroid hormone signaling during brain development: genetic dissection in mouse

Frédéric Flamant
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Thyroid hormone (T3) has been known for a long time to be required for brain development which activate TRα and TRβ nuclear receptors. In rodent models, histological defects are mainly observed in cerebellum. Whether T3 action during cerebellum development is due to direct regulation of gene transcription by liganded thyroid hormone receptors (mainly TRα) or also the indirect consequences of other defects and systemic disorders is currently unknown.

In order to unravel the direct and indirect effect of T3 during mouse cerebellum development we have used the CRE/loxP recombination technology to express a mutant form of the TRα1 isoform able to block T3 signaling in a controlled manner.

Whereas ubiquitous expression of this mutation recapitulates most if not all features of congenital hypothyroidism in the post-natal mouse cerebellum, restricted expression indicate that in some neuronal cell types, the action is cell autonomous whereas in other, indirect effects, perhaps mediated by neurotrophic factors or cell contacts, are involved.

Meet the Expert Sessions

ME1

How to optimize the management of thyroid associated orbitopathy

P Perros
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Thyroid associated orbitopathy affects approximately a third of patients with Graves' disease. It is responsible for significant symptoms, poor quality of life, and if neglected can cause blindness. Early diagnosis of Graves' ophthalmopathy is paramount as is early detection of sight-threatening disease. All but the mildest cases are best referred to centres who provide multidisciplinary care and have the experience and expertise on managing this condition. Treatment must be tailored to the individual and timed appropriately. The mainstay of medical treatment is high dose steroids. Orbital irradiation has a role in pateints with dysmotility. Surgical treatment has a lot to offer in patients with sight-threatening disease and to improve functional and cosmetic outcomes.

ME2

Laproscopic and cortical sparing surgery in adrenal tumors

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Abstract unavailable.

ME3

Clinical: adolescent and adulthood gynecomastia

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Gynecomastia (Gm) is defined as increased mammary gland size in male and must be distinguished from lipomastia. Mammary tissue is present in children of both sexes. The gland does not develop when androgens (A) prevail over estrogens (E). In men molar ratio of plasma testosterone to estradiol is about 300 to 1. Any deviation from this ratio, be it through diminished A or increased E, can lead to Gm. Testes maturation requires both A and E and palpable mammary tissue is present in about 40% of pubescent boys that disappears within 2–3 years (adolescent Gm). It may occasionally be persist until adulthood (persistent adolescent Gm) without clinical significance, except for psychogenic discomfort. Gm may appear in ageing male. In any age, Gm may indicate tumor with supranormal E secretion. Leydigyoma, embryonic carcinoma, teratocarcinoma, chorioncarcinoma and bronchial carcinoma lead either directly or via elevated hCG secretion to increased E production by Leydig cells. Palpation and ultrasound of testes are obligatory. Gm may associate congenital adrenal hyperplasia and adrenal tumors. Klinefelter syndrome, other primary or secondary hypogonadisms, diseases of androgen target organs (Reifenstein syndrome, perineal hypospadias, intersexuality), liver cirrhosis, terminal renal failure may be associated with Gm. In thyrotoxicosis, Gm result from increased production of sex hormone binding globulin and decreased bioavailability of A. In large unilateral Gm mammography is needed for diagnosis of a possible mammary cancer (1% of all breast cancers). Different drugs (including anti-androgens) may induce or exacerbate Gm. In 50% of cases idiopathic Gm is diagnosed. Recently CYP19 gene polymorphism with high aromatase activity has been attributed to incidence of Gm. Therapy should target underlying cause. In idiopathic Gm an anti-estrogen tamoxifen (10 mg b.i.d.) is suggested. If, after 3 months of treatment no improvement has occurred or if patient desires primarily a surgical correction, gynecomastectomy is advocated.

ME4

Treatment of osteoporosis

Juraj Payer
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Osteoporosis affects more than 75 million people in Europe, United States and Japan, and more than 4.5 million osteoporotic fractures occur in Europe and United States. The aim of treating osteoporosis is to reduce risk of fractures and to improve quality of life of patients with preexisting fractures. Several interventions to prevent and reduce fracture risk are being recommended. These include adequate intake of calcium (at least 1000 mg per day), vitamin D (at least 800 mg per day), muscle strengthening, exercise, avoiding nicotine, alcohol and other osteoporosis risk factors and treating disorders leading to osteoporosis.
The most commonly used osteoporotic drugs are antiresorptives. They include bisphosphonates (alendronate, risedronate, ibandronate used orally weekly or monthly and ibandronate used also intravenously yearly), selective estrogen receptor modulators (raloxifene), calcitonine and in the past also hormone replacement therapy (because of higher risk of cardiovascular events and breast cancer used sporadically). Teriparatide and parathormone are osteoanabolic drugs and strontium ralenate has dual antiresorptive and osteoanabolic effect. All these drugs have shown to reduce risk of vertebral and some also nonvertebral fractures. Indication for most of the drugs (except teriparatide and parathormone, which have special prescription criteria) is in Europe T score < -2.5 S.D. and a osteoporotic fracture. Lately WHO developed a FRAX algorithm, which allows to estimate 10 year fracture probability and to individualize the treatment selection for each patient. For monitoring the treatment bone mineral density and bone turnover are used. The average duration recommended for the treatment is 5 years, in osteoanabolics 18 months.
Early onset of powerful treatment can now effectively reduce the number of osteoporotic fractures.

ME5

Thyroid dysfunction induced by Amiodarone (focused on prevalence, predisposing factors, treatment)

Wilmar Wiersinga
Academic Medical Center, Amsterdam, The Netherlands.

Abstract unavailable.

ME6

Growth in childhood and adolescence as function of hormonal activity and nutrition

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A prerequisite milieu for normal growth is an intact hormonal system, adequate nutrition and normal physical activity. Any arm of this triangle, if interrupted, may change the normal growth pattern. Timing and interval range of disruption of this homeostasis, may affect the outcome up to irreversible results. The 3 polynomial growth model of Karlberg helps to detect, past irreversible growth retardation and in foresee and prevent growth deterioration
A normal function of the endocrine system from the gene to the receptor is dependent on an adequate supply of precursors, building materials, and functioning enzymes. Any substance, chemical that has the right configuration can activate a cascade of events in a different way from the expected normal function acting as an endocrine disruptor. On the other side of the spectrum, nutritional deficiency may result in hormonal deficiency.
We will use iron as an example for a nutritional basis of a hormonal dysfunction. Iron deficiency may be the basis of an uncontrolled hypothyroidism, this specific deficiency results in malfunctioning of all the steps of the TRH-TSH-T4 axis. The last step of the iron dependent thyroid peroxidase may prevent efficient production of thyroid hormone. Without proper replacement of iron, thyroxin replacement may be inefficient and reaching euthyroidism may be a difficult task. Iron deficiency may be the tip of the iceberg of nutritional deficiency. In such a patient, subnormal Vitamin A levels may interfere with iron supplementation, since vitamin A has an important task in iron absorption. Hypothyroidism may be severe in patients with a combination of iron and vitamin A deficiency. During puberty as a result of subnormal vitamin A supply, puberty might be delayed and growth hormone levels may be lower than expected. Thus adequate nutrition is a prerequisite for a normally functioning hormonal system for proper growth and puberty.
Physical activity may have an effect on nutritional needs but also an effect on metabolic systems. In children and adolescents, IGF-I levels may be low despite adequate supply of calories in first stages of training. While sedentary life style and caloric overload may change pubertal progression and growth.
In a world of super specialty, clinical cases teach us that endocrinology and nutrition have many very important meeting points of interest.

ME7

Menopausal hormone therapy and cardiovascular disease: the women's health initiative (WHI) randomized placebo-controlled hormone trials

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The Women's Health Initiative (WHI) placebo-controlled trial of conjugated equine estrogens (CEE) plus medroxyprogesterone (MPA) in 16 608 post-menopausal women, aged 50–79 at randomization, was stopped after an average of 5.6 years of follow-up, due to increased risks, including increased coronary heart disease (CHD), stroke, and pulmonary embolism (PE), compared to health benefits. The WHI trial of CEE only in 10 739 women with prior hysterectomy, also aged 50–79, was also stopped early, after an average of 7.1 years of follow-up, because of excess stroke and no cardiovascular benefit. There was, however, a suggestion of a cardioprotective effect of CEE in women who had been aged 50–59 years at the time of randomization, which had not been seen with CEE + MPA. This prompted a cross-sectional study of coronary artery calcification (CAC) in 1064 CEE trial participants aged 50–59, 1.3 years after stopping study pills, which showed a significantly lower mean CAC score among women who had received CEE for a mean of 7.4 years, compared to placebo. Analyses designed to explore whether the effects of hormone therapy (HT) on risk of cardiovascular disease varied by age or years since menopause, which combined all the data from the two WHI trials, revealed a (non-significant) trend of decreased CHD risk in women who had initiated HT closer to menopause compared to the increase in CHD risk among women more distant from menopause. In contrast, the risk of stroke was elevated regardless of years since menopause. The concept that beneficial cardiovascular effects of HT occur when the therapy is initiated before atherosclerosis develops, referred to as the 'timing hypothesis', has been a subject of debate in recent years and will be discussed. Worth noting, follow-up of CEE + MPA trial participants over an average 2.4 year period after women stopped study pills revealed no difference in overall risk of cardiovascular events or CHD by initial treatment.

ME8

Cushing's syndrome: pitfalls in diagnosis and management

Xavier Bertagna
Hôpital Cochin, Paris, France.

Drug interactions, intercurrent pathological states may interfere with routine diagnostic tests. Authentic hypercortisolic states can be present without Cushing's syndrome, such as in depression, anorexia nervosa, alcoholism, pregnancy. Mild forms of Cushing's syndrome, or fluctuating cases are other usual pitfalls.
When the diagnosis of Cushing's syndrome is firmly established there are still many potential pitfalls in the etiological diagnosis of Cushing's syndrome: Cushing's disease mimicking an autonomous adrenal tumor, severe Cushing's disease mimicking the classical ectopic ACTH syndrome, mild ectopic ACTH syndrome mimicking the classic Cushing's disease, and the cases of ACTH-independent Cushing's syndrome where the two adrenals are involved (AIMAH, and PPNAD).
Management of patients through pituitary surgery, adrenal surgery, anticortisolic drugs offer many other situations with potential pitfalls.
All these situations will be approached through case presentations.

ME9

New drugs in the management of the obese patients

Felipe F Casanueva
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In the prevalence of obesity has increased worldwide reaching 30% of the adult population in some countries. Direct and inferential evidences show that this excess of body fat is associated with adverse health consequences, and that even a modest 5 to 10% weight loss results in substantial improvement in health. Considering the limited efficacy of the so-called 'life style' interventions based on diet plus exercise in the obese subjects, and that bariatric surgery is indicated only for morbid obesity, it appears that drug therapy would be the only available method to tackle the problem at large scale. Until now, pharmacological obesity treatment options are limited, however, new anti-obesity drugs acting through the central nervous system pathways or the peripheral adiposity signals are under clinical development. One promising approach is the use of peptides that influence the peripheral satiety signals and brain–gut axis, like the GPL-1 analogs. However, considering that any anti-obesity drug may probably affect one or several of the systems that control food intake and energy expenditure, it is unlikely that a single pharmacological agent will be effective for a striking treatment of obesity. Thus, the future strategies to tackle obesity would need to take into account that an effective weight loss will most probably require a coadministration of medications that act through different mechanisms.

ME10

Nonclassical congenital adrenal hyperplasia (CAH)

Catherine Dacou-Voutetakis & Maria Dracopoulou-Vabouli
Unit of Endocrinology, Diabetes and Metabolism, First Department of Pediatrics, 'Aghia Sophia' Children's Hospital, Athens University Medical School, Athens, Greece.

CAH results from an enzymatic defect in the synthesis of cortisol from cholesterol in the adrenal cortex. It is a monogenic disorder transmitted as an autosomal recessive trait. More than 90% of the cases are caused by a deficiency of 21-hydroxylase (21OHD). Patients with 21OHD are categorized into 2 main forms: classical (salt wasting and simple virilizing) and nonclassical (NC).
The frequency of the NC form varies in the different populations and the method of detection (hormonal, molecular) ranging from 1:100 to 1:1000. The NC form of CAH is looked for in subjects with premature adrenarche, increased acne, hirsutism, menstrual disorders, fertility problems or abortions, adrenal or testicular adenomas. A number of cases are completely asymptomatic, especially men, and are discovered either when investigating the family members of an index case or by serendipity.
Concerning genotyping, three point mutations have specifically been associated with NC CAH: V281L, P30L and P453S. In compound heterozygotes, the phenotype is determined by the least deleterious mutations. The overall data indicate that NC genotypes do not always predict phenotype. Thus in certain cases mild, unexpected virilization may be detected.
The management of patients with NC CAH includes genetic counseling especially in prospective or current pregnancy and the administration of glucocorticoids in symptomatic subjects. In asymptomatic patients incidentally diagnosed, there is a real dilemma since the patient has no clinical problem but there is a potential risk for adrenal or testicular adenoma or polycystic ovarian disease. In such cases a consensus document does not recommend treatment. One may suggest that if follow-up can be ensured the patient may remain without therapy. It must be emphasized however that there is not as yet evidence-based recommendation for the management of such cases and individualization is required.

ME11

The relative value of 25(OH)D and 1,25(OH)2D measurements

Paul Lips
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After synthesis in the skin or intake with the diet, vitamin D3 is hydroxylated in the liver to 25-hydroxyvitamin D (25(OH)D), and subsequently in the kidney to 1,25-dihydroxyvitamin D (1,25(OH)2D). The major vitamin D store is the circulating 25(OH)D. The serum 25(OH)D concentration is the measurement of choice to assess the vitamin D status. It is relatively stable and not directly influenced by hormones or calcium in the diet. The half life of serum 25(OH)D is around 25 days. Serum 25(OH)D should be assessed in patients suspected of vitamin D deficiency or insufficiency and patients with osteoporosis. The active metabolite, 1,25(OH)2D should be measured in case of disorders of 1α-hydroxylation of 25(OH)D, existing in renal failure, vitamin D dependent rickets type 1 and hypophosphatemic rickets where 1α-hydroxylase activity is decreased or absent, and vitamin D receptor defects as in vitamin D dependent rickets type 2 where 1α-hydroxylase activity is increased. Serum 1,25(OH)2D is under negative feedback control by serum calcium and phosphate. Its formation in the kidney is stimulated by parathyroid hormone (PTH). A high calcium diet or calcium supplements will decrease serum 1,25(OH)2D and immobilisation has similar effects. The half life of serum 1,25(OH)2D is around 8 h. While the renal hydroxylation of 25(OH)D is tightly regulated, the extrarenal hydroxylation in activated macrophages is not. Extrarenal formation of 1,25(OH)2D occurs in granulomatous diseases such as sarcoidosis, tuberculosis and inflammatory bowel disease, and lymphoproliferative diseases. In these disorders, serum 1,25(OH)2D

may be elevated resulting in hypercalcemia and hypercalciuria. The measurement of serum 1,25(OH)2D in case of vitamin D deficiency is not very relevant. It usually stays within the normal reference range because the increase of serum PTH stimulates the renal hydroxylation of 25(OH)D. However, serum 1,25(OH)2D may fall to subnormal levels in case of severe vitamin D deficiency, where the synthesis of 1,25(OH)2D becomes substrate-dependent. When comparing groups of severely vitamin D deficient and replete patients, mean serum 1,25(OH)2D usually is lower in the former than in the latter group, but this is more important for research than for patient care. In conclusion, the measurement of serum 25(OH)D is important to assess vitamin D status and to exclude vitamin D deficiency or insufficiency. Serum 1,25(OH)2D should be measured in selected metabolic diseases associated with decreased or increased 1α-hydroxylase activity or vitamin D receptor defects.

ME12

Primary aldosteronism

John Connell
BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK.

Primary aldosteronism is the most common secondary cause of hypertension. Less than 50% of patients with the disorder have a solitary aldosterone producing adenoma. In the most common presentation, patients present with bilateral hypersecretion of aldosterone. The aetiology of this is uncertain. Studies within our own group have suggested that there is an underlying genetic predisposition to develop hypertension with a raised aldosterone to renin ratio (ARR) associated with variation in the gene encoding aldosterone synthase (CYP11B2). More recent studies have suggested that the development of aldosterone excess is a digenic phenomenon with variations in CYP11B2 and in the neighbouring gene (CYP11B1) that encodes 11ß-hydroxylase. The combination of polymorphisms, which are inherited as a single haplotype block in Caucasian subjects, leads to reduced efficiency of 11ß hydroxylation and excess of aldosterone production. We have proposed that, over a lifetime, this may predispose subjects to develop hypertension with relative aldosterone excess.
Regardless of the aetiology, identification of Primary Aldosteronism depends on detection using a simple screening procedure such as measurement of the ARR. Confirmation of diagnosis is had, thereafter, by appropriate sodium loading manoeuvres followed by lateralisation using imaging and adrenal vein sampling. Therapeutic strategies, including laparoscopic adrenalectomy, and medical approaches using specific mineralocorticoid receptor antagonists will be discussed.

ME13

New pharmaceutical contraceptive approaches

Philippe Bouchard
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The pharmaceutical armamentarium available for contraception is remarkable and extraordinary progress has been achieved since 1960, when the first hormonal contraceptive, Enovid, was approved in the USA. However, the demand for new methods, for improvement of existing methods, and easier availability, remains extraordinary. Indeed, the number of unintended pregnancies is still too high, averaging 40% of all pregnancies. In addition, 50% of these pregnancies are followed by abortion, many of those being unsafe. The situation is further complicated by the decreased interest of the Pharma industry. Improvement of existing methods includes OCs given non stop with bleed free regimens. There is also a need for improvement of the choice offered to women, and the multiplication of methods among which women can choose the method they like best: intrauterine devices which blocks menstruation without systemic exposure, vaginal contraceptive rings with 17 beta estradiol, and patches/transdermal hormonal contraception containing friendly steroids. Although the state of reproductive research has not permitted to target oocyte fertilization or implantation as a contraceptive method, several leads show great future: (1) the replacement of ethinyl estradiol by estradiol, which will allow the reduction VTE events, and tolerance, (2) the development of new estrogens such as estetrol, and (3) the development of the progesterone receptor modulators (PRMs). This class of product is remarkable since it is devoid of metabolic and coagulation side effects. PRMs block the LH surge and prevent implantation without suppressing endogenous estradiol production. The end result of their administration is a bleed free method available as a daily continuous regimen. Other regimens include emergency contraception, where better results have been achieved in comparison with levonorgestrel. The development of this method will allow the introduction of the first product on the market next year. Even though long term efficacy as well as endometrial safety will have to be assessed, this class of compounds looks very promising, because of their safety and efficacy but also because in animal models, PRMs prevent the development of breast cancer. Finally, the most difficult task will be to develop, in parallel, or in association, a contraceptive method, which at the same time, will protect against sexually transmitted infections.

ME14

Molecular biology for clinicians

John Kopchick
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In this lecture, fundamental concepts in the area of molecular biology will be presented. These include biology's and biotechnology's central dogma; the 'human genome project'; the discordance between human gene number and corresponding protein number; and gene cloning techniques. Also presented will be procedures used to determine gene number and location (Southern blotting) and levels of gene expression at the RNA (Northern blotting, reverse transcription/polymerase chain reaction, micro-array/gene chip) and protein (Western blotting, proteomics) level. Additionally, three examples describing the cloning of genes/cDNAs and production of the respective recombinant therapeutic proteins will be offered. Finally, functional genomic concepts and protocols will be discussed including production of transgenic and gene-disrupted (knocked out) animals as well as methods to down regulate gene expression using antisense, ribozyme, or small inhibitory RNAs. The lecture will stress the 'basics' of the various protocols with clinical examples cited.

ME15

Diagnosis and treatment of type 2 diabetes mellitus in childhood

Neslihan Gungor
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Type 2 diabetes mellitus (T2DM) has historically been considered an exclusive disease of adulthood until late 1970's when reports of increased prevalence in pediatric age group emerged in the literature. The concerning increase in the rate of diagnosis of T2DM in children and adolescents has continued parallel to the increasing rates of obesity. The disease is not specific to the United States, it has been recognized as a global problem.
T2DM of youth is a heterogeneous disease from a pathophysiology perspective. Both insulin deficiency and insulin resistance are the key components of pathogenesis, and their variable proportions alter the delicate balance between these two parameters. This reflects to the clinical presentation and the treatment needs to be tailored accordingly.
This review will address T2DM as a relatively new and significant disease of the pediatric age group. The objectives are:
1. To provide an overview of T2DM in youth, with emphasis on:
   –Characteristics and pathophysiology
   –Diagnosis, differential diagnosis
   –Risk factors, epidemiology
2. To discuss treatment goals and options, with reference to clinical presentation.

ME16

Abstract unavailable.

Clinical Highlights

Hot topics: Clinical

HTC1

Reduction in incidence of Type 2 diabetes by lifestyle modification in a Middle Eastern urban population: Tehran Lipid and Glucose Study

Hadi Harati, Farzad Hadaegh, Laleh Ghanei & Fereidoun Azizi
Research Institute for Endocrine Disorders, Prevention of Metabolic Disorders Research Center, Tehran, Islamic Republic of Iran.

Aims
Lifestyle modification has been shown to prevent development of Type 2 diabetes in high risk American, European and Asian populations. We wanted to test whether such intervention could have similar results in a white Middle Eastern population.
Methods
A total of 8212 non-diabetic subjects $\geq$20 years were selected by cluster random sampling method in the cross-sectional phase of the Tehran Lipid and Glucose Study (TLGS) from 1999 to 2001. A subsample of 3098 subjects was then selected as the lifestyle modification and the remaining 5114 subjects as the control group in the interventional phase of the TLGS from 2002 to 2005. Interventions were aimed at lifestyle modification through improving nutrition and dietary pattern, increasing physical activity levels, and smoking cessation. Fasting and 2-hours plasma glucose as well as other major diabetes risk factors were measured at baseline and follow-up examinations.
Results
After median follow-up of 3.5 years, 4747 subjects (2993 and 1754 respectively from the control and intervention group) completed the follow-up examination. Mean age and BMI of the participants were 41 ± 12 years and 26.5 kg/m^2 respectively and 58% were females. The intervention group had significantly lower rate of increase in major diabetes risk factors (Table 1). The incidence rate of Type 2 diabetes was significantly lower in the intervention versus control group (8.2 and 12.2 per 1000 person-years respectively, $P<0.001$). The hazard ratio of developing Type 2 diabetes in the intervention group after adjustment for all the major risk factors was 0.34 (95% CI: 0.25–0.47, $P<0.001$). To prevent one case of diabetes, 25.0 individuals in the whole population, 5.7 in those with impaired glucose tolerance and 6.7 in those with BMI $\geq$25 kg/m^2 had to participate in the intervention program.
Conclusion
Lifestyle modification could result in a significant decrease in the incidence of Type 2 diabetes in an Iranian urban population. This effect was independent of the level of major diabetes risk factors.

Table 1. Comparison of risk factor changes overtime between treatment groups.

Risk factors	Percent of change after 3.5 years	
	Control	Intervention
Body weight	3.2 (0.1)	2.5 (0.1)*
BMI	2.6 (0.2)	2.4 (0.3)
Waist circumference	5.7 (0.1)	4.5 (0.2)**
Systolic blood pressure	−1.0 (0.1)	−0.5 (0.3)
Diastolic blood pressure	−2.8 (0.2)	−2.1 (0.3)
Fasting plasma glucose	3.3 (0.2)	0.6 (0.4)**
Two-hours plasma glucose	10.6 (0.3)	5.4 (0.3)**
Triglyceride	6.2 (0.4)	1.2 (0.4)**
HDL-cholesterol	−7.6 (0.2)	−6.1 (0.2)

Data are mean (S.D.) of percent of change over time. *, **: represent $P<0.01$ and $P<0.001$ respectively in comparison to the control group.

HTC2

Primary hyperparathyroidism (Nationwide cohort study): an increased risk of cancer and decreased survival

Sujoy Ghosh[1], Andrew Collier[1], David Clark[2], Tarik Elhadd[1] & Iqbal Malik[1]
[1]NHS Ayrshire & Arran Information Services Division, The Ayr Hospital, NHS National Services Scotland, Scotland, UK; [2]Information Services Division, NHS Ayrshire & Arran, NHS National Services Scotland, Scotland, UK.

Introduction
- The incidence of primary hyperparathyroidism (PHPT) is ~3.5/100 000 per year
- The incidence is on the rise due to increase in 'routine biochemical testing'
- Has been thought to be a 'relatively harmless' disorder.
- Some patients tend to undergo surgery, while others are conservatively treated and most followed up for a short period before discharge from follow up.

Aims
- Determine if risk of cancer is increased in patients with primary hyperparathyroidism (PHPT)
- Determine if mortality is increased in patients with primary hyperparathyroidism.

Methodology
- A cohort of patients with PHPT between 1981 and 2007 was identified from the Scottish morbidity records and linked to the Scottish cancer registry and Scottish mortality records.
- Patients with pre-existing cancers (or developing cancer within one year of diagnosis of PHPT) were excluded from the analysis of subsequent cancer risks.
- The prevalence of new cancers and deaths was identified in these patients.
- The results were then compared with the risk of cancer and mortality in the general population living in Scotland.

Outcome/results
- Total number of patients diagnosed with PHPT: 3039
- Females: 2350 (77.3%), Males: 689 (22.7%)
- Mean age of patients: 63.5 years
- Person years at risk of cancer: 22 710
- New cases of cancer: 440/2706
- Standardised incidence rate of cancer: (SIR): 2.026, 95% CI (1.841–2.224), $P<0.001$
- Person years at risk of death: 24 186
- Number of deaths: 1601
- Standardised Incidence Rate of deaths: (SIR): 3.085, 95% CI (2.936–3.240) $P<0.001$

Conclusions
- Individuals with PHPT have a two fold increased risk of developing subsequent cancer and a three fold increased risk of death as compared to general population.
- The findings of this study have enormous implications for management and follow up of patients diagnosed with primary hyperparathyroidism.

HTC3 – ESE Young Investigator Award

Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival

Guillaume Assié[1,2,7], Aurélien de Reyniès[6,7], David Rickman[6,7], Frédérique Tissier[1,3,7], Lionel Groussin[1,2,7], Fernande René-Corail[1,7], Bertrand Dousset[4,7], Xavier Bertagna[1,2,7], Eric Clauser[1,5,7] & Jérôme Bertherat[1,2,7]
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Diagnosing malignancy and assessing the prognosis of adrenocortical tumors is challenging. The aim is to identify molecular predictors of malignancy and of survival.
Patients and methods
Of 153 unilateral adrenocortical tumors were studied by microarray ($n=92$) or RT-qPCR ($n=148$). A 2-gene predictor of malignancy was built using the disease-free survival as the end-point in a training cohort ($n=47$), then validated in an independent validation cohort ($n=104$). The best candidate genes were selected using Cox models, and the best gene combination was validated using the log-rank test. Similarly, for malignant tumors, a 2-gene predictor of survival was built using the overall survival as the end-point in a training cohort ($n=23$), then tested in an independent validation cohort ($n=35$).
Results
Unsupervised clustering analysis discriminated the malignant and benign tumors, and identified two groups of malignant tumors with different outcome. Predictors based on gene expression levels were determined. The substraction DGL7-PINK1 was the best predictor of disease free survival (log-rank $P\approx10$–12), could overcome the uncertainties of intermediate pathological Weiss scores, and remained significant after adjustment to the Weiss score ($P<1.3\times10$-2). Among

the malignant tumors, the substraction BUB1B-PINK1 was the best predictor of overall survival ($P<2\times10\text{-}6$), and remained significant after adjusting for MacFarlane staging ($P<0.005$).
Conclusion
Gene expression analysis unravels two distinct groups of adrenocortical carcinomas. The molecular predictors of malignancy and of survival are reliable and provide valuable independent information in addition to pathology and tumor staging. These original tools should provide important improvements for adrenal tumors management.

HTC4 – ESE Young Investigator Award

Functional analyses of four novel ret germline mutations: juxtamembrane mutations display the highest level of autophosphorylation

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Germline activating mutations of the RET proto-oncogene are associated with inherited medullary thyroid cancer (MTC) and can be also detected in about 10% of apparently sporadic MTC cases. In the present study, 4 novel RET mutations, located in the extracellular domain (A510V, E511K and C531R) and in the intracellular juxtamembrane region (L666N), all identified by the genetic screening on sporadic MTC cases, are firstly reported and functionally characterized. RET Plasmids carrying Ret9-WT (the short isoform of protoRet gene) and RET mutants, obtained by site-direct mutagenesis, were transiently transfected in HEK cells. Ret9-C634R (the protoRet gene containing a MEN2A causing mutation) was used as positive control. The tyrosine phosphorylation level was evaluated by immunoprecipitation and Western blot analyses. The extracellular variants A510V, E511K and C531R were found to harbour an autophosphorylation higher than Ret9-WT, but significantly lower than Ret9-C634R. Differently, the L666N variant, located 8 residues downstream the transmembrane domain displayed a high kinase activity, similar to that observed with the Ret9-C634R mutant and consistent with a strong transforming activity.

In conclusion, functional analyses on four novel germline RET mutations are reported. Consistent with previous data on a complex mutation, the L666N variant is associated with a high constitutive activation indicating that alterations in the juxtamembrane region can strongly activate RET in a ligand independent manner and be associated with a phenotype of intermediate-high severity. Therefore, we advocate strict follow-up since early age for carriers of mutations in this novel 'hot' region. Finally, present data confirm the need to routinely perform the genetic screening for RET in apparently sporadic MTC and to extend the molecular analyses to regions other than the cysteine residues and other classical hot spots.

HTC5 – ESE Young Investigator Award

Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant

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Objective
To assess if weekly administration of 40 mg pegvisomant (PEG-V) improves quality of life (QoL) and metabolic parameters in acromegalic patients with already normal age-adjusted insulin-like growth factor-I (IGF-I) concentrations during long-acting somatostatin analog (SSA) treatment.
Design
Prospective investigator-initiated, double blind, placebo controlled, cross-over study. Twenty acromegalic subjects received for two consecutive treatment periods of 16 weeks either PEG-V or placebo, separated by a wash-out period of 4 weeks. Efficacy was assessed as change between baseline and end of each treatment period. QoL was assessed by the Acromegaly Quality of Life Questionnaire (AcroQoL™) and the Patient-assessed Acromegaly Symptom Questionnaire (PAQ5™).
Results
The AcroQoL ($P=0.008$) and AcroQol physical ($P=0.002$) improved significantly after PEG-V was added. The addition of PEG-V also significantly improved the PAQ5 ($P=0.038$) and the single PAQ5 questions, perspiration ($P=0.024$), soft tissue swelling ($P=0.036$) and overall health status ($P=0.035$). No significant change in *Z*-score of IGF-I ($P=0.34$) or the *Z*-score of serum IGF-I bioactivity ($P=0.667$) was observed during addition of PEG-V. The improved QoL in the AcroQoL physical was well correlated with weight loss ($r=0.46$, $P=0.04$) but not with change in *Z*-score of (bioactive) IGF-I. Transient liver enzyme disturbances were observed in five subjects (25%) and two patients also suffered from diabetes Mellitus.
Conclusion
Improvement in quality of life was observed without significant change in (the bioactivity of) IGF-I after the addition of 40 mg pegvisomant weekly to monthly long-acting SSA therapy in acromegalic patients who already did normalize in their IGF-I on SSA monotherapy. These data question our current insight in how to assess disease activity. Moreover, it questions the current step-up approach of medical treatment in which pegvisomant only has a role when SSA therapy has failed normalizing IGF-I.

Basic Highlights

Hot topics: Basic

HTB1

Characterization of a mouse model with mutagenesis induced hyperaldosteronism

Ariadni Spyroglou[1], Sibylle Wagner[2], Jenny Manolopoulou[1], Constanze Hantel[1], Martin Reincke[1], Martin Bidlingmaier[1], Martin Hrabé de Angelis[2] & Felix Beuschlein[1]
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Although primary aldosteronism (PA) is considered to be the most prevalent cause of secondary hypertension the underlying genetic mechanisms have been elucidated only for the rare familial forms of the disease. In an attempt to define novel genetic loci involved in the pathophysiology of PA a phenotype-driven mutagenesis screening after treatment with the alkylating agent *N*-ethyl-*N*-nitrosourea was established for the parameter aldosterone. The aldosterone values of more than 2800 F1 offspring of chemically mutated inbred C3HeB/FeJ mice were measured and compared to aldosterone levels from untreated animals. Persistent hyperaldosteronism (defined as levels +3 S.D. over the mean of untreated animals) upon repeated measurements was present in eight female and 1 male F1 offspring. Further breeding of these affected female animals gave rise to F2 pedigrees from which eight lines with different patterns of inheritance of hyperaldosteronism could be established. Affected animals served for a detailed phenotypic characterization which revealed low renin values, an increased aldosterone to renin ratio (unaffected: 1.83 ± 0.54 pg per ml per ng per ml per h versus affected: 5.9 ± 1.63 pg/ml per ng per ml per h, $P < 0.01$) and low potassium (unaffected: 5.44 ± 0.05 mmol/l versus affected: 4.78 ± 0.23 mmol/l, $P < 0.05$) in line with the presence of primary aldosteronism. In addition, the investigation of their cardiac phenotype showed increased collagen deposits and subsequently cardiac fibrosis (picric acid positive areas unaffected: $0.63 \pm 0.02\%$ versus affected: $2.00 \pm 0.14\%$, $P < 0.01$). Histological examination of their adrenal glands revealed a thicker zona glomerulosa (zona glomerulosa/zona fasciculata ratio unaffected: 0.23 ± 0.01 versus affected: 0.39 ± 0.02, $P < 0.001$) without evidence of adrenal tumors. On the molecular level affected animals showed a significant increase of Cyp11b2 expression (unaffected: $100 \pm 8\%$ versus affected: $649 \pm 76\%$, $P < 0.001$) which was accompanied by a significant downregulation of the genes Smoc, MTUS and Wnt4 in comparison to unaffected littermates. Ongoing SNP analysis will allow defining causative mutations to elucidate the molecular mechanisms of autonomous aldosterone secretion in the individual lines.

HTB2

Characterization of human adult stem cell populations isolated from subcutaneous and visceral adipose tissue

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White adipose tissue acts as an endocrine organ that secretes a variety of adipokines and coordinates a number of biological processes such as energy homeostasis, neuroendocrine and immune functions. Recent studies demonstrated that abundant adipose tissue depots (particularly visceral adipose tissue), by producing inflammatory cytokines, contribute to chronic low-grade inflammation processes which may underlie the pathogenesis of metabolic disorders such as obesity, atherosclerosis, insulin-resistance and diabetes. Functional differences in adipose tissue seem associated with the regional distribution of fat depots, in particular in subcutaneous and visceral omental pads.
The aim of our study was to obtain a human cell model that provides an useful system for the *in vitro* investigation of the pathophysiological processes leading to differentiation of mature adipocyte. For the first time we isolated human *a*dipose-derived adult *s*tem *c*ells from visceral and subcutaneous abdominal fat (V-ASC and S-ASC, respectively) from the same subject. Flow cytometry immunophenotyping shows that plastic culturing selects homogeneous cell populations of V-ASC and S-ASC sharing typical markers of mesenchymal stem cells. Electron microscopy, electrophysiological analysis of cell currents and quantitative real time RT-PCR analysis of the expression of stemness markers confirm the mesenchymal stem nature of both V-ASC and S-ASC. Similarly to S-ASC, when cultured in the appropriate inducing media, V-ASC can differentiate not only towards adipogenic, osteogenic and chondrogenic lineages, but also towards muscle and neuronal cells, as demonstrated by immunofluorescence, quantitative real time RT-PCR and electrophysiological analyses, suggesting the multipotency of such adult stem cells.
In conclusion both visceral and subcutaneous adipose tissues are a source of pluripotent stem cells with multi-germline potential. However, the visceral rather than the subcutaneous adipose-derived adult stem cell populations could represent a more appropriate *in vitro* cell model for investigating the molecular mechanisms implicated in the pathophysiology of metabolic disorders such as obesity.

HTB3

Functional relevance of MC3R and GHSR heterodimerization in hypothalamic weight regulation

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By a systematic approach we investigated the interaction of a selective number of GPCRs that are expressed in the *arcuate nucleus* and known to play an essential role in hypothalamic weight regulation. Based on the results of a sandwich ELISA and fluorescence resonance energy transfer (FRET) approach we report the interaction of the melanocortin three receptor (MC3R) and the growth hormone secretagogue receptor (GHSR) which are coexpressed on arcuate NPY/AgRP neurons. Furthermore, we demonstrated a co-localization of the heterologously expressed receptors on the cell surface of living cells by confocal laser scanning microscopy. Heterodimerization of unrelated receptors is well acepted today and examples implicate profound functional consequences. It is known that MC3R couple to the Gαs whereas GHSR couple to the Gαq signaling pathway. However, here we observed that co-expression of MC3R and GHSR profoundly increase cAMP-accumulation after melanocortin challenge, that is higher compared to MC3R activation alone. In-depth characterization of the new signalling properties of the MC3R/GHSR heterodimer revealed the activation of Gαi in the presents of both endogene agonists.
In summary, our results indicate a cross talk between the signaling pathways of the two hypothalamic receptors and adds to the understanding of the complexity of weight regulation. Maybe these findings provide an explanation for snacking between meals and the dicision to eat a second slice of the cake because cAMP rising in the NPY/AgRP neurons supported the expression of the neuropeptide Y.

HTB4

Mice deficient for the Sam68 RNA binding protein are protected from dietary obesity and insulin resistance

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The Src substrate associated in mitosis of 68 kDa (Sam68) is a KH-type RNA binding protein that has been shown to regulate several aspects of RNA metabolism. The tyrosine phosphorylation of Sam68 was shown to negatively regulate its RNA binding activity and hence it was termed a Signal Transduction Activator of RNA (STAR) protein. Although KH-type RNA binding domains are known to mediate specific protein-RNA interactions, their RNA targets remain elusive. Nevertheless, Sam68 has been shown to regulate HIV Rev nuclear export pathway, the constitutive transport element and alternative splicing. To define the physiological role of this RNA binding protein, we generated Sam68 deficient mice (Richard *et al.* 2005, PLOS Genetic 1:e74). Previously we reported that the Sam68 deficient mice were protected from age-induced bone loss (Richard *et al.* 2005, PLOS Genetic 1:e74) and delayed breast tumorigenesis (Richard *et al.* 2008 Oncogene 27:548). Here we report that homozygote $Sam68^{-/-}$ mice have a lower body and adipose tissue weight despite normal insulin sensitivity. These mice are protected against dietary obesity owing to deregulation in lipid metabolism associated with adipogenesis defects. Moreover, they remain insulin sensitive when placed on high-fat diet. We observed that the expression of peroxisome proliferator-activated receptor-γ (PPARγ) is reduced in $Sam68^{-/-}$ mice. Consistent with these results, differentiation ability of 3T3-1L preadipocyte cells depleted of Sam68 is attenuated for adipocyte differentiation. These results suggest that the Sam68 RNA binding protein is a novel regulator of adipogenesis and represents a new target to treat type II diabetes and obesity.

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HTB5

Mutations in a novel exon of the LH receptor gene cause male pseudohermaprhoditism

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Objectives
Male pseudohermaphroditism, or Leydig cell hypoplasia (LCH), is an autosomal recessive disorder in individuals with a 46, XY karyotype, characterized by a predominantly female phenotype despite the presence of testicular structures. It is caused by mutations in the luteinizing hormone/chorionic gonadotropin receptor gene (LHCGR), which impair either LH/CG binding or signal transduction. However, molecular analysis has revealed that the LHCGR is apparently normal in about 50% of patients with the full clinical phenotype of LCH. We therefore searched the LHCGR for novel genomic elements causative for LCH.
Methods and results
In the present study we have identified a novel, primate-specific bona fide exon (exon 6A) within the LHCGR gene. It displays composite characteristics of an internal/terminal exon and possesses stop codons triggering nonsense-mediated mRNA decay (NMD) in LHCGR. Transcripts including exon 6A are physiologically highly expressed in human testes and granulosa cells, and result in an intracellular, truncated LHCGR protein of 209 amino acids. We sequenced exon 6A in 21 patients with unexplained LCH and detected mutations in four patients. Functional studies revealed a dramatic increase in the expression of the mutated internal exon 6A transcripts, indicating aberrant NMD. These altered ratios of LHCGR transcripts result in the generation of predominantly nonfunctional LHCGR isoforms, thereby preventing proper expression and functioning.
Conclusions
The identification and characterization of this novel exon identifies a new regulatory element within the genomic organization of LHCGR, important for receptor regulation at the transcriptional level. Mutations in exon 6A can be causative for disorders of sexual development.
Supported by the German Research Foundation (GR 1547/6-2)

Debate

What to do next when Metformin does not work in Type 2 Diabetes?

D1.1

What to do next when metformin does not work in diabetes Type2? add SU

Valdis Pirags
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Monotherapy with metformin is widely accepted as the second step in type 2 diabetes treatment after the failure to achieve glycemic goals with therapeutic education and lifestyle modification, including correction of diet and physical exercise habits. However, most of patients need combination therapy in first three to four years after metformin initiation. Obviously the efficacy of antidiabetic drugs used as add-on to metformin monotherapy could be different from those observed in monotherapy. In most of short-term comparative studies sulphonylureas showed greater reduction of HbA1c than other oral antidiabetics, and had a similar efficacy as insulin. However, the sustainability of this combination is limited to first two to three years and depends from the baseline HbA1c value. Concern about the cardiovascular safety of metformin and sulphonylurea combination is still under debate, but several large outcome studies didn't show increased risk of this therapy. In conclusion, metformin and sulphonylureas are unable to prevent the long-term progression of hyperglycemia in most of type 2 diabetes patients. The tailoring of treatment to the individual patient remains the most important key to successful diabetes therapy.

D1.2

Add insulin

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People with type 2 Diabetes (T2D) develop severe chronic complication early in the course of the disease if not treated optimally. Although lifestyle intervention and metformin clearly improve metabolic control with metformin having well established safety profile, both become insufficient in most patients with T2D. To achieve the current goal of HbA1c $< 7\%$ additional medication are introduced. By current recommendations, basal insulin or sulfonylurea are added to lifestyle intervention and metformin in majority of patients, with intensive insulin therapy following when needed to maintain the target HbA1c. Alternatively, pioglitazone or GLP-1 agonist may be added to lifestyle and metformin in selected patients.
Data demonstrating the importance of regulating postprandial blood glucose early in the course of T2D may require modified clinical algorithms. Prandial insulin may be preferable for regulating postprandial blood glucose early in the course of T2D. Similarly, GLP-1 agonist may be beneficial in younger people with early stage T2D where weight reduction and regulation of the postprandial blood glucose can be primary goals.
Diversified clinical recommendations focusing on distinct sub-groups of people with T2D are warranted with more focus on regulating postprandial blood glucose, along with additional clinical trials to verify the emerging concepts.

D1.3

What to do next when metformin does not work in diabetes Type 2? add incretin

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Near-normoglycaemia shold be reached as safely as possible. It should be considered, that at lower HbA1c concentrations, the proportional contribution of postprandial glucose to HbA1c is greater than at higher HbA1c values.
Sulfonylureas, glinides and insulin are associated with an increased risk for hypoglycaemia and weight gain. Therefore, these agents should not be considered in first line for the combination therapy in overweight patients with Type 2 diabetes and metformin monotherapy failure. Sulfonylureas and glinides as insulin secretagogues act glucose-independently and have a disadvantage compared to the novel incretin based therapies that are safe regarding hypoglycaemia and weight development. The only advantage of the sulfonylureas may be their low cost, but this has to be outweighed against the costs for more frequent blood glucose testing and the costs caused by severe hypoglycaemic events. Insulin can be dosed in a manner to lower glycaemic parameters to any desired goal, but also has the above mentioned limitations regarding weight and hypoglycaemias.
Acarbose has lowered cardiovascular events in IGT and in Type 2 diabetic patients. Gastrointestinal side effects are a barrier to a broad use of this compound.
Glitazones are also associated with weight gain and with fluid retention. Cardiovascular safety and the incidence of bone fractures have been discussed recently in spite of the positive cardiovascular data of the PRO-Active study.
A safe antihyperglycemic treatment not leading to hypoglycaemia and weight gain may be favourable, especially in patients with HbA1c values in the range below 7.5%, where postprandial hyperglycemia contributes to a higher degree to the HbA1c reduction. Here, the incretin based therapies may become an attractive treatment option especially for overweight patients with Type 2 diabetes.
In general, however, we will need long-term intervention studies to investigate the durability of the effect of the novel drugs and their effect on vascular outcomes and hard endpoints. These studies will have to be very large and will need to have a long duration to clarify the open questions that still remain.

Oral Communications

Endocrine Tumours

OC1.1

Optimizing time of prophylactic surgery in ret gene carriers on the basis of serum calcitonin

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Multiple endocrine neoplasia type 2 (MEN 2) is characterized by the presence of medullary thyroid cancer (MTC) and other benign pathologies. RET mutations are responsible of this disease and their screening is a very sensitive tool for the identification of gene carriers (GC).
Aim of this study was to verify the relevance of the basal and pentagastrin (Pg) stimulated serum calcitonin (CT) in the decision to perform TT in GC. We reviewed data of 65 GC found among 807 subjects screened for RET mutations. Twenty GC were negative for both basal and stimulated CT and, following our indications, did not undergo surgery. Thirty-five patients underwent TT on the basis of detectable levels of basal and/or stimulated CT. Twenty-one cases had an undetectable basal serum CT while 14 cases had detectable basal CT (15–922 pg/ml). All cases with undetectable basal CT levels or if detectable less than 60 pg/ml showed only C cell-hyperplasia ($n=5$) or microfoci of MTC without node metastases ($n=22$). Only cases with basal CT higher than 60 pg/ml ($n=8$) showed either small MTC associated with node metastases ($n=4$) or bigger MTC with or without node metastases ($n=4$). Six GC with positive Pg-test refused TT and 4 are under evaluation. The correlation with the RET mutation showed that all GC with a cysteine mutation had a detectable basal and/or a Pg stimulated CT while no cysteine mutations were found among the 20 GC with undetectable values of basal or stimulated CT.
In conclusion, our data indicate that basal and stimulated serum CT plays an important role in taking the decision to perform TT in GC: the positivity of the Pg-test can safely suggest when TT should be performed and avoid to treat GC at very young age when surgical complications are more frequent and more difficult to manage.

OC1.2

Long-term outcome of laparoscopic versus open adrenalectomy for adrenocortical carcinoma

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Introduction
Surgery is the established first line treatment in adrenocortical carcinoma (ACC). For benign adrenal tumours, laparoscopic adrenalectomy (LA) has become the treatment of choice. However, the role of LA in ACC remains highly controversial. Data from the German ACC Registry were used to evaluate the technical feasibility of LA in ACC and to compare the long-term outcome after LA with the results of open adrenalectomy (OA).
Methods
Out of 521 patients in the German ACC Registry, we identified 135 patients with ACC stage I–III, a tumour size ≤ 10 cm, and a follow up time of at least 6 months. In this group 27 patients underwent LA. These patients were matched with regard to tumour size (mean diameter 7.0 vs 6.5 cm), tumour stage, adjuvant therapy, age, sex, and endocrine activity with 27 patients who underwent OA. Median follow-up was 35 months (6–138 months).
Results
The 5-year overall survival (OS) of the entire cohort of 54 patients was 62%. Time to recurrence (TTR) and OS did not differ significantly between LA and OA (TTR median 22 vs 15 months; HR 1.2; $P=0.5$; OS 40 vs 34 months; HR 1.7; $P=0.2$). In 11/27 patients, laparoscopic surgery had to be converted to an open procedure. Conversion had no negative impact on OS ($P=0.4$).
Conclusions
This is by far the largest series on LA in ACC and the first including matched controls. In contrast to previous concerns, our study indicates that LA in ACC patients with a tumour size ≤ 10 cm does not lead to earlier recurrence or inferior survival when compared to OA. Our data justify a randomized trial in selected patients with ACC comparing the two surgical approaches. However, due to the rarity of ACC such a trial is challenging and unlikely to be performed in the foreseeable future.

OC1.3

131I-Iodometomidate radiotherapy for metastatic adrenocortical carcinoma: first clinical experience

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Subject
Treatment options for adrenocortical carcinoma (ACC) are still unsatisfactory. We could recently demonstrate that several patients with ACC exhibit a high and specific uptake of 123I-Iodometomidate (123I-IMTO). Therefore, we investigated if 131I-IMTO holds potential for radiotherapy in ACC.
Methods
Dosimetry with 40 MBq I-131-IMTO was performed over 5 days in 7 patients and calculated activities were administered on a compassionate use basis in 6 patients with ACC.
Results
Follow up data are available in 3 patients so far. The bone marrow proved to be the critical organ. Postulating that the bone marrow dose does not exceed a tolerable dose of 2 Gy, high therapeutic activities of up to 20 GBq were calculated. Patients received between 7 and 20 GBq 131I-IMTO as treatment dose. A high and lasting uptake was observed in almost all metastases. Corresponding to the values calculated from dosimetry, tumour doses up to 41.3 Gy were reached. Elimination of I131-IMTO from whole body showed a half life of 20 h. In all patients treatment was very well tolerated. Transient thrombocytopenia and leucopenia was observed in most patients. At follow up patient 1 showed a decrease of the size of those lesions that had shown tracer uptake. Patient 2 demonstrated a lasting decrease in ^{F18}FDG-uptake, however metastatic lesions slightly increased in size. Patient 3 had stable disease after 10 weeks follow up.
Conclusions
This is the first report of radiotreatment of metastatic ACC with 131I-IMTO. Due to the high specificity of tracer uptake high activities can be achieved within the target tissue, comparable to those achieved by other radionuclide treatment regimens. Treatment is well tolerated. However, this method has to be further evaluated to better estimate its clinical value in treatment of ACC.

OC1.4 – ESE Young Investigator Award

MicroRNA expression profiling and target prediction in adrenocortical tumors

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Introduction
MicroRNAs (miRNA) are non-coding RNA molecules involved in the posttranscriptional regulation of gene expression. MiRNAs bind mRNA molecules at their 3′ untranslated regions and induce translational repression or target degradation. MiRNAs play important roles in the pathogenesis of several neoplasms. There are no reports, however, on the possible involvement of miRNAs in the pathogenesis of adrenocortical tumors.
Objective
To study simultaneous miRNA and mRNA expression patterns in normal, hormonally inactive, cortisol-secreting benign and malignant adrenocortical tissues (ACC), and to identify major pathogenetic pathways by bioinformatics analysis.
Methods
Thirty-two tissue samples were studied approved by the Ethical Committee of the Hungarian Health Council. MiRNA and mRNA expression profiling was performed in 16 samples by TLDA Human MiR Panel and whole genome microarray platform, respectively. Results were further validated and sample sizes were extended by qRT-PCR. Tissue-specific miRNA target prediction was

achieved by an integrative bioinformatics method. Ingenuity Pathway Analysis (IPA) was used as a system biology approach.
Results
Twenty-six miRNAs with significant expressional differences were identified. Further validation was performed for 14 miRNAs. Expression of miR-214, miR-375 and miR-511 were significantly lower, whereas that of miR-184, miR-210 and miR-503 was significantly higher in ACCs in comparison with their normal and benign counterparts. By performing parallel mRNA expression profiling, we tried to achieve a tissue specific target prediction approach. Pathway analysis of the predicted targets with inverse expressional alterations as compared with their potential regulator miRNA revealed the possible involvement of cell cycle damage at G2/M checkpoint (CDC25, RPRM), along with already described mechanisms (e.g. TOP2A, CCNB2) in the pathomechanism of ACC.
Discussion
MiRNA expression patterns are significantly different in normal and neoplastic adrenocortical tissues. Beside their possible pathogenetic relevance, miRNA patterns may also be exploited in diagnostics, e.g. as an adjunct to histological diagnostics or for the determination of prognosis.

OC1.5

Bone morphogenetic protein 2 and 5 are down-regulated in adrenocortical carcinoma and modulate adrenal cell proliferation and steroidogenesis

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Bone morphogenetic proteins (BMPs) have been demonstrated to impact tumorigenesis in a variety of tumors. As for the adrenal cortex, quantitative real time PCR analyses revealed down-regulation of various BMPs (e.g. BMP2, BMP5) in tissue samples from adrenocortical carcinoma and adrenocortical tumor cell lines in comparison to normal adrenal glands. Other members, by contrast, as seen for BMP6 remained unaltered. Integrity of BMP dependent pathways in the investigated cell lines could be demonstrated by activation of the Smad1/5/8 pathway with subsequent increase of ID protein expression upon incubation with BMP2 or 5. On a functional level, BMP incubation resulted in inhibition of cell proliferation and viability in a dose- and time-dependent manner. Notably, BMP2 (50 ng/ml) and BMP5 (100 ng/ml) treatment also reduced viability of cells, which were co-incubated with the insulin-like growth factor (IGF1, 13 nM), a crucial mitogen of the adrenal and activator of the AKT pathway, by 17 and 40%, respectively. We further analyzed potential cross-signalling of BMPs with IGFs and detected a BMP dependent reduction of AKT phosphorylation under baseline conditions and under IGF co-stimulation. Furthermore, BMPs influenced steroidogenic function, whereas BMP treatment reduced MC2-R and steroidogenic enzyme expression which was accompanied by reduced aldosterone, cortisol and DHEA-S secretion. Notable, effects were more pronounced under forskolin co-treatment. Moreover, *in vitro* demethylation treatment resulted in re-activation of BMP dependent pathways with concomitant modulation of steroidogenesis. Taken together, we demonstrate that loss of expression of members of the BMP family of ligands is a common finding in adrenocortical tumors and we provide evidence that BMP dependent pathways are likely to be involved in modulation of the malignant and functional phenotype of adrenocortical cancer cells.

OC1.6

Implication of the Wnt/β-catenin signaling pathway activation in the adenoma and in the malignant adrenocortical cancer

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The commonly expressed multifunctional protein and protooncogene β-catenin displays important functions in cell–cell adhesion and in the Wnt signaling pathway. Transforming activity of the Wnt/β-catenin pathway is thought to be involved in the development of a variety of human cancers and many β-catenin target genes have been shown to play critical functions in tumors. Therefore, the aim of this project was to determine the expression levels and cellular distribution of active β-catenin and its target genes cyclin D1 and NrCAM a neuronal cell adhesion molecule in adrenocortical tumors. In this study, we performed the immunochistochemistry (IHC) for 13 and 31 patients with adenocortical adenoma (ACA) and carcinoma (ACC), respectively to evaluate the expression levels and subcellular localization of cyclin D1 and NrCAM. At the invasion front of 30/31 ACC cases strong active β-catenin expression was found in the nuclei and cytoplasm of tumor cells and weak expression at the central part of the tumor. Under these conditions, β-catenin can function as a transcription factor and thus activate target genes, among them cyclin D1-cell cycle progression protein and neuronal cell adhesion molecule NrCAM – protein involved in the adhesion and signaling. Cyclin D1 low (>5%) to absent in all adrenal tumors. In normal adrenal gland distinct expression of NrCAM was seen in the central part of the cortex and weak on the periphery. In the ACA moderate cytoplasmic NrCAM l was seen through the tumor whereas the in ACC expression level of NrCAM protein was very strong at the invasion front of the cancer in contrast to central part with faint reactivity, suggesting its translocation. Moreover, NrCAM expression was not related to the primary tumor stage or the size. These data suggest that NrCAM, neuronal cell adhesion molecule could be implicated in the pathogenesis and behavior of adrenal tumors.
Grant CMKP 501-1-1-22-01/07.

Diabetes & Obesity

OC2.1 – ESE Young Investigator Award

A novel gene therapy strategy involving immune-modulation relinquished lymphocyte infiltration into islet grafted sites in STZ-induced diabetic rats

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Background
Because type 1 diabetes (T1D) results from the T cell-mediated destruction of the insulin-producing pancreatic beta cells, the depletion of the autoreactive T cells via apoptosis represents a viable strategy for the prevention of autoimmune diabetes. The functional role TNF Related Apoptosis Inducing Ligand (TRAIL), a novel member of TNF superfamily, in autoimmune diabetes remains unknown. To understand this, TRAIL function was counteracted by an injection of soluble TRAIL receptor into NOD mice, which enhanced the degree of autoimmune inflammation in pancreatic islets and facilitated the onset of diabetes. Second, the delivery of multiple low-doses of STZ into TRAIL-deficient mice resulted in a higher degree of islet inflammation and an earlier onset of diabetes. All these results suggested that exogenous TRAIL expression in pancreatic islets may have beneficial results in the setting of type 1 diabetes by virtue of its potential to retaliate against the assault by CTL.
Methods
Fluorometric measurements revealed optimum doses of adenovirus vectors to transduce pancreatic islets. Ad5hTRAIL vector was used to overexpress TRAIL in islet cells. Cytotoxicity of TRAIL overexpression was assessed using Annexin V staining. Ad5hTRAIL or AdLacZ-transduced rat pancreatic islets were transplanted under the kidney capsule of STZ-induced diabetic rats. The diabetic status after islet transplantation was followed up for 90 days.
Results
No adverse event of TRAIL overexpression was detected in islet cells *in vitro*. Forty mg/kg per BW of STZ derived from the consideration of both blood glucose levels and survival rates, successfully induced T1D in rats. Histopathologic analysis of our transplantation set up demonstrated that non-infected and AdLacZ-infected islet grafts were heavily infiltrated with mononuclear cells following transplantation. In contrast, Ad5hTRAIL infected islets displayed non/minimal mononuclear cell infiltration.
Conclusion
Only adenovirus mediated TRAIL gene delivery suppressed lymphocyte infiltration in islet transplanted sites and prolonged normoglycemia in STZ induced diabetic rats.

OC2.2

Thymocyte migration is impaired in NOD mice: combined role of extracellular matrix and chemokines

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The NOD (non-obese diabetic) mouse remains the best experimental model of type I diabetes. We have previously described several thymic dysfunctions in these animals, including the presence of giant perivascular spaces (PVS) with arrest of mature T cells (including Treg cells) and partial impairment on fibronectin/VLA-5-dependent NOD thymocyte migration. Herein, we further studied the role of extracellular matrix (ECM) ligands, alone or in combination with the chemokine CXCL12 in NOD thymocyte migration. Intrathymic contents of CXCL12, fibronectin and laminin were evaluated by immunohistochemistry and the expression of corresponding receptors was assessed by flow cytometry. Thymocyte migration was assessed by transwell chambers and transendothelial migration evaluated through an endothelial cell monolayer. NOD thymocytes expressed much lower VLA-5 than C57BL/6 thymocytes. This defect was particularly severe in $CD4^+$ thymocytes expressing Foxp3, thus in keeping with the arrest of $Foxp3^+$ cells within the NOD giant PVS, as defined by immmunohistochemistry. Accordingly, lower percentages of NOD Treg cells were observed in the spleen and subcutaneous lymph nodes. We also observed an enhancement in CXCL12, laminin and fibronectin deposition and co-localization in the NOD thymus. Furthermore, we detected altered expression of the CXCL12 receptor CXCR4 and the laminin receptor VLA-6, as well as enhanced migratory capacity of NOD thymocytes towards these molecules, combined or alone. Moreover, transendothelial migration of NOD thymocytes was diminished in the presence of exogenous fibronectin. Our data unravel the existence of multiple cell migration-related abnormalities in NOD thymocytes, comprising both down- and up-regulation of specific migratory responses. It remains to be demonstrated if these events are correlated to the appearance of autoimmunity in NOD mice.

OC2.3

Central ghrelin administration reduces starvation-induced inflammation in rats

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The aim of the study was to investigate the influence of intracerebroventricular (ICV) injections of ghrelin on secretion of pro-inflammatory (TNF, IL-1, IFN-γ) and anti-inflammatory cytokines (TGF-β) in starved rats. Male Wistar rats (4 weeks old, 12 per group) were fed *ad libitum* or starved by caloric restriction (40% of chow consumed by their *ad libitum* fed controls from previous day) for 4 weeks. Afterwards, half of the animals in each group received ICV injections of ghrelin in PBS (0.15 nmol in 5 µl of PBS, daily for 5 consecutive days) or PBS alone. Rats were sacrificed two hours after the last injection, their blood was collected and serum concentrations of cytokines, ACTH and corticosterone were measured by ELISA. The concentrations of the pro-inflammatory cytokines TNF, IL-1 and IFN-γ were significantly increased in starved compared to rats fed *ad libitum* ($P<0.01$), while the levels of the anti-inflammatory TGF-β did not significantly differ between the two groups ($P>0.05$). The ICV application of ghrelin significantly reduced the blood levels of all three pro-inflammatory cytokines ($P<0.05$), while not affecting those of TGF-β. The observed anti-inflammatory effect of ICV applied ghrelin in starved rats was accompanied by activation of the hypothalamo–pituitary–adrenal (HPA) axis, reflected in the increase in serum levels of both ACTH and corticosterone ($P<0.05$). These results indicate that central ghrelin application might suppress starvation-induced systemic inflammation through activation of HPA axis and subsequent release of the anti-inflammatory corticosterone.

OC2.4

Mc2 receptor in adipocytes is significant for lipid composition and regular lipolysis

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The melanocortin system is significant for energy homeostasis and receptors have distinct tissue specific expression. The melanocortin 2-receptor (Mc2r) transmits ACTH dependent signalling in the adrenal cortex. Increased expression in adipocytes during differentiation indicates relevance for lipid homeostasis. Mc2r activation in adipocytes results in increased lipolysis, however, implication compared to norepinephrine (NE) stimulated lipolysis is unknown. To further define functional significance of Mc2r dependent pathways for adipocyte physiology we used an *in vitro* system of stably expressing shRNA in order to knock down Mc2r expression in differentiated 3T3 L1 adipocytes. Using the pSiren retro-virus system 2 of 4 tested shRNA sequences reduced Mc2r expression in differentiated adipocytes by at least 75%. Knock-down (kd) cell lines showed less lipid accumulation. In parallel, ACTH and NE stimulated lipolysis were substantially reduced (Control versus Kd as compared to respective baseline: 1 nM ACTH, 174 ± 22 vs $108\pm9\%$, $P=0.028$; 10 nM ACTH, 231 ± 29 vs $147\pm8\%$, $P=0.027$; NE 1 µM, 560 ± 100 vs $155\pm26\%$, $P=0.007$) demonstrating functional significance of Mc2r kd on the lipolysis pathway. The expression of differentiation markers like PPARγ2, aP2 and preadipocyte marker Pref1 was not significantly different between groups, as well as the expression of Elovl5 and Elovl6, fatty acid synthase, and fatty acid desaturase 1 and 2. Interestingly, the expression of stearoyl-Coenzyme A desaturase 1 and 2 was significantly reduced in kd cells (21 ± 8 vs $100\pm13\%$, $P=0.01$ and 32 ± 3 vs $100\pm15\%$, $P=0.046$). Gas chromatography was used to analyse lipid composition. Preliminary results indicate changed distribution of saturated vs. unsaturated fatty acids. In summary, Mc2r might play an important role in regular lipid accumulation. Moreover, changes in lipid composition indicate that Mc2r function has an impact on saturation of fatty acids.

OC2.5

Regular aerobic activity attenuates caspase-3 activity, oxidative stress, and progression of diabetic nephropathy in *db/db* mice, independent of hyperglycemia

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Diabetic nephropathy, the leading cause of end-stage renal disease, is characterized by a pro-apoptotic and pro-oxidative environment. The mechanisms by which lifestyle interventions, such as exercise, benefits diabetic nephropathy are unknown. We hypothesized that exercise inhibits early diabetic nephropathy via attenuation of the mitochondrial apoptotic pathway and oxidative damage. Type 2 diabetic *db/db* and normoglycemic wild type mice were exercised for an hour everyday at a moderate intensity for 7 weeks, following which renal function, morphology, apoptotic signalling and oxidative stress were evaluated. Exercise reduced body weight, albuminuria, and pathological glomerular expansion in *db/db* mice independent of hyperglycemic status. Changes in renal morphology were also related to reduced caspase-3 (main effector caspase in renal apoptosis), caspase-8 (main initiator caspase of the 'extrinsic' pathway) activities and TNF-α expression. A role for the mitochondrial apoptotic pathway was unlikely as both caspase-9 activity (initiator caspase of this pathway) and expression of regulatory proteins such as Bax and Bcl-2 were unchanged. Kidneys from *db/db* mice also produced higher levels of superoxides and had greater oxidative damage concurrent with downregulation of superoxide dismutase (SOD) 1 and 3. Interestingly, although exercise also increased superoxides, there was a concurrent upregulation of multiple SODs that likely inhibited lipid (hydroperoxides) and protein (carbonyls and nitrotyrosine) oxidation in *db/db* kidneys. In conclusion, exercise can inhibit progression of early diabetic nephropathy independent of hyperglycemia. Reductions in caspase-3 and caspase-8 activities, with parallel improvements in SOD expression and reduced oxidative damage, may underlie the beneficial effects of exercise in diabetic kidney disease.

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Reproduction/Stress/Endocrine Disruptors

OC3.1

Gapapentin for the treatment of hot flushes in women with natural or tamoxifen-induced menopause: a systematic review and a meta-analysis

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Context
Evidence suggests that gabapentin, a γ-aminobutyric acid analogue, is effective in the treatment of hot flushes in women with natural or tamoxifen-induced menopause.
Objective and design
To investigate the efficacy and tolerability of gabapentin for the treatment of menopausal hot flushes, a systematic review of all pertinent trials and a meta-analysis of those that were randomized placebo-controlled (RCT) were performed.
Data sources and extraction
Literature search was conducted independently by two reviewers through MEDLINE, EMBASE, CENTRAL (all three from inception to June 2008); the perusal of references from relevant studies; a personal contact with experts. Percent (%) change in hot flush frequency and composite score were used as primary outcome measures. Dropout rates and incidence of common adverse effects, such as dizziness, somnolence and fatigue, were also investigated. Seven trials, reporting data on 821 subjects, were reviewed and four RCTs included in the meta-analysis.
Data synthesis
Women assigned to gabapentin demonstrated a significantly greater percent (%) reduction in both hot flush frequency (WMD (95% CI)=23.7 (16.5 to 31.0), $P<10^{-6}$) and composite score (WMD (95% CI)=27.3 (21.2 to 33.2), $P<10^{-6}$), yet with significant between-study heterogeneity ($I^2=97.8$ and 95.6%, respectively). Dropouts due to adverse effects were more frequent in womens randomized to gabapentin compared to controls (Relative Risk (RR) (95% CI)= 2.1 (1.1 to 3.9), $P=0.02$, $I^2=0\%$). Risk of 'dizziness/unsteadiness' and 'fatigue/somnolence' clusterings was also found significantly higher in the treatment group (RR (95% CI)=6.9 (3.2 to 15.1), $P<10^{-6}$, $I^2=63.1\%$ and RR (95% CI)=4.8 (2.2 to 10.3), $P<10^{-4}$, $I^2=0\%$, respectively).
Conclusions
A 20–30% reduction in hot flushes frequency and severity could be anticipated with the use of gabapentin compared to placebo, although data across studies seem too heterogeneous to provide a reliable summary effect. Further investigation is needed to provide conclusive evidence. The clusterings of dizziness/unsteadiness and fatigue/somnolence are the more common adverse effects associated with gabapentin, that can lead to reduced compliance.

OC3.2

Variants in the *ACVR1* gene are associated with AMH levels in women with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is characterized by anovulation, hyperandrogenemia, and polycystic ovaries. Although the etiology of PCOS is poorly understood, the common denominator is a disturbance in the selection of the dominant follicle. TGFβ family members, such as anti-Müllerian hormone (AMH) and bone morphogenetic proteins (BMPs), suppress FSH sensitivity. Therefore their signaling pathway may contribute to the aberrant follicle development in PCOS women. We have investigated the role of ALK2, a type I receptor for AMH and BMPs, in PCOS using a genetic approach.
Seven single nucleotide polymorphisms (SNPs) in the *ACVR1* gene, encoding ALK2, were genotyped in a large cohort of Caucasian PCOS women ($n=359$). A cohort of 30 normo-ovulatory women and a population-based cohort of 3543 postmenopausal women served as controls.
Allele frequencies for the seven *ACVR1* tagging SNPs were similar in PCOS women and controls. However, polymorphisms rs1220134, rs10497189 and rs2033962 were associated with AMH levels in PCOS women ($P=0.001$, 0.002 and 0.007, respectively). For each of these polymorphisms, carriers of the minor allele had respectively 30, 70 and 34% higher AMH levels compared with carriers of the major allele. Polymorphism rs10497189 was also associated with follicle number ($P=0.001$). Adjustment for follicle number revealed that the association with AMH levels was, in part, independent of follicle number (rs1220134, $P=0.007$). Consistent with the individual markers in haplotype block 1 (rs1220134 and rs10497189), the haplotypes TT and AC of this block were associated with serum AMH levels ($P=0.001$ and 0.002, respectively) and follicle number ($P=0.01$ and 0.001, respectively). No associations were observed between the different *ACVR1* genotypes and LH, FSH, androgen and estradiol levels in the PCOS cohort.
In conclusion, genetic variation within *ACVR1* is associated with follicle number and AMH levels in PCOS women, suggesting that ALK2 signaling contributes to the disturbed folliculogenesis in PCOS patients.

OC3.3 – ESE Young Investigator Award

Variable phenotype of PROKR2 and PROK2 mutations in central hypogonadism

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Idiopathic central hypogonadism (ICH) is a rare and heterogeneous disease due to defects of GnRH secretion or action. Depending on the association with a normal or defective sense of smell, ICH could be respectively identified as normosmic ICH (nICH) or Kallmann's syndrome (KS). Recent experimental evidences indicate the involvement of the new PROK2/PROKR2 pathway in GnRH neuron maturation and function and mutations affecting these two genes have been described in some ICH cases. We analyzed by direct automatic sequencing the genes encoding the ligand PROK2 and its related G protein-coupled receptor (PROKR2) in a series of ICH patients: 19 KS (16M, 3F) and 31 nICH (26M,5F). Only one case is familiar, while all other presented as sporadic. We found 4 new (15fsX45, V158I, T260M, V334) and 1 known mutation (20fsX43) in PROKR2 gene and 1 new mutation (G62D) in PROK2. These variations are present in the heterozygous state in the patients according to the reported mechanism of haploinsufficiency. While most of the carriers of these mutations exhibited typical ICH manifestations, two of them presented a particular phenotype. The nonsense mutation 15fsX45 was found in a nICH male patients, who was diagnosed with delayed puberty at 18 years and was then put on testosterone treatment. After 6 years, the medication was discontinued for revaluation and the patient presented a reversal of the ICH phenotype, with a spontaneous normal secretion of LH/FSH and testosterone. The mutation 20fsX43 was instead found in a 58 years old man who was referred to us for obesity accompanied by loss of libido. He reported a normal pubertal development at 13 years and fathered 2 daughters.
At physical examination, he presented signs of normal sexual development and testes volume. Biochemically, he had a typical nICH hormone profile in the absence of any traumatic brain injury and hypothalamic–pituitary lesion at MRI. These two particular cases demonstrate the extreme variability in the expression of PROKR2 heterozygous mutations and the existence of ICH cases with adult onset that have a recognized genetic origin.

OC3.4

Reference ranges for sex hormone-binding globulin and free testosterone index in adult men

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Objective
The majority of circulating testosterone is bound to sex hormone-binding globulin (SHBG), but also to albumin and cortisol-binding globulin. The remaining part is free-circulating testosterone unattached to serum proteins, which represents the active form of the hormone. A common measurement of the free testosterone is the calculated free testosterone index (FTI)=100*(total testosterone/SHBG).

Testosterone is the principal male sex hormone and is involved in the regulation of fertility, libido, and muscle mass. The objective of the present study was to calculate age-specific reference ranges for serum SHBG and free testosterone index (FTI) using quantile regression.
Methods
From the cross-sectional population-based Study of Health in Pomerania (SHIP) 806 healthy men were included in the analyses. Serum testosterone and SHBG levels were measured using a competitive chemiluminescent enzyme immunoassay on an Immulite 2500 analyzer. All data were weighted to adjust for non-response and reflect age–sex distribution of the European adult population.
Results
The use of quantile regression provided exact reference ranges. Nearly nearly 5.0% of subjects (equal for SHBG and FTI: above 2.3%; below 2.4%) with SHBG levels as well as FTI values outside the reference range were detected.
Conclusion
The present study established age-specific reference ranges for serum SHBG and FTI levels. Previous studies of our research group (1, 2) regarding reference ranges showed that quantile regression should be preferred to calculate reference ranges because a better concordance to original data is possible due to no distribution assumption are required and the robustness against outliers. These results confirmed this suggestion.

OC3.5

The xenoestrogen bisphenol A inhibits postembryonic vertebrate development by antagonizing gene regulation by thyroid hormone

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Bisphenol A (BPA) is an environmental estrogen that is globally used in the production of plastics. Studies on BPA as an endocrine disruptor have focused on its estrogenic activity *in vitro* and in adult animal models. Recently, the National Toxicology Program at the US National Institutes of Health raised concerns for the neural and behavioral effects of BPA in fetuses, infants, and children at the currently allowed human exposures. However, the difficulty in studying uterus-enclosed mammalian embryos has hampered the analysis of the effects of BPA on vertebrate development. *In vitro* studies have suggested that BPA can bind to and antagonize thyroid hormone (T3) activation of T3 receptor (TR). Because small perturbations in T3 homeostasis can severely and adversely affect human health and development, we propose the use of *Xenopus laevis* metamorphosis as a model to study the effect of BPA on T3-dependent vertebrate development. Amphibian metamorphosis requires T3 and encompasses the postembryonic period in mammals (a few months before and several months after birth in human) when T3 action is most critical. We show here that BPA inhibits T3-induced metamorphosis of *Xenopus laevis* by blocking T3-dependent gene regulation pathway. Importantly, microarray analysis also indicates that most of the genes affected by BPA are T3-response genes, suggesting that BPA mainly affects T3- but not the estrogen-signaling pathways during metamorphosis. Our finding that this endocrine disruptor well known for its estrogen-like activity *in vitro* functions to inhibit T3-pathway to affect vertebrate development *in vivo* thus not only implicates potential deleterious effects of BPA on human embryonic development but also demonstrates the importance of studying endocrine-disruption in a developmental context *in vivo*.

OC3.6

Cortisol as a prognostic marker of outcome in acute ischemic cerebrovascular events

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Background
Stroke is the second commonest reason of mortality worldwide and a major cause of long term disability. Early prediction of outcome is important for allocation of therapeutic strategies. Endocrine alterations of the hypothalamus–pituitary–axis (HPA) are one of the first stress-induced alterations after cerebral ischemia. We evaluated the prognostic value of cortisol for outcome in acute ischemic cerebrovascular events.
Method
In an observational study including 362 patients with an ischemic stroke cortisol was measured on admission. We compared its prognostic accuracy to the National Institute of Health Stroke Scale Score (NIHSS) and to other known predictors with respect to functional outcome (as assessed by the modified Ranking scale) and mortality.
Results
Patients with an unfavorable outcome and non-survivors had higher cortisol levels on admission compared to patients with a good outcome and survivors, respectively (582 nmol/l (439–727) vs 444 nmol/l (318.5–585.5) and 681 nmol/l (573–1082) vs 466 nmol/l (337–598); $P<0.0001$ and $P<0.0001$). The area under the receiver operating characteristics (ROC) curve to predict mortality for cortisol with an AUC of 0.81 (0.76–0.86) was in the range of the NIHSS with an AUC of 0.85 (0.8–0.89). Cortisol had a higher prognostic accuracy as compared to glucose (AUC 0.59 (0.53–0.66), $P=0.002$), white blood count (WBC) (AUC 0.66 (0.53–0.67), $P=0.004$) and Charlson co-morbidity index (CCI) AUC 0.59 (0.53–0.65), $P=0.007$). In univariate logistic regression analysis, cortisol was a significant predictor of death and functional outcome with unadjusted ORs of 1.004 (95% CI 1.003–1.006) and 1.003 (95% CI 1.001–1.004). After adjusting for age, blood pressure, CRP, glucose, temperature and gender, cortisol remained an independent outcome predictor with adjusted ORs of 1.005 (95% CI 1.002–1.008) and 1.002 (95% CI 1.001–1.004).
Conclusion
Cortisol is a prognostic marker to predict functional outcome and death in patients with an ischemic stroke, comparable to the NIHSS.

Acromegaly/IGF1/Type 2 Diabetes

OC4.1

High-dose octreotide LAR in patients with acromegaly inadequately controlled by conventional somatostatin analogue therapy: a randomized, controlled trial

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Objective
In acromegaly, 25–50% of patients remain uncontrolled with conventional somatostatin analogue (SA) therapy. Evidence suggests that response may be improved by increasing the dose or frequency of administration of SAs. This study evaluated the efficacy and safety of octreotide LAR administered at a high dose or high frequency in patients with acromegaly.
Methods
This was a 24-week prospective, multicenter, randomized, open-label trial in patients with active acromegaly despite $\geq$6 months' conventional maximal-dose SA therapy. Patients had baseline GH $>$ 2.0 μg/l, elevated IGF-I for age/sex-matched controls and had a $\geq$50% reduction in GH during previous SA treatment. Patients were randomized to receive high-dose (60 mg/28 d; $n=11$) or high-frequency (30 mg/21 d; $n=15$) octreotide LAR for 24 weeks. The primary endpoint was change from baseline in GH and IGF-I at week 24. Secondary endpoints included IGF-I normalization, tumor shrinkage, safety and tolerability.
Results
In the high-dose group only, a significant change from baseline was seen for mean GH (−28%; $P=0.046$) and IGF-I (−27%; $P=0.023$). In the high-frequency group, changes from baseline in mean GH (+6.4%) and IGF-I (−4.7%) were not statistically significant. Significantly more patients in the high-dose group achieved a reduction in IGF-I at week 24 than those in the high-frequency group (91 vs 53%; $P<0.05$). IGF-I normalization or GH $<$ 2 μg/l occurred with only the high-dose regimen (IGF-I 36 vs 0%, $P=0.022$; GH 27 vs 0%, $P=0.06$). The proportion of patients experiencing tumor shrinkage was similar in the high-frequency and high-dose groups (14 vs 11%). Both regimens were well tolerated.
Conclusion
High-dose octreotide LAR (60 mg/28 d) is effective and well tolerated in patients with active acromegaly inadequately controlled with conventional SA therapy. These results suggest that in selected patients uncontrolled on conventional doses of SAs, high-dose octreotide LAR should be tried before switching to other treatment modalities.

OC4.2

Both insulin resistance and insulin secretion are involved in the pre-diabetes of acromegaly

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In acromegalic patients growth hormone (GH) excess induces insulin resistance but whether this is sufficient, in the face of normal insulin secretion, for pre-diabetes (impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)) to occur is a matter of debate.
Aim
To assess the relative role of insulin resistance and insulin secretion in the pre-diabetes of acromegaly.
Methods
One hundred and twenty-four patients with acromegaly (78 women, 46 men, mean age 50±11 years) addmited to our department were included in the study. Plasma glucose, GH and insulin levels were measured basal and 30, 60 and 120 min during a 75 g oral glucose tolerance test (OGTT). Insulin resistance was assessed by HOMA-IR index (fasting plasma glucose (FPG) (mg/dl)*fasting plasma insulin (FPI) (mU/l)/22.5*18). Basal and stimulated insulin secretion was assessed using HOMA-B% index (FPI (mU/l)*20)/(FPG (mg/dl)/18–3.5)) and insulinogenic index (IGI) (Δ insulin(30′–0′) (mU/l)*100/glucose(30′) (mg/dl) respectively. The local Ethic Committee approved the study.
Results
According to ADA criteria, there were 49 subjects with pre-diabetes (30 IFG, 11 IGT and 8 combined glucose intolerance). Seventy-five subjects had normal glucose tolerance (NGT). There were no significant differences between pre-diabetes group and NGT group regarding age (53±13 vs 48.7±11 years, P=NS), sex (53 vs 69.3% women, P=NS) and nadir GH in OGTT (18±17 vs 12.3±17 ng/ml, P=NS). The pre-diabetes group had a significantly higher HOMA-IR index (4.6±3.1 vs 2.6±2.1, $P<0.001$) and lower HOMA-B% index (159±108 vs 236±257, $P=0.02$) than NGT group. IGI did not differ between the two groups (39±48 vs 48±43, P=NS) but IGI/HOMA-IR was signifficantly lower in pre-diabetes group (9.7±8 vs 24.5±26, $P<0.001$). Nadir serum GH correlated with HOMA-IR index ($r=0.35$, $P<0.001$) but not with HOMA-B% or IGI.
Conclusions
Our data suggest that reduced basal and stimulated insulin secretion, reflecting the failure of β-cells adaptation to increased, GH-induced insulin resistance, leads to pre-diabetes in acromegaly.

OC4.3

Homologous and heterologous *in vitro* regulation of pituitary receptors for somatostatin (SST), growth hormone (GH)-releasing hormone (GHRH) and ghrelin in a non-human primate (*Papio anubis*)

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Secretion of GH by pituitary somatotropes is primarily stimulated by GHRH and ghrelin and inhibited by SST through the activation of specific receptors (GHRH-R, GHS-R and Sstr1-5, respectively). However, we have previously shown that SST, at low doses, can also stimulate GH release, directly and specifically, in primary pituitary cell cultures from baboons (*Papio anubis*) and pigs. To determine whether these primary regulators of GH release can also regulate directly the expression of their receptors (GHRH-R, GHS-R and Sstr1-5) in primates, primary pituitary cell cultures from baboons were treated for 4 h with 10^{-8} M of GHRH or ghrelin, or with high (10^{-7} M) and low (10^{-15} M) doses of SST and GH release and expression levels of all receptors were assessed by ELISA and real-time-PCR. Results show that GHRH and ghrelin decreased the expression of their respective receptors (GHRH-R and GHS-R) while both peptides increased Sstr1, did not affect Sstr2 and only GHRH decreased Sstr5 mRNA levels. These effects of GHRH and ghrelin on receptor expression were mimicked by forskolin (adenylate cyclase activator) and TPA (PKC activator) respectively, indicating the regulation of receptor-isoform levels by GHRH and ghrelin involved distinct signaling pathways. In contrast, high SST doses did not alter GH release but increased expression of Sstr1, 2 and 5, and did not alter GHRH-R and GHS-R levels. Interestingly, low SST doses increased GH release and increased Sstr1 but decreased Sstr5 and GHRH-R mRNA levels, similar to that observed for GHRH. Taken together, our data show for the first time in a primate model that the primary regulators of somatotrope function (GHRH, ghrelin and SST) exert both homologous and heterologous regulation of their own receptor synthesis which is dose- and isoform-dependent, and would involve distinct signaling pathways.

OC4.4

Development of a novel anti-IGF1 receptor immunoliposomal cancer therapy with enhanced therapeutic efficiency

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Overexpression and aberrant activation of the insulin-like growth factor system plays a key role in tumor cell proliferation and tumorigenesis in many human tumors. Different therapies targeting IGF1-receptor (IGF1-R) have been developed and currently, some of these agents are evaluated in preclinical and early clinical trials with promising results. Moreover, recent studies have demonstrated that combined treatments with doxorubicin, enhance the efficiency of anti-IGF1-R therapies. To merge these therapies in one formulation we coupled a monoclonal IGF1-R blocking antibody (1H7) to sterically stabilized liposomal doxorubicin (SSL-DXR). Flow cytometry analysis demonstrated high and significant cellular association of SSL-DXR-1H7 in comparison to SSL-DXR or unspecific IgG coupled SSL-DXR with human neuroendocrine tumor cells BON (44.2±1.6 vs 0.5±0.3 vs 0.8±0.3%; $P<0.0001$). Moreover, the lack of cellular association at 4 °C together with visualization of intracellular fluorescence of 1H7 coupled rhodamine-PE labeled liposomes by fluorescence imaging verified the otherwise rarely achieved event of liposomal internalization after binding to the target cell. *In vivo,* pharmacokinetic experiments with BON tumor xenografts in NMRI nude mice confirmed increased doxorubicin accumulation in the tumors after treatment with SSL-DXR-1H7 (157±6%) in comparison to liposomes conjugated with unspecific IgG (100±18%, $P<0.05$). In a therapeutical study with large scale tumor bearing mice significant effects on mean survival time (days) were only detectable after treatment with SSL-DXR-1H7 (31.5±2.2; $P=0.0084$) in comparison to untreated controls (8.0±0.6) and compared with all other treatments (free 1H7, 20±0.8; $P=0.0069$; SSL-DXR-IgG, 20±1.4; $P=0.0062$; SSL-DXR, 22±1.8; $P=0.0169$; SSL-DXR + free 1H7, 23±2.4; $P=0.0344$). Further flow cytometry analyses (SSL-DXR-1H7 versus plain liposomes) with the tumor cell lines MCF7 (64.9±2.3 vs 0.04±0.1%; $P<0.0001$), Kelly (21.8±2.9 vs 0.02±0.009%; $P=0.002$) and DU145 (31.9±0.7 vs 0.06±0.02%; $P<0.0001$) indicate the suitability of the established therapy also for other human cancer entities as breast carcinoma, neuroblastoma and prostate carcinoma.

OC4.5

Regulation of obestatin levels during unacylated ghrelin infusions in normal and type 2 diabetic patients

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Background
Obestatin, a ghrelin gene product was recently isolated but important questions remain regarding its regulation and its physiological effects. The aim of the present study was to evaluate the effect of unacylated ghrelin (UAG) on obestatin levels in normal subjects and patients with type 2 diabetes (T2D).
Methods
Five normal subjects and 5 T2D patients were included in this study. Unacylated ghrelin (3 μg/kg per hour) or saline were continuously administrated i.v. for 5 h and a standardized lunch was served after 2 h of infusion (0 min). Blood samples were collected at −15, 15, 30, 45, 60, 90, 120, 150 and 180 min and obestatin, insulin, glycemic and FFA levels were assayed.
Results
Before meal, UAG or saline treatments did not induce a significant change in individual groups. However, obestatin levels were significantly increased in normal subjects versus T2D patients ($P=0.04$) following UAG pre-treatment. In postprandial conditions, a significant decrease in obestatin levels was observed at times 60, 90, 120, 150 and 180 min in normal subjects during saline infusion. In

addition, nadir and AUC values were both significantly elevated during treatment with UAG versus saline in normal subjects ($P<0.05$). Likewise, during treatment with UAG, peak, nadir and AUC values were significantly higher in normal subjects versus T2D patients ($P<0.001$).
Conclusion
This study is first to describe that the postprandial reduction of obestatin is abolished by a UAG infusion in normal subjects but not in T2D patients. Overall the present results suggest that UAG upregulates obestatin expression from the ghrelin gene only in normal subjects. Further studies will be needed to describe the mechanisms underlying the regulation of obestatin in normal subjects and T2D patients.

OC4.6

Cardiovascular effects of chronic Sildenafil treatment in men with type 2 diabetes

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In type 2 diabetes (T2DM), cardiomiopathy is characterized by an impairment of diastolic performance resulting in ventricular hypertrophy and dilatation. Heart remodelling leads to an increase in its angle of torsion, measurable by innovative cine-magnetic resonance imaging (MRI). To evaluate the impact of phospho-diesterase 5 inhibitors (PDE5i) on cardiovascular performance in T2DM, we designed a randomized, placebo-controlled, double blind (subject/outcome assessor) study on chronic treatment (3 months) with high dose of Sildenafil (100 mg/in 3 daily doses). The study has been registered at US NIH clinicaltrial.gov (identifier NCT00692237). We have enrolled 50 diabetic men (35–75 years), metabolically controlled; 27 subjects have already ended the study; 2 patients drop out the study (1 for dyspepsia, 1 for non-compliance). Safety monitoring was taken monthly at follow-up visits. Primary outcome is the analysis of left ventricular torsion (cineMRI). Secondary outcomes reveals: (1) a significant improvement of heart remodelling parameter: end diastolic volume, ejection fraction and hypocynetic areas. (2) A significant improvement of cardiovascular risk parameters: reduction of *post prandial glycemia* from 178±49 to 156±48; *HbA1c* from 7.8±1 to 7.1±0.9; *waist to hip ratio* and increase of *HDL cholesterol* from 39±7 to 43±9. (3) A significant reduction of *P selectina* on activated platelet–monocytes interaction (cytofluorometry), involved in atheromatous process. 4) A significant reduction of *systolic* (136±12 to 131±12) and *diastolic blood pressure* (78±9 to 76±7) (Holter 24 h). Our study documents the safety of prolonged chronic sildenafil treatment on the adaptative endothelial changes affecting cardiovascular response in T2DM.

Thyroid: Basic and Clinical

OC5.1

Neurological and behavioral phenotypes in mice lacking *Mct8*-mediated neuronal T3 uptake

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Thyroid hormone transport into cells critically depends on plasma membrane transport proteins. One of these, monocarboxylate transporter 8 (MCT8), is mutated in patients suffering from a form of X-linked mental retardation, the Allan-Herndon-Dudley syndrome. These patients are characterized by abnormal thyroid hormone and TSH plasma levels indicating a role for MCT8 in the regulation of the thyroid hormone axis. Mice lacking the *Mct8* gene replicate the thyroid hormone abnormalities observed in the human patients. However, no neurological deficits have been described in *Mct8*-deficient mice. We have subjected *Mct8*-deficient mice to a comprehensive immunohistochemical, neurological, and behavioral screen. Unlike earlier reports, we have found several behavioral abnormalities in *Mct8* mutant animals. Aspects of the behavioral phenotype are both compatible with alterations observed in hypothyroidism and hyperthyroidism. We thus hypothesized that in *Mct8*-mutant mice, subsets of neurons exist in a hyperthyroid and hypothyroid state, respectively. In order to analyze this discrepancy, we characterized thyroid hormone transporters expressed in mouse cortical neurons using pharmacological tools and identified the T3 transporters expressed by mRNA analysis. Among the T3 transporters expressed in primary cortical neurons, *Lat2*, a L-type amino acid transporter, was co-expressed with *Mct8* both in isolated neurons and in the mouse brain during development. In contrast, *LAT2* is expressed in human brain during gestation, but in a spatio-temporal pattern not overlapping with *MCT8*. We speculate that lack of functional complementation by *LAT2* in developing human neurons may precipitate the devastating neurodevelopmental phenotype in *MCT8*-deficient patients, while *Mct8*-deficient mouse neurons are functionally complemented by *Lat2*.

OC5.2

Selenium and iodine determination from single murine thyroid lobes by TXRF-spectroscopy

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Iodine (I) and selenium (Se) are two essential trace elements for regular thyroid gland functioning and thyroid hormone metabolism. Most Europeans are only marginally supplied with both elements through their daily nutrition. The mammalian organism has developed efficient and highly complex mechanisms to control I and Se uptake, metabolism and recycling. The thyroid gland is equipped with the necessary transporters and metabolizing enzymes for I uptake and bioconversion into thyroid hormones. In addition, it appears extremely efficient in controlling its exceptionally high Se status by as yet poorly defined pathways. Se in the thyroid gland is pivotal for its defense against highly toxic peroxides generated continuously during thyroid hormone biosynthesis, and for the expression of active deiodinase isozymes which control thyroid hormone activation and inactivation. A number of genetically or chemically modified mouse models have been generated to study thyroid gland physiology and thyroid hormone biosynthesis. Until now, quantification of Se and I concentrations in the thyroid gland, peripheral tissues or murine serum samples has been hampered by the small amounts of material which is available from single mice. To circumvent these limitations, we have tested total reflection X-ray fluorescence (TXRF) spectroscopy as a very sensitive method to measure the elementary composition of a single murine thyroid lobe (approx. 2 mg wet weight). In a first group of animals, we found an average content of 223 ppb (Se) and 543 ppm (I) which is inside the expected physiological ranges. As a proof of concept, we compare Se concentrations in small (thyroid, pituitary, adrenals) and large (liver, kidney, spleen) organs from Se-deficient and regular mice with their serum Se concentrations. Our results indicate that the TXRF-spectroscopy represents a fast and reliable method to determine trace element status from minute amount of tissue or serum and might become an important technique for mouse experiments.

OC5.3

The role of *N*-linked oligosaccharides on the function of thyrotropin: development of new agonists and antagonists

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Introduction
Thyrotropin (TSH) and the gonadotropins (FSH, LH, hCG) are a family of heterodimeric glycoprotein hormones composed of two noncovalently linked subunits, α and β. hTSH, heterodimer was converted to a biologically active single-peptide chain, by fusing the common α subunit to the carboxyl-terminal end of hTSHβ subunit in the absence (hTSH$\beta\alpha$) or presence of a ~30 aminoacid carboxyl-terminal peptide from hCGβ (CTP) as a linker (hTSHβCTPα). Ligation of CTP to the carboxyl-end of hFSH, hCGα subunit and to hTSH resulted in increasing the biological activity and longivity *in vivo*.
Objectives
Investigation the role of the *N*-linked oligosaccharides of α and β subunits on secretion and function of hTSH using the single chain variant, hTSHβCTPα.
Methods
Two deglycosylated variants were prepared using site-directed mutagenesis and gene transfer; one lacks both *N*-linked oligosaccharide chains on α subunit (hTSHβCTPα_{1+2}), and the other lacks also the *N*-linked oligosaccharide chain on β subunit of the single chain (hTSHβCTPα (deg). The single-peptide chain variants were expressed in CHO cells.
Results
Absence of *N*-linked oligosaccharides on α or β subunits does not affect the secretion of the variants. These results indicate that the signal for the secretion exists in the single peptide chain is independent of the oligosaccharides. hTSH variants lack of the oligosaccharide chains is less potent than hTSHβCTPα on cAMP accumulation and T_3 secretion in human cultured thyroid follicles. Moreover, both deglycosylated variants compete with normal hTSH and hTSI in a dose dependent manner *in vitro* and *in vivo*.

Conclusions
The *N*-linked oligosaccharides are not important for receptor binding, but they are critical for bioactivity of TSH *in vitro* and *in vivo*. This variant, behaves as potential antagonist, who may offer a novel therapeutic strategy in the treatment of Grave's disease, the most common form of hyperthyroidism.

OC5.4

Selenium supplementation fails to improve thyroid hormone metabolism in subjects with SBP2 gene mutations

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Selenium (Se) is an essential trace element needed for the biosynthesis of selenoproteins. Biosynthesis of such proteins depends on the selenocysteine incorporation sequence binding protein 2 (SBP2), which represents a key trans-acting factor during the translation process. We recently described children with mutations in SBP2 gene who displayed an abnormal thyroid function test(s) and reduced selenoprotein concentrations. Now we aimed to improve selenoprotein biosynthesis and thyroid hormone metabolism in SBP2 deficient subjects by supplementing an organic and inorganic Se form.
Three affected and two unaffected siblings received daily doses of 100, 200 or 400 μg selenomethionine-rich yeast and 400 μg sodium selenite for one month each. Serum was drawn at baseline and after supplementations. Thyroid function tests, extracellular glutathione peroxidase activity, Se and selenoprotein P concentrations were determined. Selenomethionine-rich yeast increased serum Se concentrations in all subjects irrespective of genotype. Sodium selenite was effective in increasing the selenoprotein P concentration in normal and to a lesser degree in affected subjects. Both forms failed to increase the glutathione peroxidase activity or to correct the thyroid function abnormalities in the SBP2 deficient individuals indicating that impaired deiodinase expression was not corrected. No adverse side-effects were observed.
We conclude that total serum Se concentrations in SBP2 deficient subjects respond to selenomethionine supplementation but this effect is not indicative for improved selenoprotein synthesis. Se is obviously not a limiting factor in the SBP2 deficient individuals when regular daily Se intake is provided. The phenotype does not depend on the daily Se intake and therefore would likely have a similar presentation in the different geographical areas of the world. These findings might help to identify and diagnose more individuals with selenoprotein biosynthesis defects who might present at young age with characteristic thyroid function test abnormalities, growth retardation, and reduced Se and selenoprotein concentrations.

OC5.5

Graves patients with high sCTLA-4 level are at risk of severe ophthalmopathy

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Objectives
Graves' disease (GD) is an autoimmune disease caused by combination of environmental and genetic factors. The *CTLA-4* gene is a candidate gene for conferring susceptibility to thyroid autoimmunity. Increased serum level of soluble isoform of CTLA-4 molecule (sCTLA-4), resulting by alternative splicing, was found in some autoimmune diseases. The role of this molecule in the pathomechanism of autoimmunity has not been defined.
The principal aim of the study was to test clinical utility of sCTLA-4 estimation as well as to study factors influencing serum concentration of this molecule in GD accompanied by Graves ophthalmopathy (GO).
Patients and measurements
The serum sCTLA-4 concentrations were determined using specific ELISA assay in 102 GD patients and 83 controls. g.319C > T, c.49A > G, CT60G > A, Jo31G > T and g.*642AT(8_33) in *CTLA-4* gene was established by minisequencing assay. GO was assessed according to the Clinical Activity Score (CAS) and to 4-stage classification of severity.
Results
Serum sCTLA-4 concentration was significantly higher in GD as compared with control (8.3 vs 2.39 ng/ml, $P = 0.000001$). Increased level of sCTLA-4 was found in patients with severe GO as compared with controls (10.2 vs 2.39 ng/ml, $P = 0.000001$) as well as with patients with non-severe GO (10.2 vs 7.1 ng/ml, $P = 0.05$). Patients with *Jo31G > T*[G] phenotype had significantly elevated serum sCTLA-4 level as compared with [G-] ($P = 0.006$). sCTLA-4 concentration was not related to thyroid status or thyroid hormones levels.
Conclusion
To the best of our knowledge, this is the first clinical study searching for a relationship between sCTLA-4 concentration and the clinical expression of GO and thyroid status. Our results confirm an association of sCTLA-4 with the GD. sCTLA-4 is a sensitive marker of the disease and appears to be related with the severity of eye changes and with disease-bearing Jo31G > T[G] phenotype but not to thyroid function. Increased concentration of sCTLA-4 may indicate GD patients at risk of severe GO.

Paediatric Endocrinology/Bone

OC6.1

Pseudohypoparathyroidism type Ia and GNAS epigenetic defects: clinical evaluation and molecular analysis in 40 patients with Albright's hereditary osteodystrophy

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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 PTH and additional hormones, while PHP-Ib patients do not have AHO and hormone resistance is limited to PTH and TSH. Recently, methylation defects have been detected in 5 patients with PHP-Ia, indicating a molecular overlap between the two forms.
The aim of this study was to screen for methylation defects patients with clinically diagnosed PHP-Ia and to investigate the presence of correlations between the molecular findings and AHO severity. We investigated differential methylation of GNAS regions and STX16 microdeletions in genomic DNA from 40 patients (28 females, 12 males) with clinical diagnosis of sporadic PHP-Ia, i.e. AHO with multi-hormone resistance, with no mutations in Gsα-coding *GNAS* exons. Molecular analysis showed GNAS cluster imprinting defects in 22 of the 40 PHP-Ia patients analyzed. No STX16 deletion was detected. No correlation was found between the severity of AHO and the presence or absence of Imprinting defects. In conclusion, we report the largest series of the literature of patients with PHP-Ia and confirm the existence of an overlap between molecular and clinical features of PHP-Ia and PHP-Ib. These data provide new information on this rare disease and emphasize the clinical heterogeneity of genetic defects within the GNAS locus.

OC6.2

Recent decline in age at breast development and prolongation of puberty duration in girls

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Objective
Recent publications showing unexpectedly early breast development in American girls created debate worldwide. However, secular trend analyses are often limited by poor data comparability among studies done by different researchers in different time periods and populations. Here, we present new European data, systematically collected from the same region and by one research group at the beginning and end of the recent 15 year period.

Methods
Two thousand and ninety-five girls aged 5.6 to 20.0 years were studied in 1991–93 (1991-cohort, $n=1100$) and 2006–08 (2006-cohort, $n=995$). All girls were evaluated by palpation of glandular breast, measurement of height and weight and blood sampling (estradiol, LH and FSH). Age distribution at entering pubertal stages B2–B5, PH2–PH5 and menarche was estimated for the two cohorts.
Results
Onset of puberty, defined as mean estimated age at attainment of glandular breast tissue (Tanner stage B2+), occurred significantly earlier in the 2006-cohort (estimated mean age 9.86 years (95% confidence interval 9.70–10.01) when compared with the 1991-cohort (10.88 (CI 10.69–11.06)), $P<0.0001$. The difference remained significant after adjustment for BMI. Estimated ages at menarche were 13.42 (CI 13.24–13.60) and 13.13 (CI 12.95–13.31) in the 1991- and 2006-cohorts, respectively ($P=0.023$). The time between B2 and menarche increased from 2.54 years to 3.38 years. Serum FSH and LH did not differ between the two cohorts at any age interval, whereas significantly lower estradiol levels were found in 8–10 year old girls from the 2006-cohort compared to similarly aged girls from the 1991-cohort.
Conclusion
We found significantly earlier breast development and increased duration of the pubertal transition among girls born more recently. Alterations in reproductive hormones and BMI did not explain these marked changes, suggesting that other factors yet to be identified may be involved.

OC6.3

Ghrelin and obestatin levels in normal weighted and obese prepubertal children

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Introduction
Three peptides, acylated ghrelin (AG), unacylated ghrelin (UAG) and obestatin are derived from a common prohormone, preproghrelin by posttranslational processing, originating from endocrine cells in the stomach. Circulating ghrelin levels are decreased in obese subjects and increased by fasting and in patients with anorexia nervosa, but the physiological role of the three peptides is poorly understood in particular in childhood.
Aim
In order to understand the biological implications of these three peptides, we measured AG, UAG, obestatin, IGF-I, cortisol, TSH, prolactin, glucose, insulin, AST, ALT, and ALP levels at fasting in 25 normal weight (NW) and 35 obese (OB) prepubertal children.
Results
AG (8.62 ± 1.10 vs 21.66 ± 5.8 pg/ml; $P<0.005$), UAG (25.91 ± 3.16 vs 63.36 ± 8.20 pg/ml; $P<0.0001$) and obestatin (0.163 ± 0.040 vs 0.655 ± 0.134 ng/ml; $P<0.007$) levels were lower in OB when compared to NW children. The levels of the three peptides were positively correlated each others ($P<0.004$). AG levels were negatively correlated with height, height-SDS, weight and BMI ($P<0.01$), and positively with AST ($P<0.002$). UAG levels were negatively correlated with age, height, height-SDS, weight, BMI, ALP, IGF-I, cortisol, glucose, and insulin levels ($P<0.01$), and positively with AST ($P<0.01$). Obestatin levels were negatively correlated with height, BMI, and glucose ($P<0.03$). In the regression analysis, the best predictors were: 1) obestatin (β: 0.753) for AG; 2) IGF-I (β: -0.707), AG (β: 0.405), and glucose (β: -0.368) for UAG; 3) AG (β: 0.853) and glucose (β: -0.448) for obestatin.
Conclusions
OB children show lower levels of AG, UAG and obestatin. The evaluation of the two forms of ghrelin demonstrates a peculiar relationship between UAG levels and metabolic parameters. On the other hand, obestatin seems to be a regulator of AG circulating form.

OC6.4

Appearance matters: the impact of perceived altered appearance as a result of Klinefelter's syndrome on psycho-social functioning

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Background
Klinefelter's syndrome (KS) is a genetic condition affecting men with the potential to severely reduce their testosterone levels and affect their physical appearance. Being a syndrome there are a variety of different symptoms which individuals may experience to a great or lesser extent. While much research has been directed at understanding the cognitive impact of KS much less research has been undertaken considering the psycho-social impact of living with the condition. This research, requested by the Klinefelter's Syndrome Association (KSA), addresses this issue.
Method
A questionnaire was sent to the 300 members of the KSA. Items in the questionnaire covered demographic and medical information as well as the physical characteristics of the participants. Standardised questionnaires were included including measures of general anxiety and depression (HADS); social anxiety and avoidance (DAS-24); self-esteem (RSE); and quality of life (WHOQoL-Bref). Sixty-two questionnaires were returned from participants aged between 18 and 74 years (mean 43.69).
Results
Men who identified themselves as having gynaecomastia as a symptom of KS ($n=37$) were significantly more socially anxious ($P<0.003$) and had reduced quality of life ($P<0.03$) than those that did not. Similarly, those men who identified themselves as having a lack of facial and/or body hair ($n=38$) were significantly more socially anxious ($P<0.03$) than those that did not. Having a small penis as a result of KS significantly affected levels of depression ($P<0.002$), self-esteem ($P<0.001$), social anxiety ($P<0.01$) and quality of life ($P<0.02$).
Conclusion
These data suggest that men with KS can experience high levels of emotional distress and significantly reduced psycho-social functioning due to their visible differences, experienced as a result of their condition.

OC6.5

Cortical bone size is associated with serum sex hormone-binding globulin levels in healthy men at the age of peak bone mass

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Background
In elderly men, fracture risk is independently associated with higher serum sex hormone-binding globulin (SHBG) levels[1]. Previously, we observed lower free estradiol (E_2) and higher SHBG levels in a three-generational family study of men with idiopathic osteoporosis[2].
Objective
To investigate associations between serum SHBG levels and cortical bone size at age of peak bone mass.
Design
Six hundred and seventy-seven healthy male siblings aged 25–45 years were recruited in a cross-sectional, population-based study. This study is part of SIBLOS a broader study designed to investigate determinants of sex steroid levels and peak bone mass in men.
Methods
Cortical bone parameters at the radius and tibia (66% site, 2/3 of bone length from distal) were assessed using peripheral quantitative computed tomography (XCT2000, Stratec GmbH). In a morning blood sample, testosterone (T), E_2 and SHBG levels were measured using immunoassays. Cross-sectional relations were investigated using linear mixed-effects modeling analyses.
Results
After controlling for age, weight and height, SHBG levels were positively associated with total and cortical bone area and mineral content, and with peri- and endosteal circumference at both radius and tibia ($\beta=0.09$–0.17; $P=<0.001$–0.016). No associations with volumetric bone mineral density or cortical thickness were found. These findings remained significant after adjusting for T and/or E_2 levels.
Conclusions
This study demonstrated that higher serum SHBG levels are associated with larger bone size and thus more favorable indices of bone strength in healthy men at the age of peak bone mass, which seems in contrast with observations in elderly men and men with idiopathic osteoporosis. The underlying mechanism is presently unknown, but the possibility of a differential role of SHBG depending on sex steroid status might be considered.
References
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OC6.6

Reduced selenoprotein P expression affects bone formation

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Selenium (Se) is an essential trace element and is important for maintaining health and preventing disease. Se exhibits its biological function as the 21st proteinogenic amino acid selenocysteine (Sec). Different *cis* and *trans* acting factors are necessary for incorporation of Sec into proteins. Se can be transported and stored as Sec in selenoprotein P (SePP), a liver-derived protein which contains up to 10 Sec residues per protein in humans. Thus, SePP functions as both a systemic Se-transporter and a local Se-storage protein.

There are several hints demonstrating that Se and selenoproteins have an impact on bone physiology. Mutations in the Sec insertion sequence binding protein 2 (SBP2) and nutritional Se deficiencies are known to induce growth retardation in human patients and rats, respectively. Bone mineral content (BMC) and bone mineral density (BMD) have been shown to depend on the Se status in rodents. In humans, Se deficiency is known to be associated with Kashin-Beck disease, an endemic osteoarthropathy.

Since SePP is the central factor controlling Se status, we investigated SePP mRNA and the importance of SePP gene expression for bone physiology. In mice, SePP mRNA was strongly expressed in the developing limb buds. For functional analysis, SePP wildtype and SePP knockout mice were fed with regular diet and their tibia were analyzed by micro computer tomography (μCT). In male SePP-KO mice, the ratio of mineralized bone volume per total bone volume (BV/TV) was significantly reduced to 60% of wildtype. We conclude that impaired SePP expression results in reduced Se concentrations in the developing bone leading to impaired bone formation in male mice. Whether these results also apply for female mice or for humans remains to be investigated.

Poster Presentations

Adrenal

P1

Hematoma: unusual presentation of adrenal masses

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Introduction
Adrenal hematomas are very rare entities. They occur often associated with: trauma, anticoagulation, coagulopathy, septicemia, pregnancy complications or tumors. When none of these predisposing factors is present, diagnosis and treatment can become a real challenge.
Case report
A 19-years-old woman presented with complaints of asteny, loss of appetite, loss of 10 kg in a month, and pain in right lumbar and abdominal regions. No other complaints, such as increase of pilosity, menstrual irregularities, headache, palpitations or sweating. No previous diseases; no chronic medication. When subjected to palpation of right abdominal quadrants, a mass was found that revealed hard to define. Patient was then submitted to an abdominal computed tomography (CT) which revealed in place of right adrenal, an expansive formation of oval morphology, regular edges and well defined limits, with 7×6, 5×5 cm, homogenous texture, only with thin parietal calcifications which seemed like a complex cystic lesion. The most likely hypothesis was a residual hematoma. All laboratory findings were normal (hemogram with platelets, coagulation, renal and liver function, thyroid function, baseline hormone levels of pituitary, gonadal function, urinary free cortisol, ACTH-cortisol rhythm). Patient was submitted to a right adrenalectomy and no complications were developed. The final pathological diagnosis of excised specimen was compatible with an organized adrenal hematoma. Three weeks later the patient was admitted to our department for re-evaluation. At the time was completely asymptomatic; hormonal evaluation was normal. Abdominal CT showed only a right adrenalectomy. Study was made to rule out coagulopathy. Patient was then scheduled a hematology appointment.
Conclusions
When faced with an image on the adrenal gland compatible with a hematoma, it's necessary to rule out underlying adrenal tumors (through hormonal evaluation) and to research the existence of predisposing factors for the formation of a hematoma. Only the pathological study of excised specimen allows a definitive diagnosis.

P2

Von-Hippel Lindau disease and pheochromocytoma – case report

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Aims
VHL disease is an autossomal dominant syndrome that affects one in every 36 000 live births. The diagnosis is based on clinical criteria and the detection of the mutation on VHL gene. Most cases are diagnosed during the 2nd decade of life; this syndrome includes pheochromocytomas with the following characteristics: extraadrenal location, bilaterality, multifocal lesions, age of onset <30 years and discrete manifestations of catecholamine overproduction.
The authors present the case of a pheochromocytoma diagnosed in a patient with Von-Hippel Lindau disease.
Methods
We present the case of a 42-year-old female patient with Von-Hippel Lindau disease manifested by retinal angiomas (bilateral amaurosis at age 17), and cerebellar hemangioblastomas (ataxia at age 28). The patient was admitted at our clinic after detection of two right adrenal nodules with 2 and 3 cm each, and a hipodense mass on the left kidney.
The patient complained of headaches and occasional palpitations; she had high blood pressure diagnosed 11 years ago controlled with two antihypertensive drugs. She denied previous hypertensive emergencies. There was no family history of high blood pressure or pheochromocytoma.
Our investigation revealed
–Orthostatic hypotension;
–5473 nmol/day of normetanephrine (laboratory range: 480–2424 nmol/day) in 24 h urine sample;
–Area of increased uptake in radionuclide scintiscan with I131-MIBG in the right adrenal.
Right adrenalectomy was performed revealing two nodules of 2, 5 and 3 cm. Histology revealed two capsulated pheochromocytoma with low mitotic index, no vascular invasion and positivity for chromogranin, synaptofisin, NSE and S-100. Genetic study was requested but results are unavailable until now.
Conclusions
In this case we emphasize the age of diagnosis (later than usual), the presence of hypertension without paroxysms, the presence of orthostatic hypotension and the finding of multiple adrenal lesions.

P3

Effect of dietary protein on post-prandial salivary cortisol and androgen levels in healthy women volunteers

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Objective
Macronutrients such as protein and fat have been postulated to elicit post-prandial effects upon cortisol and testosterone concentrations. This pilot study was conducted to test the hypothesis that a meal high in protein content can elicit a positive effect on post-prandial cortisol levels whilst producing a negative effect on DHEA and testosterone levels.
Methods
In 13 healthy female volunteers (aged between 19–29 years; BMI ranged from 19.3 to 24.7 kg/m^2), salivary cortisol, DHEA and testosterone were measured in saliva samples taken before and after two meals, one of high protein content (65% energy as protein); and the other of low protein content (12% energy as protein) in a randomised crossover design. Saliva samples from each participant at regular intervals on the day of intervention were collected (09.00 a.m. till 17.00 p.m). Steroid hormone concentrations were measured using specific and sensitive ELISA methods. Measures of mood, appetite and psychological well-being were also recorded.
Results
An acute and significant meal-dependent increase in salivary cortisol was detected at approximately 90 mins post-prandially which was dependent upon the proportion of protein in the meal ($P=0.05$ in absolute cortisol levels and $P=0.04$ in the difference at 90 min and 150 min versus premeal value). In addition, midday meal rich in protein appears to affect the latency of the post-prandial cortisol peak. No significant difference was elicited in DHEA levels following the two midday meals, but there was a trend increase following the low protein meal and a decrease in the high protein meal ($P=0.06$). No overall difference in testosterone levels, however a significant decrease between waking and 2 h post ingestion of low protein meal only was found ($P=0.01$).
Conclusion
This investigation suggests a role for macronutrients such as protein in influencing the transient daily steroid hormone levels and their subsequent implications with metabolic homeostasis and hormone-dependent disease.

P4

Correlation between development of testicular adrenal rest tumors and genotype in children, adolescents and adult males with congenital adrenal hyperplasia

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Background
Previous studies have demonstrated a high overall prevalence of testicular adrenal rest tumors (TART) in adults with congenital adrenal hyperplasia (CAH), whereas little is known about the prevalence in children. The aim of this study was to determine the presence of TART according to age and genotype.
Design
Retrospective study, tertiary University centre.
Patients and methods
In 47 male patients (age 2.6–40.3 years) with CAH due to 21-hydroxylase deficiency a testicular ultrasound was performed to evaluate the presence of TART and CYP21A1P genotypes were identified and divided in four groups according to the severity of the mutation (predicted activity of the 21-hydroxylase); null, A, B and C.
Results
TART were detected in 26 patients (overall prevalence of 55%). For the 23 patients younger than 18 years TART were present in 11 (48%). The youngest patient having TART was 7.5 years old. The presence of TART was highly

dependent of the specific CYP21A1P genotype: 26 of 35 patients (74%) with the most severe mutations (group null and A) had TART whereas none of 12 patients with the milder mutations (group B and C) had TART.
Conclusion
TART are frequently present in patients with severe CYP21A1P mutations in contrast to those having milder mutations. They may present in early childhood suggesting that testicular ultrasound should be included in the clinical follow-up of CAH children, especially those with severe CYP21A1P mutations.

P5

Abstract withdrawn.

P6

Unbiased stereological study of silymarin effects on adrenocortical structure of dexamethasone treated hamsters

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Adrenal cortex is an essential portion for life and its function can be affected by many chemical agents and drugs. This study was to investigate effect of silymarin, a flavonoid, on adrenocortical structure of male dexamethasone treated hamsters. In this study, 20 young adult male golden hamsters were randomly allocated to four groups: control group which received no drug; group two which received 7 mg/kg dexamethasone; group three which received 100 mg/kg silymarin; group four which received 7 mg/kg dexamethasone and 100 mg/kg silymarin. All animals were injected IP for seven consecutive days and conducted in accordance with humane care and ethical animal welfare. At the eighth day, the animals were euthanized and the adrenal glands were quickly removed, weighed and fixed in buffered formalin. The samples were processed by routine and standard paraffin embedding and serially sectioned in 5 μ thickness. The total volume of adrenal gland, adrenal cortex and cortical zone were estimated by Cavalieri's point-counting principle using Weibel's multipurpose test grid M_{42}. Total number of adrenocortical cells in each zona was estimated by stereological methods. At least, statistical analysis was performed by ANOVA with LSD test to evaluate the means.
The results showed that the adrenal gland mass of dexamethasone treated hamsters was significantly decreased in comparison to animals that received dexamethasone with silymarin. It was also obtained that there are no significant difference in the zona glumerolosa volume and cell number among examined groups. The volume of the cortex, zona fasciculata, zona reticularis and cell number of these regions were significantly reduced in dexamethasone treated hamsters compared to controls ($P<0.05$), whereas in group four, this reduction was not observed. Finally it can be concluded that silymarin seem to be a suitable protective drug for side effect of glucocorticoid therapy in adrenal glands.

P7

Use of diurnal rhythm in salivary aldosterone to discriminate between bilateral adrenal hyperplasia and aldosterone producing adenoma

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Differential diagnosis between bilateral adrenal hyperplasia (BAH) and aldosterone producing adenoma (APA) in aldosteronism remains challenging in many cases due to the high prevalence of incidentalomas during imaging techniques, the limited sensitivity of orthostatic testing and the technical difficulties of adrenal vein sampling (AVS).
We investigated circadian variation in salivary aldosterone (SA) in patients with APA ($n=22$) and BAH ($n=20$). In 12 APA patients, we also compared plasma aldosterone (PLA) during orthostatic testing (4 h) to diurnal changes in SA. Interfering medication was discontinued before sampling (mineralocorticoid receptor antagonists 4 weeks; ACE inhibitors, AT2, beta-blockers, 1 week). Patients underwent MRI/CT scan and APA was defined by successful AVS and/or cure after operation. Paired saliva samples were collected between 0800–1200 and 2000–2400 h. SA was measured using an in-house fluorometric assay, salivary cortisol by luminescence immunoassay (IBL, Hamburg).
Overall, mean ($\pm$S.E.M.) SA in APAs decreased from morning (146 ± 17 pg/ml) to evening (92 ± 13pg/ml, $P=0.015$), while in BAHs levels remained unchanged or increased (morning: 94 ± 16 pg/ml; evening: 107 ± 20 pg/ml ($P=0.75$). In the APA group, SA decreased in 18 patients (81.8%), was unchanged in one, and increased in three (13.6%). In 12 of these APA patients, PLA during posture testing was also available. PLA decreased in five, was unchanged in one and increased in six cases following posture. In these 12 patients SA decreased in 10 and increased only in two between morning and evening. Cortisol decreased diurnally in both groups as expected, excluding the influence of stress on evening aldosterone levels.
Compared to the 4-hour posture test, which shows increases in PLA in a significant number of APAs, the long-term diurnal decrease monitored by SA presents a more constant finding. Although a direct comparison between posture testing and SA measurements in patients with BAH is pending, our preliminary data suggest that SA could be a supporting tool in discrimination between BAH and APA.

P8

Effect of universal somatostatin analog (SOM230) on primary adrenal cell cultures

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Introduction
Somatostatin (SST) is a widely distributed polypeptide that modulates the endocrine and exocrine secretion, cellular proliferation and differentiation, and apoptosis via G protein-linked receptors. Our previous studies showed high SSTR expression in adrenocortical carcinoma (ACC), aldosterone producing adenoma (APA) and in cortisol producing adenoma (CPA). Only very few information are available about the effectiveness of SST analogs in adrenal tumors.
Objective
The aim of this study was to evaluate the effect of SOM230 on hormonal secretion and apoptosis in human adrenal carcinoma cell line, H295R, and in primary cell cultures from adrenal tumors.
Material and methods
For our studies we collected three APA and two ACC primary cell cultures (one obtained from a cortisol secreting adrenocortical carcinoma and the other from a non secreting adrenocortical carcinoma). Cortisol and aldosterone concentrations in the medium were measured by RIA. The apoptosis evaluation was performed by immunofluorescence using MitoPT kit both in H295R cells and in the two ACC primary cell cultures.
Results
Our data showed an inhibition of hormonal secretion after the treatment with SOM230 both in H295R cells and particularly in adrenal primary cell cultures. Nanomolar concentrations of SOM230 reduced the cortisol secretion in ACC primary cell culture and also reduced the aldosterone secretion in APA primary cell cultures. Furthermore, SOM230 at nanomolar concentrations showed a positive effect on apoptosis induction in H295R cells and in two ACC primary cell cultures.
Conclusion
SOM230 inhibitory effect on hormonal secretion in H295R and especially in adrenal primary cell cultures, the positive effect on apoptosis induction in adrenal cells suggests a possible therapeutic role of SSTR agonists in adrenal tumors.
Supported by AIRC.

P9

Replicating the normal cortisol circadian rhythm using a formulation of modified-release hydrocortisone

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Background
The adrenal glucocorticoid, cortisol, has a distinct circadian rhythm regulated by the brain's central pacemaker. This cortisol rhythm acts as a secondary messenger to peripheral tissues and loss of the rhythm is associated with increased morbidity and mortality. This is a specific problem in adrenal insufficiency and congenital adrenal hyperplasia (CAH). Based on pharmacokinetic modelling we have developed a modified-release formulation of hydrocortisone (MR-HC) to test whether it can replicate normal cortisol rhythm.
Methods
Using historical data from normal subjects ($n=33$) we have defined the parameters of the physiological circadian rhythm of cortisol. We have then tested our MR-HC in healthy volunteers ($n=28$), defined its pharmacokinetic characteristics, compared these to physiological cortisol levels and modelled an optimal treatment regimen to replace circadian cortisol levels.
Results
The key cortisol concentration and time variables in the physiological cortisol profile were defined as: peak 400 nmol/l (95% reference range 296–540), acrophase (time of peak) 0832 h (95% CI 0759–0905 h), nadir <50 nmol/l (95% reference range 35–64), time of nadir 0018 h (95% CI 2339–0058 h), and quiescent phase 1943–0531 h. All MR-HC formulations tested demonstrated delayed and sustained release with MR-HC 15 mg having a mean (S.E.M.) C_{max} 457 (38.4) nmol/l at 7.41 (0.57) hrs after drug. Bioavailability of MR-HC 5, 10, 15 & 30 mg was 100, 79, 86, & 69% that of immediate-release hydrocortisone. By pharmacokinetic modelling we revealed that MR-HC 15–20 mg at 2300 h and 10 mg at 0700 h could replicate normal cortisol circadian rhythm.
Conclusion
We have shown that a modified-release formulation of hydrocortisone has the potential to imitate the physiological cortisol rhythm. The future of hydrocortisone replacement lies in the use of physiological therapy for patients with adrenal insufficiency and congenital adrenal hyperplasia.

P10

The cortisone to cortisol conversion in autoimmune Type 1 diabetes

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The aim of the study was to obtain data about peripheral metabolism of cortisol. We compared diabetics with low response (LR), and with normal response (NR) during low dose ACTH test, and a control group (C).
Twelve diabetics were investigated; LR ($n=6$), NR ($n=6$), age 44 ± 10 year (mean $\pm$ S.D.), age at diagnosis of DM1 28.5 ± 10 year, disease duration 15 ± 8 year, BMI 24.5 ± 2.7 kg/cm^2, HbA1c 7.2 ± 1.2%. The control group had six healthy subjects; age 27 ± 6 year, BMI 21.7 ± 2.3 kg/cm^2. Neither group showed any clinical signs of adrenal disorders and adrenal autoimmunity.
The study was approved by the local Ethical Committee.
At 23:00 on day 1, subjects were given 1 mg dexametasone orally. The following morning, cortisone acetate (25 mg) was administered orally. Serum cortisol (F) and cortisone (E) were then measured at 30 and 60 min intervals, respectively, for 240 min.
In diabetics the initial increase of F was delayed compared to C; the maximum was observed at 90th and 120th min (in C at 60th min). The following decrease of F was much slower in diabetics than in C but the levels of F in this phase were significantly higher then in C ($P<0.05$). When we compared LR to NR patients, the initial increase of F was delayed till 150th min in NR contrary to 120th min in LR. After that the levels of F in both groups decreased, but the concentrations of F were significantly higher in NR.
In conclusion, we found distinct difference in cortisone to cortisol conversion between diabetics Type 1 and controls. These result may contribute to better understanding of tissue glucocorticoid metabolism in diabetics Type 1 with latent adrenal insuficiency.

P11

Are there some alterations of salivary cortisol dynamics in obese patients?

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The metabolic syndrome resembles Cushing's syndrome in several clinical aspects. Main characteristic of both syndromes is obesity assessed by the body mass index (BMI) or waist circumference (WC).
The aim of this study was to investigate cortisol dynamics in 20 healthy volunteers (BMI <27 kg/m^2; 13 females, 7 males, age $42.1+12.39$ years) and 20 obese patients (BMI ≥ 27 kg/m^2; 13 females, 7 males, age $49.25+15.52$ years). Screening tests included: 1) late-night (23:00 h) salivary cortisol; 2) morning to late-night (08:00/23:00 h) salivary cortisol ratio; 3) salivary cortisol response to 1 mg overnight dexamethasone suppression (post-DXM), and 4) basal morning to post-suppression (08:00 h/post-DMX) salivary cortisol ratio. Results are given in following Table.

	Normal controls	Obese patients	*P*
23:00 h	3.83	6.11	0.0016
08:00/23:00 h ratio	5.23	3.76	0.0232
Post-DXM	2.39	6.45	0.0001
08:00 h/post-DMX	8.45	4.28	0.0003

In obese patients, late-night (23:00 h) salivary cortisol as well as the post-dexamethasone level was significantly higher than in age and gender-matched healthy controls. Consequences of these alterations in cortisol dynamics were significantly lower morning to late-night (08:00/23:00 h) as well as basal morning to post-suppression (08:00 h/post-DMX) salivary cortisol ratios observed in obese patients.
It could be concluded that some subtle alterations in salivary cortisol dynamics exist in obese patients. This observation is in accordance with the literature data on the incidence of subclinical Cushing's syndrome in patients with Metabolic Syndrome that is not negligible.

P12

Short term regulation of aldosterone secretion after stimulation and suppression experiments in mice

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Aldosterone is synthesized acutely upon stimulation of the renin-angiotensin-aldosterone system from the cells of the zona glomerulosa. Several enzymes are involved in this steroidogenic process including the steroidogenic acute regulatory protein (*StAR*), P450 side chain cleavage enzyme (*Cyp11a1*) and aldosterone synthase, the product of the gene Cyp11b2.
We investigated the short time transcriptional regulation of these genes in wild type mice at 10 min intervals for one hour following application of angiotensin II (ANGII) and sodium chloride in comparison to sham injections. The aldosterone response after each stimulus was quantified through measurement by a fluorescent immunoassay. Using Real-Time PCR a fast upregulation of adrenal *Cyp11b2* expression (53 ± 5% increase over baseline expression; $P<0.05$) could be observed 10 min after sham injection which was accompanied by a transient increase in aldosterone secretion (baseline, 88 ± 10 pg/ml vs 10 min, 228 ± 23 pg/ml; $P<0.005$) while *StAR* (20 min, $233\%\pm47\%$; $P<0.05$) and *Cyp11a1* (20 min, $363\%\pm31\%$; $P<0.001$) upregulation was delayed and more sustained. ANGII caused an increase of *StAR* and *Cyp11a1* expression similar to that observed after sham injection while *Cyp11b2* upregulation was more pronounced (10 min, $236\pm39\%$) and reflected ANGII induced stimulation of aldosterone output. Sodium challenge was followed by a sustained reduction of all three genes examined (*Cyp11b2* 20 min, $-63\pm6\%$) which was accompanied by significant

suppression of aldosterone secretion detectable after 60 min. These data suggest that acute regulation of aldosterone synthesis is accompanied by fast transcriptional modulation of steroidogenic enzymes. Parallel changes of *Cyp11b2* and aldosterone levels are in line with the concept of *Cyp11b2* being a rate limiting step for aldosterone output.

P13

Autoimmune adrenal insufficiency: Addison's disease versus idiopathic isolated secondary adrenal insufficiency

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Studies on autoimmune Addison's disease (AD) have a long tradition, while knowledge on idiopathic isolated secondary adrenal insufficiency (SAI), which also seems to be caused by autoimmunity, is not so popular. Our presentation aimed at comparing two groups of patients: 1) 238 patients with AD (183 women, F/M = 4.3), aged 8–61 years at time of diagnosis and 2) 301 patients with SAI (269 women, F/M = 8.4), aged 17–87 years at time of diagnosis. Maximum time of duration: 44 years in AD, 18 yrs in SAI. Hyperpigmentation in AD was the main clinical sign differentiating the both groups of patients; in AD clinical features were more dramatically manifested. Adrenal crisis was more frequent in AD while in SAI threatening adrenal crisis was not a rare finding. Cortisol levels were low in both groups (lower in AD), however they rose significantly only in SAI after stimulation with synthetic ACTH. High ACTH levels in AD were in contrast with low ACTH values in SAI. Biochemical analyses revealed that hyperkalemia was typical for AD, while hyponatremia was more characteristic for SAI. Additional autoimmune disorders were present in 79% of AD and in 67% in SAI. The main autoimmune diseases in AD: thyroid diseases – 34%, premature ovarian failure (POF) – 14%, IDDM and vitiligo – 13%, while in SAI – 36, 4, 3 and 4% respectively. Thyroid autoantibodies were found in 72% in AD and in 60% in SAI. Adrenal autoantibodies were detected in 55% of 115 AD patients under study, while pituitary autoantibodies in 34% out of 65 patients with SAI. In AD group 33 women became pregnant and delivered healthy children, which contrasted with only one patient with SAI (mainly due to a higher age in SAI). Conclusions: apart of clinical picture and hormonal pattern the main differences between the both groups concerned age at time of diagnosis, F/M ratio, type of electrolyte disturbances, frequency of adrenal crisis, frequency of POF, IDDM and vitiligo.

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P14

Prediction of metabolic syndrome by low serum testosterone levels in men: results from the Study of Health in Pomerania

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Aim
The aim of this analysis was to determine whether low serum testosterone (T) and dehydroepiandrosterone sulfate (DHEAS) levels predict the development of metabolic syndrome (MetS) in men.
Methods
Data were obtained from the Study of Health in Pomerania (SHIP), a population-based prospective cohort of adults aged 20–79 at baseline. Analyses were conducted in 1004 men without baseline MetS, defined by National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines. T and DHEAS were categorized by age-specific quartiles. To assess the association between sex hormone levels and MetS relative risks (RR) and 95% confidence intervals (95% CI) were estimated from Poisson regression models.
Results
After a median follow-up time of 5.0 years, 480 men (47.8%) had developed MetS. T levels decrease with increasing number of MetS components. T levels in the lowest quartile predicted MetS (RR 1.37, 95% CI 1.14–1.66), particularly among men aged 20–40 years (RR 2.03, 95% CI 1.28; 3.23). Low DHEAS levels were not related to incident MetS after adjustment for age, smoking, alcohol consumption, physical activity, body mass index, and self-related health (RR 0.95; 95% CI 0.75; 1.21).
Conclusions
Low T but not DHEAS predict development of MetS in a population-based cohort of men. Assessment of T in young and middle old men may allow early interventions in the general population.

P15

Prediction of fatal stroke by high serum aldosterone levels

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Background
There is increasing evidence that aldosterone exerts deleterious, blood pressure independent effects on cerebral blood vessels. We aimed to evaluate whether high aldosterone levels are related to fatal stroke.
Methods
Serum aldosterone concentrations (SAC, pg/ml) were measured in 3073 Caucasian patients, who were routinely referred to coronary angiography. We calculated Cox proportional hazard ratios for death due to fatal stroke according to SAC quartiles.
Results
During a median follow-up time of 7.75 years 769 patients died, including 37 individuals with a diagnosis of fatal stroke (ischemic or hemorrhagic). After multivariate adjustment for age, sex, common vascular risk factors including arterial blood pressure and statin use, the hazard ratio for death due to fatal stroke was 2.78 (95% CI 1.02–7.58; $P = 0.046$), when comparing patients within the highest SAC quartile (median SAC 16.8) to the lowest SAC quartile (median SAC 3.4).
Conclusions
High aldosterone levels are associated with death due to fatal stroke independent of multiple vascular risk factors. Our results suggest that mineralocorticoid blocking strategy might be beneficial for prevention of fatal cerebrovascular events.

P16

Effects of castration and testosterone treatment on adrenal activity of a saharan gerbil, *Meriones libycus* in breeding and non breeding season

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In the Saharan gerbil *Meriones libycus*, live trapped in the south west of Algeria, castration, carried out during the breeding season (Winter–Spring), induced, 50 days later, important modifications in adrenal gland activity. The adrenal weight and cortex volume increased by 11.7% and 25.0% respectively, with hypertrophy of reticularis zona (+57%, $P < 0.001$) and hypotrophy of fasciculata zona (−13.4%, $P < 0.001$) while glomerulosa exhibited only small height variations. However, the histological study showed significant increases in cellular height and nucleus diameter of reticularis cells. In the fasciculata zona, the height cells also increased (11.4%; $P = 0.04$) whereas nucleus diameter decreased by 11% ($P = 0.003$). On the other hand, adrenal content of cortisol decreased by 30% (absolute values; $P = 0.4$) or 50% (relatively to adrenal or body weights; $P = 0.2$). Plasma cortisol also decreased (−83%, $P = 0.3$) in the castrated males.
Testosterone replacement was performed by twice daily of s. c. injections of 75 µg sesame oil diluted testosterone enanthate, during 7 days in both 50 days castrated gerbils during breeding season and intact animals during resting season

(autumn). This treatment induced adrenal hypertrophy due to that of cortex volume and fasciculata and reticularis zona in all testosterone treated animals. However, the adrenal cortisol content increased in all animals, whereas plasma cortisol values were restored in castrated ones (breeding season) and continue to decrease in non castrated (non breeding season).
This study suggests that testosterone affects the adrenal structure and activity, particularly in cortisol production by inhibiting or stimulating its secretion either directly via androgen receptor or via hypothalamic–pituitary–adrenal axis. Then, testicular androgens seemed to be implicated, at least in part, in the endogenous determinism of annual cycle of adrenal activity in this desert species.

P17

Gitelman syndrome: clinical presentation and genetic analysis of 27 patients with hypokalemia caused by renal potassium wasting

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Gitelman syndrome (GS) is a recessive salt loosing tubulopathy caused by mutations in the *SLC12A3* gene encoding the thiazide-sensitive Na^+-Cl^- cotransporter, and characterized by secondary hyperaldosteronism, hypokalemic alkalosis, hypomagnesemia and hypocalciuria.
The aim
Of the work was to investigate 27 adult patients with hypokalemia due to renal potassium wasting after exclusion of diuretics abuse, vomiting or diarrhea.
Methods
Clinical and biological data were recorded, and genetic analysis of *SLC12A3* gene performed for each patient.
Results
Of 15 patients had two pathogenic mutations of *SLC12A3* defining a true GS, two patients one single mutation, and nine no pathogenic mutation, but gene polymorphism in 6/9 cases. Patients with true GS were 35 ± 15 years old at time of diagnosis. Symptoms (dizziness, paresthesia, tetany or nycturia) were present in 60% of cases. Complications of GS were found in five patients: two with chondrocalcinosis, two with growth delay, and one with syncope related to cardiac arrhythmia. Four patients had high blood pressure, while GS is usually associated with low blood pressure. All patients with true GS had hypokalemia (mean $\pm$ S.E.M.: 2.8 ± 0.3 mEq/l) and hyperreninism (except for one patient), while hypomagnesemia (absent in 8/15 patients) and hypocalciuria (absent in 2/7 explored patients) were inconstant. Hypokalemia was mild in patients without pathogenic mutation of *SLC12A3*: 3.5 ± 0.3 mEq/l, and hyperreninism lacking in most cases. Follow-up of patients with true GS during 9 ± 6 years was marked by a mild decrease of renal function (2.8 ml/mn per year of creatinin clearance), correction of hypokalemia above 3 mEq/l in 73% of patient with treatment, and mild elevation of blood pressure.
Conclusion
In adult patients referred for hypokalemia, GS should be evoked especially in case of hyperreninism that is a constant feature in patients with a confirmed genetic diagnosis. High blood pressure does not set the diagnosis aside. Mild hypokalemia are sometimes associated with heterozygous mutation of the *SLC12A3* gene.

P18

Bilateral adrenal incidentalomas – a clinical study of 1710 patients observed at a single endocrinological centre

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In the last 18 years 1710 patients with incidentally found adrenal tumours = adrenal incidentalomas (AI), 1300 women (F/M = 2.8), aged 10–87 years, were registered at our department. In 338 of them (20%) bilateral tumours were detected. This study aimed at analyzing character of bilateral tumours and defining methods of their management. The size: 1.0–13 cm (in the group of 1710 patients: 1.0–23 cm) most of them up to 3.5 cm. Hormonal and imaging studies were performed in all the patients. High density of the tumour in the native phase of CT with delayed washout and the size >4.0 cm were considered as important indications for surgery. Of 66 patients with bilateral AI were treated by surgery for oncological or endocrinological purposes (mainly pre-Cushing's syndrome). In case of malignancy bilateral operations were performed, in other cases unilateral adrenalectomy was usually done, basing on individual indications (choosing the tumour greater in diameter or with a more evident progression or presenting higher density on CT).
Pathomorphology
The most frequent malignant tumours were metastases and lymphomas, more rare was cancer (four patients); non-malignant tumours – adenomas, adrenal hyperplasia and pheochromocytomas. Pre-Cushing's syndrome was diagnosed the most frequently in adrenal hyperplasia; sometimes both hyperplastic adrenals presented subclinical cortisol hypersecretion. Following removal of an adrenal adenoma causing subclinical Cushing's syndrome secondary insufficiency of the remaining adrenal was observed. Replacement therapy with hydrocortisone in gradually reduced doses was withdrawn within 3–6 months in a majority of these patients.
The remaining patients, not qualified for surgery, have been carefully observed (imaging and hormonal examinations have been performed).
Conclusions
Endocrinological or oncological indications for surgery were recommended in 20% of patients with bilateral AI; the most frequent causes were adenomas, adrenal hyperplasia, pheochromocytomas, metastases and lymphomas.
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P19

Adipokine levels in patients with adrenal incidentaloma

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Patients (pts) with adrenal incidentaloma (AI) may show an increased incidence of several cardiovascular risk factors, as obesity, hypertension, diabetes mellitus and dyslipidemia. As previous data suggested that the increased cardiovascular risk in these pts could be in part mediated by alterations of adipokines, this study was aimed to evaluate plasma IL-6, resistin, CT-1 levels in a wide series of pts with AI.
Ninety-three pts (42 males; 51 females; aged 63.6 ± 9.1 years; BMI 28.2 ± 0.5 kg/cm^2, mean $\pm$ S.E.M.) were studied. All adrenal masses were identified as cortical adenoma. In all pts serum cortisol (F), plasma ACTH and urinary free cortisol were determined in basal conditions and after an overnight 1-mg dexamethasone (dexa) test. Plasma IL-6, CT-1 and resistin levels were measured by ELISA methods.
In pts with AI plasma IL-6 levels were greatly elevated (26.5 ± 1.3 vs 2.9 ± 0.4 pg/ml, $P < 0.001$); resistin levels were also higher, though not significantly, than in normal subjects, while CT-1 levels were similar (11.6 ± 0.6 vs 9.6 ± 0.7 ng/ml; 19.2 ± 1.0 pg/ml vs 18.7 ± 1.3, respectively). In all pts adipokine levels did not correlate with cortisol/ACTH levels (either basal or after dexa) or with other clinical (adenoma size, hypertension), metabolic (insulin resistance, diabetes mellitus), hormonal parameters (subclinical Cushing syndrome).
Interestingly, patients with visceral obesity more frequently showed a greater elevation of IL-6 (100 vs 96.6%), resistin (29.8 vs 22.5%) and CT-1 levels (8.8 vs 0%) than the remaining pts.
Conclusion
a) pts with AI may show increased levels of adipokines, apparently not related to the presence of diabetes, metabolic syndrome, insulin resistance, hypertension; b) a direct influence by the adenoma itself on cytokine production has been suggested only by experimental studies; c) whether it is plausible to consider a role for cytokines in AI, starting before cardiovascular complications arise, it will be possibly clarified by future prospective studies.

P20

Chemokine receptor expression in the adrenal cortex and in adrenocortical tumours

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Introduction
Chemokines and their receptors (CR) have been demonstrated to be involved in tumour growth and site specific metastasis. Furthermore, several cytokines have been described to modulate adrenocortical function. Therefore, we have investigated the expression pattern and functional activity of chemokine receptors and of corresponding chemokines in adrenocortical tissue.
Methods
Chemokine and CR expression was assessed by RT-PCR in normal adrenals (NAG), NCI-h295-cells, SW13-cells and adrenocortical carcinomas (ACC). CXCR4-expression levels were quantified by qPCR and immunohistochemistry in 7 NAG, 23 benign adrenocortical tumours and 167 ACC tissues (135 primary tumours, 14 metastases and 18 local recurrencies). Effects of the CXCR4 ligand CXCL12 on hormone production and signal transduction in NCI-h295-cells was assessed by radioimmunoassay and western blot, respectively.
Results
The adrenal gland expresses multiple chemokine receptors (CXCR1-6,CCR1,2,5-9,11 and CX3CR1) and chemokines (CXCL1,3,8,12 and CCL22). CXCR4 was most abundantly detected in both NAG and in adrenocortical tumours. CXCL12 led to activation of the ERK signal cascade and slightly but significantly decreased cortisol production in NCI-h295-cells. Immunohistochemistry revealed positive CXCR4 staining mainly in the outer adrenocortical zone. Conn and Cushing adenomas showed highest expression levels compared to NAG, whereas non secreting adenomas had only weak CXCR4 expression. In ACC, CXCR4 expression levels showed considerable variation with highest levels in metastases and lowest in local recurrencies.
Conclusion
Chemokines and CR are expressed both in benign and neoplastic adrenal tissue. CXCR4 is one of the most abundant CR exhibiting functional activity in NCI-h295-cells. CXCR4 expression was significantly higher in endocrine active compared to inactive tumours, suggesting that CXCR4 plays a role in adrenal steroidogenesis. However, no significant differences in expression levels between benign and malignant tumours were detected.

P21

Bilateral macronodular adrenal hyperplasia versus bilateral micronodular adrenal hyperplasia

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Bilateral macronodular or micronodular adrenal hyperplasias are related to ACTH-independent or ACTH-dependant pathologies. ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) and primary pigmented adrenocortical disease (PPNAD) are classical but rare examples of ACTH-independent pathologies, causing Cushing Syndrome. PPNAD is presented as bilateral micronodular adrenal hyperplasia.
On the other hand, inherited defects in the enzymatic steps of cortisol biosynthesis result in a decrease in cortisol biosynthesis and a consequent increase in the secretion of ACTH, thereby stimulating adrenal hyperplasia. The groups of syndromes related to enzyme deficiencies is termed congenital adrenal hyperplasia (CAH). CAH can be found as bilateral diffuse, macronodular or micronodular adrenal hyperplasia.
We present five cases with different etiologies in order to review the topic which is sophisticated.
Case 1
A 68-year-old woman with a 40-year history of hypertension was refferred for further evaluation of bilateral adrenal metastases. Endocrinologic data of the patient showed ACTH-independent hypercortisolemia, bilateral macronodular adrenal hyperplasia, type 2 DM and hypertension.
Case 2
A 43-year-old woman with a 10-year of obesity was refferred for further evaluation of adrenal adenoma. Endocrinologic data of the patient showed ACTH-independent hypercortisolemia, bilateral macronodular adrenal hyperplasia and type2 DM.
Case 3
A 20-year-old woman with a 3-year history of hypertension was refferred for further evaluation. Endocrinologic data of the patient showed ACTH-independent hypercortisolemia, bilateral micronodular adrenal hyperplasia (PPNAD).
Case 4
A 45 years-old man was referred because of ambiguous genitalia. Endocrinologic data of the patient showed 21OH deficiency, hyperandrogenism, bilateral macronodular adrenal hyperplasia. The karyotype was 46XX. He/she was diagnosed as simple virilizing form of CAH due to 21OH deficiency.
Case 5
A 35-year-old women with a 17-year history of hirsutism, and a 2-year history of hypertension was refferred for further evaluation. Endocrinologic data of the patient showed partial 11beta-hydroxylase deficiency, hyperandrogenism and bilateral micronodular adrenal hyperplasia.

P22

Role of chemokines MIP1α and MIP1β in patients with Addison's disease isolated or associated to autoimmune thyroid disease

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High levels of macrophage inflammatory proteins (MIP1α and MIP1β), related to the recruitment of Th1 and Th2 cells, respectively, have been evidenced in some organ and non organ-specific autoimmune diseases. CXCL10/IP10 has been evidenced in patients with autoimmune thyroid disease (ATD) and in Addison's disease (AD); MIP1α and MIP1β chemokines have not been so far evaluated in these diseases.
Aim
To evaluate plasmatic levels MIP1α, MIP1β and IP10 in patients with AD isolated or associated to ATD. MIP1α, MIP1β and IP-10 were evaluated in the plasma of 11 patients with AD associated to ATD (group 1), 8 patients (group 2) with isolated AD and 30 healthy controls (kit Bio-Plex Human Cytokine Assay). All patients were treated with an appropriate substitutive therapy, except 1 patient of group 1 and 2 of group 2 who had a new diagnosis of AD. The levels of MIP1α and MIP1β resulted significantly high ($P < 0.001$) in both groups with respect to controls. No significant difference was observed between the two groups. Moreover, levels of MIP1β were significantly and positively related to the titre of adrenal autoantibodies and were inversely correlated to the duration of the disease.
The presence of high levels of MIP1α and MIP1β not only in cases of autoimmune isolated AD, but also in those ones associated to ATD seems to indicate a role of these chemokines in the autoimmune pathology of these glands mediated by the recruitment *in loco* of Th1 and Th2 cells. The reduction of MIP1β, inversely correlated to the duration of the disease, seems to indicate a predominant role of these chemokines in triggering an inflammatory process by a massive recruitment of Th2 cells. Instead, the persistent high levels of MIP1α during the disease seem to favour a chronic autoimmune process by constant recruitment of Th1 cells.

P23

IL-6 and osteoprotogerin levels in subjects with non-functioning adrenal adenomas

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Data regarding cardiovascular risk in subjects with non-functioning adrenal adenoma is limited. The aim of this study is to investigate osteoprotogerin (OPG) and Interleukin-6 (IL-6) levels in subjects with non functioning adrenal incidentalomas.
Of 51 subjects without findings of hypercortisolism or other adrenal gland disorders (AI), 32 BMI-unmatched controls (C) and 20 BMI-matched controls

(BC) were enrolled. Participants underwent hormonal evaluation including morning cortisol, adrenocorticotrophic hormone (ACTH), post dexamethasone suppression test (DST), dehydroepiandrosterone sulfate (DHEAS) and urinary free cortisol. In subjects with elevated post DST cortisol (>1.8 mcg/dl), elevated UFC (>110 mcg/day), and suppressed ACTH and DHEAS levels, midnight cortisol was evaluated (normal < 7.5 mcg/dl).
AI group had increased BMI, blood pressure, waist circumference, post DST cortisol, uric acid and HOMA levels when compared with C. Blood pressure, uric acid and post DST cortisol remained significantly elevated in AI versus BC. IL-6 and OPG levels were comparable among groups (AI versus C and AI versus BC). IL-6 and OPG were not significantly correlated with hormonal parameters. IL-6 was correlated with BMI, waist circumference, triglyceride and uric acid in subjects with adrenal incidentaloma.
Despite the presence of several metabolic disturbances, subjects with AI did not feature IL-6 or OPG elevation. Exclusion of the patients with established cardiovascular events or diabetes might cause comparable levels of OPG and IL-6.

P24

Characterization of tyrosine hydroxylase expression in various adrenal tumors to confirm the diagnosis of adrenal pheochromocytomas

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Background
Tyrosine hydroxylase (TH) is the first enzyme in the pathway of catecholamine synthesis catalyzing the conversion of tyrosine to dihydroxyphenylalanine (DOPA). To establish a molecular marker for adrenal pheochromocytomas, we compared the expression in various adrenal tumors in comparison to normal adrenal glands.
Methods
Tissue from 19 pheochromocytomas (PHEO), 20 aldosterone-producing adenomas (APA), 20 cortisol-producing adenomas (CPA), and 20 non-functional adenomas (NFA) was obtained following laparoscopic surgery. Seven normal adrenal glands were obtained during autopsy. The diagnosis was confirmed by various biochemical tests, histological investigation, and clinical follow-up. Extracted RNA underwent Real Time RT-PCR using TH specific primers and probe (detection limit 3.2×10^2 copies/µg RNA (cp)). mRNA levels were normalized to GAPDH mRNA levels. ROC analysis was performed to established cut-offs with specificity of at least 95%.
Results
PHEO demonstrated higher TH expression with a median of 8.6×10^6 cp (range 7.2×10^4–4.3×10^7 cp) than detected in normal adrenal glands with a median of 1.1×10^6 cp (range 7.4×10^3–1.8×10^7 cp). In contrast, expression was significantly lower ($P<0.001$) in APA, CPA, and NFA with 2.8×10^4 cp (3.5×10^2–1.6×10^6 cp), 5.3×10^3 cp (7.5×10^2–2.5×10^5 cp), and 6.6×10^3 cp (3.2×10^2–1.5×10^7 cp), respectively. ROC analysis suggested a threshold of 1.1×10^6 cp with a sensitivity of 95% and specificity of 95%. No significant correlations were found between TH expression and nor-/metanephrine levels, chromogranin A levels or tumor size.
Conclusion
Characterization of TH expression may serve as a molecular marker to distinguish adrenal pheochromocytomas from other adrenal neoplasms. Such criteria could be used to evaluate biochemical tests for the diagnosis of these tumors.

P25

Towards an aldosterone producing cell line from an aldosterone producing adenoma

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To date, the in depth analysis of the key molecular mechanisms involved in functional autonomy and tumor formation in aldosterone producing adenomas has been hampered by the rarity of the disease and the lack of adequate tumor cell lines. Herein, we cultivated a primary cell culture of an aldosterone producing adenoma taken from a 40 year old male patient with a left sided adrenal tumor mass. The cells have been passaged 24 times and still continue to grow after nearly 11 months in a stable, cell line-like fashion. Adherent monolayer growth was observed, when the cell were cultured in serum containing media, whereas they adopted spheroid-like structures in EGF and FGF supplemented serum free media. Aldosterone output was measurable in spheroids (S) as well as in monolayer (M) cells (3107 ± 297.0 vs 706 ± 7.0 pg/ml, $P=0.01$), and could be further increased by ACTH stimulation (3382 ± 245.4 pg/ml vs 946 ± 29.2, $P=0.01$). Real-time PCR analyses revealed that in comparison to a normal (N) human adrenal gland, mRNA levels of 3ß-HSD and StAR - normalized to HPRT – were significantly lower in the cultured cells (3ß-HSD: 3.9 ± 0.13; $P<0.01$ (S) and 0.2 ± 0.003; $P<0.01$ (M) versus 1380 ± 219.6 (N), StAR: 475 ± 67.6; $P<0.01$(S) and 85 ± 22.4; $P<0.01$ (M) versus 7970 ± 740.6 (N)). However, these expression levels were similar to those measured in the established adrenocortical cancer cell line NCIh295R (3ß-HSD: 5.4 ± 0.38; $P<0.01$; StAR: 170 ± 28.7; $P<0.01$). This holds also true for P450scc, since only slight differences in mRNA expression between monolayer cells, spheroids, and NCIh295R cells could be detected (234 ± 5.1; $P<0.01$ (S) and 321 ± 9.6; $P=0.01$ (M) versus 294 ± 3.7 NCI). These results were supported by a clearly positive immunohistochemical staining for P450scc on embedded spheroids and monolayer cells, also verifying the adrenocortical origin of the cultured cells. Currently we are aiming at further defining the *in vitro* characteristics of the cultured aldosteronoma cells, especially regulatory pathways involved, the reaction to different stimuli, and eventually the ability for *in vivo* engraftment.

P26

Cardiac structure and function in patients with adrenal incidentaloma: an echocardiographic study

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Subclinical Cushing's syndrome (SCS) 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. Twenty patients and 20 sex- and age-matched healthy controls entered the study: among patients, 11 had SCS and the remaining nine 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 significantly higher in patients than in controls. At Doppler echocardiography, EF ($P<0.01$) and E/A ($P<0.01$) were significantly reduced in patients compared to controls. However, no significant difference was found in LVMi between patients and controls. In particular, both patients with and without SCS had significantly reduced EF and E/A compared to controls ($P<0.01$). A slight but not significant increase in LVMi ($P=0.099$) was found in patients with but not in patients without SCS. No significant difference in SBP and DBP was found between patients with and without SCS. In conclusion, patients with adrenal incidentaloma have an 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.

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Autoantibody screening of autoimmune gastrointestinal disorders in patients with autoimmune Addison disease

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Backrgound
Coexistent gastrointestinal pathology might alter hydrocortisone (HCT) and levothyroxine (LT4) absorption and gut transit times.

Aims
To screen patients with autoimmune Addison disease for the presence of serological markers of autoimmune gastrointestinal disorders, to compare doses of HCT and LT4 in patients with positive autoantibodies to those of patients without signs of autoimmune gastroenteropathy.
Subjects and methods
Of 62 patients with autoimmune Addison disease were investigated. Autoantibodies against tissue transglutaminase (t-TGA) were measured by CLIA, autoantibodies against gastric parietal cells (PCA) were measured by an indirect immunofluorescence method.
Results
Mean daily doses of HCT and LT4 were higher in patients (n = 13) with positive PCA and/or t-TGA – HCT 22.5 ± 5.9 vs 19.0 ± 2.9 mg ($P > 0.05$), HCT_{BW} 0.37 ± 0.08 vs 0.32 ± 0.04 mg/kg ($P < 0.05$), HCT_{BSA} 13.9 ± 3.0 vs 11.8 ± 1.3 mg/m^2 ($P < 0.05$); LT4 103.8 ± 21.4 vs 87.9 ± 28.0 μg ($P > 0.05$), $LT4_{BW}$ 1.75 ± 0.46 vs 1.47 ± 0.55 μg ($P > 0.05$), $LT4_{BSA}$ 65.4 ± 14.3 vs 54.7 ± 16.9 μg/m^2 ($P > 0.05$).
Conlusions
Our results suggest for high prevalence of serological markers of autoimmune gastrointestinal disorders in APS. Already state of PCA- and/or t-TGA positivity may underly the requirement of higher HCT and LT4 doses. Measurement of serum PCA and t-TGA should be part of the routine autoantibody screening and the diagnostic work up in Addison or APS patients with unexplained high HCT or LT4 doses.

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Prior dexamethasone suppression test predicts development of adrenal insufficiency after a 14 days' course of oral prednisone in healthy male volunteers

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Background
Neither dosage nor duration of systemic glucocorticoid therapy are reliable indicators of subsequent adrenal insufficiency. The dexamethasone suppression test is a measure of feedback sensitivity of the hypothalamic–pituitary–adrenal axis to cortisol. We hypothesized that this test predicts the later development of an adrenal insufficiency after a 14 days' course of oral prednisone in healthy volunteers.
Methods
We analyzed data from 13 healthy males from a total of 30 who will be included in this prospective observational study. After getting routine laboratory tests, including a low dose (1-μg) cosyntropin test, all participants underwent an overnight 0.5 mg dexamethasone suppression test. All subjects then took 0.5 mg/kg prednisone for 14 days. A low dose (1-μg) cosyntropin test was performed on day 1, 3, 7, and 21 after withdrawal to asses adrenal axis function.
Results
Cortisol levels after the dexamethasone suppression test significantly correlated with cortisol levels after low dose cosyntropin testing on day 7 ($r = 0.646$, $P = 0.02$). In patients with a cortisol level after dexamethason $<$ 35 nmol/l or $\geq$35 nmol/l, respectively, median cortisol levels after low dose cosyntropin testing were significantly lower on day 3 (495 nmol/l (IQR 446.5–518.75) vs 593 nmol/l (IQR 563–646), $P = 0.015$) and day 7 (494 nmol/l (IQR 474–532.75) vs 690 nmol/l (IQR 619.5–722.5), $P = 0.002$). A suppressed adrenal function occurred in 87.5 and 83.3% on days 3 and 7, respectively in participants with a cortisol level after dexamethasone $<$ 35 nmol/l as compared to 22.2 and 0%, respectively when cortisol after dexamethasone was $\geq$ 35 nmol/l ($P = 0.007$ and $P = 0.002$).
Conclusion
Cortisol levels after a 0.5 mg dexamethasone suppression test predict the development of a suppressed adrenal function at days 3 and 7 after a 14 days' course of oral prednisone. With this information a more targeted concept for the need of stress prophylaxis after cessation of steroid therapy can be envisioned.

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Glucocorticoid replacement therapy in adrenocortical insufficiency

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The effectiveness of glucocorticoid replacement therapy is based on clinical criteria, since objectives parameters are not standardized. The purpose of our study was to assess the adequacy of replacement therapy with cortisone acetate, trying to identify parameters indicating proper replacement. We studied 22 patients with adrenal insufficiency (7 primary and 15 secondary) treated with cortisone acetate (mean daily dose 36.5 +/− 2.1 mg at 0800 a.m. and 0300 p.m.), and six control subjects. Serum cortisol was evaluated in all the patients at 0800 at 0200, with drawings every hour, with parallel 24 h urinary free cortisol evaluation. The quality of life was assessed by standard questionnaires SF-36.
The 12 h trend of serum cortisol in patients did not overlap with that of controls. In patients, serum cortisol peak was noted 2 h after the morning dose and 1 h after the afternoon dose, being significantly increased compared with controls. Urinary free cortisol levels were normal in all patients. The quality of life was compromised in patients compared with controls, since greater impairment of physical or psychological well-being was associated with low serum cortisol levels recorded at 2 and 3 p.m., before to the second drug dose. Our data suggest that plasma cortisol evaluation performed 2 h after the morning dose and 1 h after the afternoon dose may be useful to monitor the replacement treatment with cortisone acetate. In particular, the morning peak reflects the physiological peak, while the afternoon peak may provide useful information on a possible underdosage, suggesting a different schedule of drug administration. In our study, impaired physical or psychological well-being is associated with low serum cortisol levels, suggesting that a three dose administration schedule might be useful to improve the quality of life.

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Study of hormonal activity and insulin resistance in patients harbouring incidentally discovered bilateral adrenal adenomas

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Background
Hormonal activity and insulin resistance (IR) in patients with incidentally discovered bilateral adrenal adenomas (BA) has not been evaluated until now. Therefore, we have investigated cortisol and aldosterone secretion and IR in 29 patients with BA, in 113 patients with unilateral adrenal adenomas (UA) and in 89 healthy subjects (C).
Methods
All subjects underwent the following investigation: 1) Low dose dexamethasone suppression test (LDDST) (0.5 mg DEX/6 h for 2 days) to access cortisol secretion, 2) NaCl (0.9%) infusion test (INF) (2lt NaCl 0.9% i.v. in 4 h) following LDDST to access aldosterone secretion, 3)2 h Oral Glucose tolerance test (OGTT) (75 g) with glucose and insulin measurements every 30 min. Homa ((fasting glucose (mmol/l)*fasting insulin (μIU/ml))/22.5) and Matsuda index{10 000/square root ((fasting glucosexfasting insulin)*(mean OGTT glucose*mean OGTT insulin)} were calculated for IR assessment.
Results
Cortisol and aldosterone cut-offs based on mean ± 2 S.D. values in CT group were calculated following LDDST and infusion test respectively. (cortisol cut-off: 34.11 (nmol/l) and aldosterone cut-off: 74.83 (pmol/l)). Autonomous cortisol or aldosterone secretion was found in 61.58 and 33.74% of patients with UA and in 65 and 28% of patients with BA respectively, whereas autonomous concomitant cortisol and aldosterone secretion was documented in 15.68% of patients with UA and 21% of patients with BA. The results of the performed tests are summarized below. ((mean ± S.E.M.), cortisol (nmol/l) aldosterone (pmol/l)).

	BA	UA	C	BAvsC	UAvsC	BAvsUA
Cortisol after LDDST	69.3 ± 10	58.1 ± 5	22.8 ± 1	$P < 0.01$	$P < 0.01$	ns
Aldosterone after INF	101.7 ± 22	78.4 ± 12	39.6 ± 2	$P < 0.01$	$P < 0.01$	$P < 0.01$
Homa index	2.2 ± 0.3	3.6 ± 0.2	4.6 ± 0.4	$P < 0.01$	$P < 0.05$	ns
Matsuda index	2.4 ± 0.2	3.7 ± 0.2	4.5 ± 0.3	$P < 0.01$	$P < 0.05$	$P < 0.05$

Conclusions
Autonomous cortisol and aldosterone secretion in patients with UA is more common than previously described. Hormonal activity of patients with BA is described for the first time. Patients harbouring BA appear to have more pronounced autonomous cortisol and aldosterone secretion and increased IR than patients with UA.

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Confounding variables for plasma metanephrines and normetanephrines may influence the diagnosis of pheochromocytomas

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Introduction
Measurements of plasma metanephrines (META) and normetanephrines (NOR) have been advocated as first-line tests for the diagnosis of pheochromocytoma. This study assessed the impact of several potential confounders, which may influence the correct diagnosis.
Methods
Eight healthy males (age 26.8 ± 1.7; BMI 23.1 ± 0.8) were tested on 6 separate days. Tests were performed in supine rest with blood sampling via i.v. canullas at 0900 h following an overnight fast. Samples were taken after venipuncture (0,15,30,60,90,120′), after caffeine (0,60′), after standard breakfast (0,60′), after physical exercise (0,15,30′), and in various body positions (upright and lying each 0,120′). Samples were centrifuged and frozen directly after collection. In addition, whole-blood and plasma samples were stored either at 4 °C or at room temperature (RT) for 0, 1, 3, 24, and 72 h. Plasma META and NOR were measured by RIA (LDN, Nordhorn, Germany).
Results
META and NOR were not significantly influenced by venipuncture. Caffeine (+23%) and food (+8%) elevated NOR significantly ($P<0.05$), while META remained stable. Physical exercise increased META (+82%) and NOR (+84%) significantly ($P<0.005$). Lying posture significantly decreased both META (−34%) and NOR (−19%) compared to standing position ($P<0.01$). In plasma, META and NOR were stable at 4 °C for 72 h, but decreased significantly, when stored at RT. In whole-blood, NOR were significantly increased after 72 h, both at 4 °C and at RT.
Conclusion
Blood samples for determination of META and NOR should be immediately centrifuged and stored at 4 °C to improve stability. Interestingly, levels of NOR increased in whole-blood, potentially due to ongoing enzymatic activity. Food, caffeine, and physical exercise should be avoided prior to sampling. Samples should be taken in a standardized posture. Significant changes due to confounders may lead to a misdiagnosis of pheochromocytomas.

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Is adrenal adenoma associated with the development of non-alcoholic fatty liver disease?

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It has been previously shown that adrenal adenomas are associated with a variety of metabolic disturbances like glucose intolerance and obesity. The aim of this study is to demonstrate the risk of non-alcoholic fatty liver disease development in subjects with adrenal incidentalomas.
Among 302 subjects referred between 2001 and 2008 for the evaluation of incidentally discovered adrenal mass, 150 subjects with appropriate data were included. Computed tomography (CT) was the initial radiological intervention. Initial hormonal evaluation included 1 mg or 2 day 2 mg dexamethasone suppression test, urinary free cortisol (UFC), 0800 a.m. ACTH and DHEAS levels. In subjects with elevated post DST cortisol (>1.8 mcg/dl), elevated UFC (>110 mcg/day), and suppressed ACTH and DHEAS levels, midnight cortisol was evaluated (normal < 7.5 mcg/dl). Urinary catecholamine excretion and aldosterone/renin ratio were also measured. Hepatosteatosis was evaluated with liver ultrasonography and or magnetic resonance imaging. ALT, AST and GGT were also measured.
There were 101 subjects with non-functioning adrenal adenomas, 40 subjects with functioning adrenal adenomas (Cushing or subclinical Cushing syndrome) and nine subjects with myelolipomas. The rate of non-alcoholic liver disease among these groups was 25.7, 22.7 and 11% respectively. After a median follow up duration of 22.5 months in a subgroup generated from study participants ($n=35$) without non-alcoholic fatty liver disease, we showed that 9% of subjects with non-functioning adenomas but 17% of subjects with sub clinical Cushing syndrome developed non-alcoholic liver disease.
Non-alcoholic liver disease, which is an important manifestation of insulin resistance is significantly associated with adrenal cortical adenomas rather than extra-cortical masses such as myelolipomas. The risk of developing non-alcoholic fatty liver disease is related with cortisol autonomy.

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A multi-institutional audit of laparoscopic adrenalectomy in Greece and the UK

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Objective
To evaluate the results of laparoscopic adrenalectomy in our institutions.
Methods
Prospectively collected data from 2000 to 2007.
Results
During the 8-year study period, 160 laparoscopic adrenalectomies were performed in 150 patients. Mean age was 49.8 years (female: 60.7%). Twenty-seven patients (18%) had previous open abdominal operations. There were 10 bilateral (6.6%) for Cushing's disease, 64 right (42.7%) and 76 left (50.7%) adrenalectomies. Diagnosis in unilateral cases was Conn's syndrome in 43 patients (30.7%), non-functioning adenoma in 35 (25%), phaeochromocytoma in 26 (18.6%), Cushing's syndrome in 20 (14.3%), metastasis in 8 (5.7%) and other in 8 (5.7%). Median tumour diameter was 4.0 cm, with 48 (30%) tumours >5.0 cm. In particular, 37 (23.1%) tumours were <3.0 cm, 75 (46.9%) were 3.0–5.0 cm, 9 (5.6%) 5.1–6.0 cm, 18 (11.2%) 6.1–7.0 cm, 14 (8.8%) 7.1–8.0 cm and 7 (4.4%) tumours were >8.0 cm. Median operative time was 130 min for bilateral and 55 min for unilateral procedures. Eleven cases (7.3%) underwent concurrent laparoscopic surgical procedures. Three adrenalectomies (1.8%) required conversion: a 10 cm phaeochromocytoma, a 4.5 cm phaeochromocytoma involving the renal artery and a 6.2 cm metastatic tumour extending to extra-adrenal tissues. Morbidity was 2.6% ($n=4$) and no mortality occurred. Median hospital stay was 48 h; five patients were discharged 5–8 h after the procedure.
Conclusions
Laparoscopic adrenalectomy, even for large tumours, is safe and effective when performed by surgeons highly experienced in laparoscopic endocrine surgery.

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The utility of the low dose dexamethasone suppression test in patients diagnosed with an adrenal incidentaloma

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Objective
The diagnosis of subclinical Cushing's syndrome in patients with incidentalomas is not always straight forward and a number of different criteria have been used. The 1 mg overnight dexamethasone suppression test has been recommended as a screening test, followed up by other tests of the hypothalomo–pituitary–adrenal axis to confirm the diagnosis. In this study we investigate whether the low-dose dexamethasone suppression test offers additional information to the overnight dexamethasone suppression test in establishing diagnosis.
Design
Retrospective, observational study.
Patients and measurements
Demographic and clinical data were collected on 137 patients diagnosed with incidentalomas. A full endocrinology work up was performed.
Results
There were 76/137 (55%) patients who had either a positive overnight dexamethasone suppression test or/and a low-dose dexamethasone suppression test. 60/103 (58%) patients were found to have a positive overnight dexamethasone test whilst 45/63 (71%) patients had a positive low-dose dexamethasone test. 29 patients had both tests done of which 20/21 patients with a cortisol level >70 nmol/l after the overnight dexamethasone test had a positive low-dose dexamethasone test, whilst the other 8 patients, all of which had a cortisol level <70 nmol/l post-overnight dexamethasone test, had a negative low-dose dexamethasone test. Correlation analysis revealed a significant positive correlation between cortisol levels for both tests ($r=0.78$; $P<0.001$). Mean cortisol levels after each test were similar (103 vs 104.8 nmol/l; $P=0.9$).
Conclusion
We have established that in patients with a cortisol level of >70 nmol/l after an overnight dexamethasone test, the low-dose dexamethasone suppression test is

usually positive and will not offer more information than the overnight dexamethasone suppression test in the diagnostic work-up of subclinical Cushing's syndrome.

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Synacten test in patients with adrenal incidentaloma

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Considering that adrenal tumors are frequent in patients with congenital adrenal hyperplasia (82% in homozygote and 45% in heterozygote), Synacten test was at first introduced as marker of congenital adrenal deficiency in patient with adrenal incidentaloma. Recent studies showed normal and exaggerated cortisol response in more than 50% of patient with nonhypersecretory adrenal incidentaloma and more than 70% of patients with adrenal incidentaloma and subclinical hypercorticism, witch can not be explained by variant form of 21 hydroxylase deficiency.
The aim of our study was to test cortisol response in patients with adrenal incidentaloma.
Of 208 patients (148 women and 60 men, mean age 55.08 ± 11.02 years and mean BMI: 27.91 ± 4.6 kg/m^2 with adrenal incidentaloma-CT/MR confirmed were admitted to our Instiute, tested and divided in two subgroups: First: 46 patients (38 women and 8 men, mean age 56.6 ± 9.25 years and mean BMI 27.83 ± 4.37 kg/m^2) with confirmed subclinical CS; Second: 162 patients (110 women and 52 men, mean age 54.66 ± 11.45 years and mean BMI 27.93 ± 4.67 kg/m^2) with nonhypersecreting adrenal tumors. Cortisol response to ACTH stimulation was tested in 110 patients. Our results shows mean cortisol value significantly higher in group with subclinical CS (Mann Whitney Test: $W=513$; $P<0.01$). AUC for cortisol during the test were significantly higher in patients with SCS (64 846, 15 vs 44 337, 59; Mann Whitney Test: $W=621.5$; $P<0.01$).
In conclusion, enhanced cortisol response to ACTH stimulation can be partially explained by altered intratumoral steroidogenesis maybe due to presence of some glucocorticoid receptor (GR) gene polymorphisms.

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Central ghrelin modulates morphology and function of adrenal cortex in male rats

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Ghrelin is a growth hormone secretagogue that plays an important role in appetite and weight regulation. We have previously demonstrated that central ghrelin stimulated pituitary ACTH synthesis and secretion. In this study we further examined the effects of intracerebroventricular (ICV) ad-mi-nistration of ghrelin on zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) of the adrenal cortex, as well as the blood concentrations of aldosterone, corticosterone and dehydroepiandrosterone (DHEA) in male rats. Rats received 1.0 μg of ghrelin dissolved in 5.0 μg of PBS ICV, whereas control rats received only ICV PBS, during five consecutive days. The rats were decapitated 2 h after the last ICV ghrelin injection. Blood samples were collected from each rat for hormonal analyses, while adrenal glands were excised and prepared for further histological and morphmetrical measurements. Ghrelin treatment significantly increased ($P<0.05$) body weight by 13% compared to controls. Absolute weight and volume of whole adrenal glands were significantly ($P<0.05$) increased by 17.9% and 19.7%, respectively, in comparison with control values. The absolute volume of adrenal cortex, *zona glomerulosa, zona fasciculate* and *zona reticularis* were also significantly ($P<0.05$) increased by 20.3, 20.9, 21.4 and 11.1%, respectively, in comparison with the corresponding controls. Serum concentrations of the aldosterone, corticosterone and DHEA were significantly ($P<0.05$) increased by 32.3, 66.5, and 28.0%, respectively. These re-sults indicate that central ghrelin acts stimulatory on growth and secretory function of all adrenocortical zone cells of adult male rats.

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Pheochromocytoma: a retrospective study on clinical presentation, management and outcomes

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Pheochromocytomas are rare, catecholamine-secreting, adrenal neoplasms. In about 25% of cases they arise in patients with germline mutations. Malignancy occurs in about 10%.
We retrospectively analysed the records of patients with histological diagnosis of pheochromocytoma submitted to adrenal surgery between 1987–2008 and followed in the Endocrinology department.
Thirteen patients were included. We evaluated age on diagnosis; clinical presentation, urinary concentration of cathecolamine metabolites; imaging at diagnosis; pre-operative management; surgical complications and clinical evolution.
Mean age was 45.2 ± 20.2 years (20–77); seven patients (53.8%) were males. Six patients (46.2%) had sustained hypertension, two presented with acute pulmonary oedema. The mean delay of diagnosis after clinical presentation of hypertension was 5.6 ± 6.2 year. Four cases (30.8%) presented as incidentalomas. Mean tumor size was 6.4 ± 3.2 cm (1.4–13.8); six cases (46%) were on the right adrenal gland and two were bilateral. Mean urinary metanephrines increase was 8.9 times from the reference range; Vanilmandelic acid was normal in three cases. The mean fenoxibenzamine dose used in the pre-operative preparation was 28.3 ± 7.5 mg. The most common surgical complication was hypotension after removal of the tumour. The surgical approach was made by laparotomy in six cases and by laparoscopy in three. Two patients had malignant sporadic pheochromocytoma, one died 9 years after surgery, the other has been followed for 21 years and has received five MIBG treatments for bone metastasis. Four patients were lost to follow-up and eight (61.6%) are in remission for 6.5 ± 5.5 years. Four of them belonged to two different families and had MEN2A. In this cohort, tumour size did not significantly correlate with urinary metanephrines ($ro=0.4$, $P=0.35$, Spearman test).
In conclusion, urinary metanephrines were the most useful method in confirming diagnosis. Incidentalomas were a frequent presentation. Establishing the prognosis of these situations remains difficult so close follow-up is required in order to prevent further complications.

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Epidemiological study of adrenal mass in our clinical centre

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Adrenal tumours form a very heterogeneous group by their origin, clinical presentation, size and biological potential.
The aim of our retrospective study was the epidemiological analysis of the patients with adrenal tumours. We analyzed the gender, age, localisation, clinical presentation, and in patients who were treated surgically- the type of the intervention, patohystological and immunohystochemical finding from the 01.01.2000 to 01.01.2008. The linear trend of the frequency of these tumours during this period was calculated.
The results
We treated 149 patients with adrenal tumour, what makes 0.025% of the population gravitating to our Clinical Centre. There were 98 (65.8%) female and 51 (34.2%) male patients, with the mean age 53.47 ± 11.9 years. The greatest percentage were the patients with afunctional tumours (77.8%; 116 patients), out of which 72 (62.1%) were the incidentalomas. Among functionally active tumours the most frequent were the Cushing syndrome 57.6% (12.7% of all tumours), pheochromocytoma 27.3% (6% of all tumours), Conn's syndrome 15.1% (3.3% of all tumours). Of 125 patients had tumours of one adrenal gland, 71 (56.8%), of the right and 54 (27.1%) of the left adrenal gland, while 23 (15.4%) patients had bilateral tumours. There were 84 patients (56.4%) with tumors less than 3 cm, 48 patients (32.2%) with tumors from 3 to 6 cm, and 17 (11.4%) greater than 6 cm. Of 51 patients (34.2%) were operated on, 31 (60.8%) with classical, and 20 (39.2%) laparoscopic approach. Immunohystochemystry showed 46 (90.2%) benign and 5 (9.8%) malignant tumours. The linear trend showed the increase in the incidence of adrenal tumours, especially in current year.
Conclusion
The results show the adrenal tumours not to be a rare disease in our surroundings and that there is an increase in the incidence during this eight years period. A functional and benign tumours are the most frequent.

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Anemia in male patients with cushing's syndrome before and after cure

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Glucocorticoids are known to exert a stimulatory action on white blood cell precursor proliferation but little is known on erythrocyte counts in patients with Cushing's syndrome.
Aim
Of the present study was to assess red and white blood cell parametres in a large series of patients with Cushing's syndrome and follow the blood cell changes after postsurgical remission or persistence of hypercortisolism.
Methods
Of 84 patients with Cushing's syndrome (67 women, 17 men, age 38.9±9.98 year) were evaluated prior to surgery and for up to 257 months (mean 47.7±2.6 months) after pituitary/adrenal surgery.
Results
Leukocytosis ($>9000/mm^3$) was detected in 46% of patients with Cushing's syndrome; leukocyte counts fell promptly after remission of disease (9800±350 vs 7200±130/mm^3 in cured patients, $P<0.05$; 9280±81 vs 8050±210/mm^3 in surgical failures, NS) with a consistent drop in neutrophils (68.9 vs 54.5%, $P<0.05$) and slight increase in eosinophils (1.1 vs 2.4%, $P<0.05$) compared with presurgical values. Red blood cell counts were in the upper normal range in women with Cushing's syndrome (Hb 13.7±0.17 g/dl; RBC 4.5±0.65×$10^6/mm^3$) whereas, unexpectedly, male patients presented with low-normal hemoglobin (14.4±0.22 g/dl) and RBC (4.5±0.13×$10^6/mm^3$) and four patients were frankly anemic (Hb <13 g/dl). Surgery *per se* was followed by a decrease in Hb levels by 1.5–2 g/dl, regardless of surgical outcome. Women cured of Cushing's syndrome rapidly restored and stabilized their Hb levels around 13.2±0.19 g/dl whereas a longer time (up to 3 years) was necessary to achieve normal RBC counts in cured men. Indeed, mean Hb levels in the middle quartiles of the normal range (15.2±0.31 g/dl) were observed on average 36 months after surgery. The recovery of RBC appeared independent of replacement therapy and was correlated to testosterone levels ($r=0.349$, $P<0.05$).
Conclusions
Male patients with Cushing's syndrome present relatively low RBC counts, possibly linked to the attendant hypogonadism, which resolve over time after surgery. This study highlights yet another unfavourable feature of men with Cushing's syndrome (Pecori Giraldi JCE&M **88**:1554–1558, 2003).

P40

Bilateral adrenal hemangioma cavernosum: NMI and a CT scanning as a complementary methods in newly detected degeneration in one gland

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Hemangiomas are the rare cases of adrenal incidentalomas, especially in their bilateral forms. Case study: male patient, 65 year, presented after the pain in right upper hemi abdomen, lasted half year. On ultrasonography there was an enlarged right suprarenal gland. Patient was on routine abdominal ultrasonography, with undetectable suprarenal glands, one year before. We concluded that something new happened in the meantime. On NMR the right suprarenal gland was 6.2×4.7 cm, with a peripheral spotty contrast enhancement and centripetal enhancement. Despite the highly suspected typical pattern of enhancement for the diagnosing of adrenal hemangioma, it was not possible distinguishing the adrenal tissue from the liver and kidney tissue making diagnosis of a benign lesion difficult. Left adrenal gland was 3.7×2.7 cm with low signal and postrcontrast patchy enhancement. Potassium, cortisol and catecholamins were in referent values. So we indicated CT scanning which clearly separated the liver from suprarenal tissue. Bone scintigraphy scans was normal. Pathohistology after operation confirmed the diagnosis of cavernosal hemangioma with cystic cavities up to 1 cm, clotting blood, fibrosis, sclerosis and calcifications within the extirpated suprarenal gland. After operation patient insisted that the remaining pain was 1/10 of that preoperatively present. Conclusion: Degenerative process in one adrenal cavernosal hemangiomas led to the development of the pain in abdomen. In this case study MRI better described tissue characteristics and CT its shapes leading to a high suspected preoperative diagnosis.

P41

Prospective evaluation of metabolic and anthropometric parameters in non-functioning adrenal adenomas

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Adrenal adenomas are characterised with metabolic disturbances. The aim of this study is to demonstrate the changes in some metabolic and anthropometric parameters after 24 months follow up in subjects with non-functioning adrenal adenomas.
Subjects referred between 2001 and 2008 for the evaluation of adrenal tumours were included. Computed tomography (CT) was the initial radiological intervention. Initial hormonal evaluation included 1 mg or 2 day 2 mg dexamethasone suppression test, urinary free cortisol (UFC), 08.00 a.m. ACTH and DHEAS levels. In subjects with elevated post DST cortisol (>1.8 mcg/dl), elevated UFC (>110 mcg/day), and suppressed ACTH and DHEAS levels, midnight cortisol was evaluated (normal < 7.5 mcg/dl). Urinary catecholamine excretion and aldosterone/renin ratio were also measured. BMI, waist circumference, blood pressure were recorded. Fasting plasma glucose, fasting insulin, lipid parameters, hsCRP and fibrinogen were measured.
Of 140 subjects with adrenal adenomas were included. There were 106 subjects with non-functioning adenomas and 34 patients with subclinical Cushing syndrome or overt adrenal Cushing Syndrome. Median follow up duration was 24 months. Mean age was 55 years and female dominance was present (104/36). We showed that, in subjects with non functioning adrenal adenomas, after a 24 months follow up, new onset hypertension was diagnosed in 19% of the normotensive subjects ($P=0.004$, Mc-Nemar) and new onset hyperlipidemia was diagnosed in 46% ($P<0.001$, Mc-Nemar) of the subjects with normal lipid values. The rate of diabetes development was 5%. We also showed that BMI, fasting glucose, total cholesterol and LDL-cholesterol increased and DHEAS levels decreased significantly after follow up in subjects with adrenal adenomas. Adrenal adenomas even non functioning ones may be associated with future metabolic risks. Close monitoring and treatment of traditional risk factors should be taken into consideration in subjects with adrenal adenomas.

P42

Metastases of renal cell carcinoma to the adrenal glands: results of surgical treatment

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Renal cell carcinoma metastases to adrenal glands are indicative of a generalized tumor process, but in cases of solitary metastases, a radical surgical treatment of these patients is possible.
The object of the present study was an estimation of the remote outcome of surgical treatment of 18 patients with solitary renal cell carcinoma metastases to adrenal glands operated on in our clinic over the period from 1999 to 2008. Among them, there were 6 men and 12 women, aged 52–77 year (mean age 65+5.7 years). In all cases, the renal cell carcinoma was verified during histologic investigation of the renal tumor. In 16 patients, adrenal metastases were unilateral, in 2 – bilateral; in 8 patients – synchronous, and in 10 – metachronous. Metachronous metastases were revealed 9–180 months later (mean 41.2 months) the radical nephrectomy. For topical diagnosis, US-scan, CT or MRI, and PET-scan were used. Adrenalectomy including tumor removal was an operation of choice (a total adrenalectomy was carried out in cases of bilateral adrenal metastases). A control group consisted of 8 patients who received only symptomatic treatment because of the multiple adrenal metastases from the renal cell carcinoma.
Follow up period after radical surgery of metastatic tumors was, on an average, 40.4+6.2 months (variation limits – 4–110 months); 11 patients are still alive, and 7 – died of tumor progression (metastases to contralateral kidney, liver, and pancreas). The 1-, 3- and 5-year overall survival, calculated by the Kaplan–Meier method, was 71.4%, 64.9% and 43.2%, correspondingly. In the control group, the index of the 1- and 3-year overall survival formed 44.4% and 0%, accordingly ($P<0.05$). The median overall survival after adrenalectomy was 67.2 months that was reliably higher as compared with that of the control group (23.2 months, $P<0.05$). The 1-, 3- and 5-year disease-free survival was 70.1, 53.1 and 27.3%, conformably. The median disease-free survival after the radical adrenalectomy was 57.1 months.

The outcome obtained allows, in our view, to recommend adrenalectomy as a method of radical treatment of patients with renal cell carcinoma metastases to adrenal glands.

P43

Iatrogenic Cushing's syndrome induced by topical corticoid application in child and adult

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Topical application of corticosteroids is frequently used in the therapy of dermatological diseases due to their anti-inflammatory and immunosuppressive effects. On the other hand, when reaching pharmacological levels, exogenous corticoids frequently lead to complete Cushing syndrome, including skin modifications, irrespective of the route of administration. We describe four cases of iatrogenic Cushing's syndrome triggered by topical application of corticoids. The first two cases are those of VM, a 4-month-old girl and DAR, a 2-year-old boy treated for eczema with clobetasolum propionate for 2 and 6 months, respectively. The third case is a 51-year-old woman, BE, who also used clobetasolum propionate and applied it to generalized lesions of psoriasis for more than 1.5 years. Both children and patient BE developed overt iatrogenic Cushing's syndrome, with centripetal obesity, typical cutaneous modifications and significant behaviour disturbances. Low morning plasma cortisol, urinary cortisol and ACTH confirmed the exogenous nature of hypercorticism. The children's growth was slowed during therapy, with significant delay seen in DAR (-3 S.D.) BE developed insulin-necessitating diabetes mellitus and hypertension. Therapy arrest provoked prolonged hypocorticism with asthenia, depression and low blood pressure in DAR and BE. The fourth patient, AN, an obese 24 year old male adult, used topical axillary application of flumetasone pivalate for hydrosadenitis. This patient developed cutaneous changes suggestive of Cushing's syndrome that were limited to the surface of the application and showed no pathological modifications of the corticotrophic axis or corticoid-related complications. Iatrogenic Cushing syndrome from topical application is common in children, but rare in adults. Individual sensitivity, local absorption capacity and the particularities of the topical corticoid used may all be responsible for the large variety of corticoid-related side effects in patients using topical corticoid therapy. Therapy should not be abruptly interrupted, but rather tapered for avoiding transient adrenal insufficiency.

P44

A rare cause of hyponatraemia presenting as acute adrenal insufficiency: bilateral adrenal haemorrhage

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We report a rare case of 37 years female of Greek and Irish extraction with a history of thalassemia trait, who presented with generalised weakness and severe loin and pelvic pain 9 days post vaginal hysterectomy and 3 days after hospital discharge. After readmission, she developed mild pyrexia (37.5 °C), hypotension, mild hyponatraemia with plasma sodium which fell from 138 to 131 mM and early signs of acute respiratory distress syndrome. A Short Synacthen test was performed and the patient was empirically commenced on stress dose of intravenous hydrocortisone.

CT scan of abdomen and pelvis revealed pelvic haematoma and bilateral adrenal haemorrhage. The infected haematoma was surgically drained and the patient was treated empirically with meropenem and gentamicin and later on with linezolid. Blood and pelvic haematoma culture failed to grow any organisms. Baseline plasma cortisol was 89 nM and 30 min post tetracosarin was 92 nM confirming adrenal insufficiency. The patient made a full recovery and was discharged on maintenance oral hydrocortisone.

The diagnosis of adrenal insufficiency in patients with bilateral adrenal haemorrhage is challenging and requires clinical suspicion due to the subtle nature of its presentation, and has previously been well documented as a post-mortem diagnosis. The combination of hypotension, hyponatraemia and generalised lethargy should lead to empirical glucocorticoid treatment until confirmation of adrenal insufficiency is made by hormonal evaluation.

Adrenal haemorrhage is a frequently fatal condition but if diagnosed, may be successfully treated. It may develop without predisposing conditions but more often occurs in patients subjected to severe stress such was in the described case. This rare case illustrates the importance of rapid onset hyponatraemia and unexplained hypotension as markers of acute adrenal insufficiency.

P45

Long-term follow-up of a 46XX patient with congenital lipoid adrenal hyperplasia due to a new mutation of the steroidogenic acute regulatory protein gene

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Congenital lipoid adrenal hyperplasia (CLAH) is a severe disorder characterized by early impairment of both adrenal and gonadal steroidogenesis, leading to early adrenal failure and male sex reversal. The most common aetiology of CLAH is mutation of Steroidogenic acute regulatory protein (StAR) gene.

Objective

We report evolution over 20 years of a 46XX patient harbouring a novel StAR gene mutation.

Methods

Clinical, hormonal and imaging data were retrospectively collected; molecular analysis was performed by direct sequencing of the StAR gene.

Results

This 46XX patient had a younger 46XY phenotypically female sibling who died of acute adrenal failure at 4 months of age. A novel homozygous 719delC StAR gene frame shift mutation was found, leading to large modifications of the C-terminal end. The child presented at 10 days of life with salt-wasting and recovered on adrenal replacement therapy. She had normal psychomotor development. Early overweight was observed despite elevated ACTH and plasmatic renin activity on high doses of hydrocortisone. Gonadal replacement therapy was initiated at age 13 year, while spontaneous onset of puberty was observed at age 12. After 2 months discontinuation of the oestroprogestative therapy, no ovulation occurred, but development of large ovarian cysts was noticed. Imaging did not show any lipid deposit in adrenals or gonads during follow-up, but right adrenal hypoplasia. Cerebral MRI showed white matter abnormalities.

Conclusion

This long-term follow-up report illustrates spontaneous evolution of 46XX patients presenting CLAH due to a StAR gene mutation. In such patients, adrenal replacement therapy should be adapted on the basis of clinical rather than biological data and gonadal substitution should not be interrupted because of risks of life-threatening ovarian cyst.

P46

Insulin hypoglycemia test in the diagnosis of subclinical Cushing's syndrome

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Although subclinical Cushing's syndrome (SCS) is an important metabolic risk factor in patients with adrenal incidentaloma, the diagnostic criteria for SCS has not been established yet. The aim of this study was to evaluate the diagnostic measures of SCS and to investigate the availability of insülin hypoglycemia test (IHT) in the diagnosis of SCS. Twenty patients with adrenal incidentaloma CT characteristics highly suggestive of benign adrenal adenoma with no overt hormonal hyperfunction were included in the study. Increase in the basal serum cortisol concentration or in the urinary cortisol excretion, absence of diurnal cortisol rhythm, insufficient suppression of cortisol with dexamethasone, decrease in the concentrations of DHEA-S or ACTH were used as diagnostic parameters for SCS. According to these parameters, patients are grouped as definite (2/20), probable (5/20) and improbable (13/20) SCS. All the patients were underwent

insulin hypoglycemia test (IHT). Maximum increase in serum cortisol concentration after hypoglycemia (ΔF) was evaluated and borderline significance ($P=0.053$) was noticed between definite SCS and the other groups. When ΔF was compared with the other diagnostic parameters of SCS, there was significant correlation only with post 3 mg DST serum cortisol concentration ($r=-0,463$; $P=0,04$). The sensitivity and specifity of IHT in SCS was calculated 42 and 84%, respectively.
In conclusion, according to these findings sensitivity and specificity of IHT are not high enough to be used as a diagnostic test for SCS and also because of the risks, IHT is not suitable in the routine practice.

P47

Carotid intima media thickness is increased and associated with morning cortisol in subjects with non-functioning adrenal incidentaloma

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Data regarding cardiovascular risk in subjects with non-functioning adrenal adenoma is limited. The aim of this study is to investigate carotid intima media thickness (IMT) as a robust indicator of atherosclerosis in subjects with AI.
Of 49 subjects without findings of hypercortisolism or other adrenal gland disorders, 34 BMI-unmatched controls (C) and 18 BMI-matched controls (BC) were enrolled. Participants underwent hormonal evaluation including morning cortisol, adrenocorticotrophic hormone (ACTH), post dexamethasone suppression test (DST), dehydroepiandrosterone sulfate (DHEAS) and urinary free cortisol. Anthropometric and metabolic parameters and carotid IMT were measured.
AI group had increased BMI, blood pressure, waist circumference, post DST cortisol, uric acid and HOMA levels when compared with C. Blood pressure, uric acid and post DST cortisol remained significantly elevated in AI vs BC. Average IMT was increased significantly in AI vs C (0.74 vs 0.68 mm, $P=0.029$) and insignificantly elevated in AI vs BC (0.74 vs 0.67 mm, $P=0.086$). IMT was correlated with age, BMI, HOMA, waist ciricumference, morning cortisol and uric acid. Morning cortisol was independently associated with HOMA levels in both AI group and all participants.
Increased IMT in non-functioning AI was a consequence of insulin resistant state associated with subtle cortisol autonomy rather than a direct effect of cortisol. The difference of IMT values between AI and BMI-matched controls and the linear association between morning cortisol and IMT favor but not exactly illuminate the direct effects of hypothalamus-pituitary-adrenal axis disturbances on vasculature.

P48

Cannabinoids and regulation of adipogenesis in differentiating 3t3-l1 preadipocytes

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Objective
Endocannabinoids (CBs) are novel lipid mediators that modulate appetitive behaviour and energy metabolism. The overactivation of the endocannabinoid system plays an important role in obesity. Endocannabinoids may regulate the lipid metabolism through their receptors (CB1 and CB2) in liver and adipose tissue. 11β-hydroxysteroid dehydrogenase (11β-HSD1) type 1 regulates the local availability of active glucocorticoid, potent inductors of adipogenic differentiation. We hypothesized that CBs are involved in adipogenesis and investigated whether this occurs via 11β-HSD1 modulation.
Materials and methods
Of 3T3-L1 cells were either differentiated with a cocktail containing dexamethasone + IBMX + insulin, or with insulin alone and treated with anandamide (AEA 100 nM–10 mcM) for 48–96 h. Gene expression was analyzed by reverse transcription followed by PCR with specific murine primers for 11β-HSD1 and beta-actin as housekeeping gene. 11β-HSD1 activity was measured as [3H]-cortisone into [3H]-cortisol conversion estimated by TLC separation and beta-scanning.
Results
At day 7 of differentiation, with cocktail and insulin, AEA stimulates 11β-HSD1 expression compared to control ($+30.4\pm0.08\%$ AEA vs C, $P=0.005$) and induces a slight increase of its enzymatic activity ($+15.3\pm3.5\%$ AEA vs C). In presence of insulin alone, AEA strongly increases 11β-HSD1 mRNA levels of three times when compared to C ($P<0.0012$). In mature adipocytes AEA increases significantly 11β-HSD1 activity ($+77.1\pm36.1\%$ AEA vs C, $P=0.0002$) and mRNA levels ($+21\pm0.51\%$ AEA vs C, $P=0.01$). Preliminary results show that selective CB1 blockade is not effective in antagonizing AEA, whereas by itself seems to affect 11β-HSD1. CB2 blockade properly antagonizes the effects on 11β-HSD1.
Conclusions
CBs influence the expression and activity of 11β-HSD1 during adipogenesis, by promoting adipocytes maturation even in cells not receiving differentiation cocktail. Therefore, CBs not only has a lipogenic effect in mature adipocytes, but also induce adipogenesis in differentiating cells. The interplays between CB1 and CB2 seem to be involved in this process.

P49

Laparoscopic resection of a large adrenal oncocytoma

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Background
Oncocytomas are predominantly benign tumors that are well described in the kidney, thyroid and salivary glands. They have also been reported in more rare sites including the pituitary and parathyroid glands, respiratory tract and choroid plexus. The occurrence of these tumors in the adrenal gland, however, represents an extremely exceptional finding.
Case presentation
We report the case of a 34 year-old male patient who presented with a right-sided renal colic. Abdominal ultrasonography revealed lithiasis of the right kidney as well as a right adrenal mass. Computed tomography scan of the abdomen demonstrated a large suprarenal retroperitoneal tumor, 6.5 cm at its largest diameter, with no lymphadenopathy and no other intraabdominal neoplastic sites. All blood and urinary tests were normal apart from elevated serum progesterone and testosterone levels. The latter finding leaded to an MRI scan of the hypophysis that revealed an adenoma. The patient underwent a laparoscopic right adrenalectomy via the lateral transperitoneal approach. A well-circumscribed, encapsulated right adrenal mass was identified. The tumor, with the perirenal fat, was dissected from the liver and superior pole of the right kidney, and completely resected. Gross inspection of the specimen during pathologic examination, revealed a grayish and soft mass, measuring $7.5\times6.5\times5.2$ cm in diameter and weighting 168 g. Immunohistochemical stains for vimentin, synaptophysin and calretinin were positive. Ultrastructurally, the neoplastic cell morphology was consistent with adrenocortical oncocytoma with the presence of myelolipoma foci.
Conclusions
Although very infrequent, oncocytomas should be included in the differential diagnosis of adrenal masses. The potential association of the presented lesion with the adenoma of the hypophysis and elevated progesterone and testosterone levels merit further study.

P50

Effects of corticosterone intake as stress-alternative hormone on broiler chickens: performance and blood parameters

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This study was conduced to determine effects of blood corticosterone (CS) increasing on some physiological parameters and performance of boiler chickens. To avoid treatment of birds with various forms of stress with administration of CS a model was developed to study of mimicked stress in chickens. Total 180 1-day old chicks of the cobb-500 strain from male sex were placed in 12 pens. CS at four

levels (0, 10, 20, and 30 mgl/1) in drinking water was provided *ad libitum* between 1 and 49 days of age. Continuous intake of CS for 49 d caused increasing in serum glucose, cholesterol, triglycerides, high and low density lipoprotein and mortality. Final body weight, total feed intake and abdominal fat deposition were decreased, whereas feed conversion ratio was constant. The relative weights of major immunobiological organs including spleen, thymus and bursa of fabricius were decreased ($P<0.05$). Numerically, weights of selected visceral organs especially liver were elevation in all groups that received higher levels of CS. Therefore, it seems that CS intake is an alternative tool and useful test for assess the effects of physical, psychological and physiological stress in researches on broiler chickens.

P51

Evaluation of cardiovascular risk factors in patients with incidentally discovered adrenal adenoma during a follow-up longer than five years

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The existing follow-up studies on patients with incidentally discovered adrenal adenoma (AA) focused almost exclusively on repeat imaging and endocrine work-up on a short period.
Of consecutive 152 patients with AA referred to our center from 1989 to 2003, we have assessed the risk of developing metabolic disorders and vascular complications in the 74 patients who did not underwent surgery (51 women and 23 men, median age 57, range 25–74 years) during a follow-up of at least 5 years (median 84, range 60–228 months). The diagnosis of AA was based on either typical CT characteristics or repeat scan after 3–6 months; median size of AA was 2.9 cm (range 1–6 cm). Comparing the clinical and biochemical characteristics at diagnosis and at the last follow-up, we observed a slight worsening of the cardiovascular risk profile that was non statistically significant considering advancing age (obesity 12.1% at diagnosis vs 14.8% at the last follow-up, hypertension 48.6 vs 60.8%, metabolic syndrome, defined according to ATP III criteria, 22.9 vs 28.3%, impaired glucose tolerance 25.7 vs 27.0%, diabetes mellitus 6.7 vs 16.2%, dyslipidemia 45.9 vs 51.3%). At diagnosis, 4 patients had a previous cardiovascular event and 5 patients had a new cardiovascular event during follow-up. There was no correlation between hormonal data and the change of the cardiovascular risk profile. An increase in AA size of >0.5 cm was reported in 9 (12.1%) patients and 2 of them had an increase >1.0 cm.
We found a high prevalence of cardiovascular risk factors at diagnosis in patients with incidentally detected AA who, however, develop new metabolic and vascular complications unfrequently in the long-term. Moreover, AA have a limited potential of growth. These data suggest that most patients with incidental AA should be managed conservatively.

P52

Expression of mTOR pathway in human adrenocortical carcinomas and *in vitro* effects of mTOR inhibitors in human adrenocortical cell lines

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Background
Adrenocortical carcinoma (ACC) is an uncommon malignancy with a still scantily understood pathogenesis and generally poor prognosis. Surgery, performed at early stages, offers the best chance for cure, but unfortunately, it is often noncurative. Medical treatment produced disappointing responses. mTOR inhibitors, such as sirolimus (S) and temsirolimus (T), are promising antineoplastic drugs in several types of carcinomas.
Methods
To evaluate whether ACC might be a candidate for treatment with S or T, we have studied the mRNA expression of mTOR, 4EBP1 and S6K, in 40 human adrenal samples (10 (ACC), 16 adenomas (ACA), 10 hyperplasia (AH), 4 normal (NA)), and in NCI-H295 and SW13 cell lines, by qRT-PCR. The effects of S and T on cell growth (after 24 h, 3 d, 6 d and 9 d) and on the induction of apoptosis (after 24 h and 3 d) were studied by DNA-measurement and the analysis of DNA-fragmentation, respectively, in NCI-H295 and SW13 cell lines.
Results
In ACC, the expression of S6K was lower than in other adrenal samples (ACC $=0.13\pm0.1$ versus ACA $=0.33\pm0.16$, AH $=0.36\pm0.13$, NA $=0.31\pm0.12$; $P<0.01$; median $\pm$ S.D.), and the expression of mTOR was significantly higher than in NCI-H295 and SW13 cell lines (0.34 ± 0.53 vs 0.18 ± 0.029 and 0.1 ± 0.02, respectively; $P<0.005$; median $\pm$ S.D.). A significant correlation was found among the mTOR, 4EBP1 and S6K mRNA levels in ACC ($P=0.01$). S and T were able to suppress the cell growth of both cell lines, in a similar and dose- and time-dependent manner. SW13 cells ($EC_{50}\cong7.5\times10^{-11}$; maximum effect $\cong90\%$ at 10^{-8}) were significantly more sensitive to treatment with S and T than NCI-H295 cells ($EC_{50}\cong10^{-8}$; maximum effect $\cong50\%$ at 10^{-5}). A slight induction of DNA-fragmentation, was observed only at the higher concentrations used.
Conclusion
The results of the current study demonstrated that sirolimus and temsirolimus inhibit the *in vitro* proliferation of ACC cell lines, suggesting that mTOR-inhibitory drugs may have a role in the treatment of ACC.

P53

Prospective evaluation of tumour size and hormone secretion in adrenal incidentalomas

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Increased use and improved technology of imaging procedures have led to increased recognition of adrenal incidentalomas. The aim of this study is to evaluate the natural course of benign adrenal tumours in terms of tumour growth and hormone secretion.
Subjects referred between 2001 and 2008 for the evaluation of adrenal tumours were included. Computed tomography (CT) was the initial radiological intervention. Initial hormonal evaluation included 1 mg or 2 day 2 mg dexamethasone suppression test, urinary free cortisol (UFC), 08.00 a.m. ACTH and DHEAS levels. In subjects with elevated post DST cortisol (>1.8 mcg/dl), elevated UFC (>110 mcg/day), and suppressed ACTH and DHEAS levels, midnight cortisol was evaluated (normal <7.5 mcg/dl). Urinary catecholamine excretion and aldosterone/renin ratio were also measured.
Three hundred and two consecutive subjects with adrenal tumours were included. Fifty-six patients had adrenalectomy because of hyperfunctioning tumours or malignant appearance on radiological interventions. Among the remaining 246 subjects, 132 participants were selected with appropriate radiological and hormonal follow up data. Mean age was: 55 years and female dominance was present (99/33). There were 98 subjects with non-functioning adenomas, 26 subjects with subclinical cushing syndrome, 5 subjects with myelolipomas and 3 subjects with primary hyperaldosteronism. Median follow up duration was 23.5 months. In 7 (5.3%) subjects, a decrease in tumour size was observed, while an increase was observed in 23 (17.4% participants). In 97 subjects with non-functioning adenomas, 3 (3.08%) patients developed subclinical cushing syndrome while pheochromocytoma or primary hyperaldosteronism were not diagnosed during follow up. Median follow up was significantly higher in subjects with tumour size increase (21 vs 32.5 months, $P<0.05$).
This prospective study demonstrates that malignancy and tumour hypersecretion do not frequently develop during short term follow up in subjects with benign adrenal tumours. Increase of tumour size is not rare and may be detected in patients with relatively longer follow up durations.

P54

Rosiglitazone interferes with human adrenocortical carcinoma growth in a xenograft mouse model

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Adrenocortical carcinoma (ACC) is a rare and aggressive tumour with a poor prognosis, characterized by radio/chemotherapy resistance. The lack of an

effective medical treatment is due to the poor knowledge of the mechanisms underlying malignant tumour transformation and aggressiveness. *In vitro* studies on the ACC H295R cell model have demonstrated that RGZ, an antidiabetic drug belonging to the thiazolidinedione ligands of PPARgamma, blocks cell proliferation/migration and induce cell differentiation/apoptosis. Moreover, PPARgamma ligands have been shown to inhibit primary tumour and metastasis growth in different cancers.
This study aim at evaluating RGZ effects in a human adrenocortical carcinoma xenograft model. Tumour xenograft was obtained by subcutaneous injection of 7×10^6 H295R cells in nude Balb/c mice. When the tumour size reached 5 mm, the animals were randomly allocated to 2 groups orally treated with 5 mg/kg RGZ ($n=9$) or water ($n=13$), 6 days a week for 31 days. Tumour volume was measured twice a week. At the end of the treatment, mice were sacrificed and tumours were split for histological/immunohistochemical or RT-PCR analyses.
A statistically significant reduction of tumour growth in the RGZ versus control group ($P=0.007$) was observed. Histological and immunohistochemical evaluation of the tumour revealed characteristics of invasiveness, richness in small vessels and mitotic figures in control group, while RGZ group tumours presented expanding and not infiltrating borders, with few vessel and many apoptotic bodies, and reduction in proliferation. Quantitative real time RT-PCR demonstrated a statistically significant reduction in the expression of angiogenic and vascular (VEGF and CD31), proliferation (BMI1) and anti-apoptotic (Bcl-2) genes as well as in the number of human H295R cells, in RGZ versus control group tumours ($P<0.05$, Student *t*-test).
In conclusion, our findings support a role of RGZ in controlling ACC proliferation and angiogenesis. Further investigations are needed to clarify the molecular mechanisms underlying RGZ anticancer effects.

Thyroid

P55

Report of twelve cases with thyroid hemiagenesis: single centre experience from Turkey

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Thyroid hemiagenesis is a rare anomaly characterized with the absence of one thyroid lobe due to a failure in embryologic development of the thyroid gland. This anomaly is more frequent in women. It usually affects left side of the thyroid. Isthmus may be detected in half of the cases. Thyroid hemiagenesis is usually diagnosed after thyroid imaging due to other disorders, which might be related or unrelated to the thyroid gland. In this paper, we report clinical features of twelve patients with thyroid hemiagenesis who were diagnosed during the period of five years in Dokuz Eylul University Hospital Endocrinology Clinic.

P56

Severe obesity accompanied with subclinical hypothyroidism

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Obesity is the modern medical problem. According to the WHO expert panel the prevalence of obese population worldwide is 300 million and overweight is 750 million.
In the first trimester of 2007, we studied the obese patients in the Iv. Javakhishvili State Medical University's Department of Endocrinology. The objective of our research was to study the course of subclinical hypothyroidism adjunct with severe obesity, its diagnostic criteria and treatment. Ninety-four patients were studied, with age range 14–53 years. The duration of subclinical hypothyroidism was from 6 to 18 months. There were 72 women and 22 men, who underwent following clinical, laboratory and topical diagnostic investigations: glucose tolerance test, biochemical analyses of blood, electrolytes, coagulation test, urine test, thyroid and abdomen ultrasound assessment, ECG, Laboratory evaluation of following hormones: TSH, FT3, FT4, leptin.
Fifty-five of 95 patients were diagnosed to have BMI more than 40; 4 patients BMI exceeded 30; 27 patients were diagnosed to have subclinical hypothyroidism.
The treatment of the patients included individual low calorie diet, physical activity in combination with potassium iodide and levothyroxine in some cases. The doses of levothyroxine were decreased along with the loss of weight and normalization of TSH. Received results were positive.

P57

The psychovegetative status at patients with postoperative primary hypothyriodism, compensated by levothyroxyn

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Purpose
To reveal changes of the vegetative status, namely vegetative maintenance, vegetative reactance, and infringement of emotional status at patients with postoperative primary hypothyroidism (concerning central or total craws), the compensated treatment by levothyroxyn.
Materials and methods
Sixteen persons are included in research with primary hypothyroidism with various etiology, compensated by levothyroxyn. The ages – 36–58 years, duration of diseases – 1–16 years. For an estimation of the psycho-vegetative status A M Vejna's technique, an index of Kerdo, a refex of Danini-Ashnera, a hospital scale of alarm and depression, a scale of alarm of Hamilton, a scale of depression of Hamilton were used.
Results and discussion
Sixteen persons with postoperative primary hypothyroidism have been carried out psycho-vegetative tests. At research of an initial vegetative status prevalence of parasympathetic department of nervous system is noted. At all investigated patients alarm symptoms, disturbing condition are revealed. Depression had more expressed character. On the basis of the received data at patients with the operated thyroid gland, influence of parasympathetic department of vegetative nervous system prevails. Estimating changes of psycho-emotional status: the prevalence of depressions over the disturbing is noted.

P58

The influence of thyroxine replacement therapy on bone mineral density in hypothyroid subjects

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Recent studies have suggested that subjects receiving thyroxine replacement therapy are in potentially increased risk of osteoporosis. We set out to measure bone mineral densities by ultrasound bone densitometry (UBMD) in three groups: group A of post-menopausal women ($N=25$) mean age 65 (±14), group B of women in generative period ($N=20$) mean age 41(±11), and group C ($N=18$) male mean age 54, receiving thyroxine replacement therapy during two years at least. The mean value of TSH was 2.5 mU/l, and the limit for *T* score was -2.5 S.D.
Results
Group A has a mean value of *T* score -2.67 S.D. (±0.7), although mean value of *T* score in group B was 1.8 S.D. (±0.6) and in group C was -0.6 S.D. (±0.5).The difference between group A and group B, and group A and C was significant, $P<0.001$, between group B and C there was no significance.
Conclusion
These results suggests that thyroxine has potentially effect on increase of bone turnover in post-menopausal women, but there is no similar effect in women in generative period and males on thyroxine replacement therapy, it is possible due to lack of protective oestrogen action on bone turnover in post-menopausal period.

P59

Real-time elastography and contrast-enhanced ultrasound for the assessment of thyroid nodules

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Background and aims
The aim of the present study was to evaluate real-time elastography (RTE) and contrast-enhanced ultrasound with Sonovue (CEUS) for the differentiation of benign and malignant thyroid nodules using cytologic/histologic analysis as reference method. While first studies have reported excellent results of RTE for the differentiation of thyroid nodules, data on CEUS of the thyroid are rare.
Methods
RTE was classified as: score 1 = soft, 2 = predominantly soft, 3 = predominantly hard, 4 = hard nodule. Power Doppler perfusion (PD) was classified as: pattern 1 = no/minimal perfusion; 2 = perinodular; 3 = little intranodular; 4 = hyperperfusion. Fourty-six nodules of 45 patients were included in the present study. All examined nodules were $\geqq 1$ cm in size and non-functioning or hypo-functioning on radionuclide scanning. FNA was performed in 41 nodules, 13 of which received an operation due to a suspicious or non-diagnostic result. Five patients received primary operation.
Results
Five patients had to be excluded due to non-diagnostic FNA. Therefore, 41 nodules in 40 patients were available for analysis. FNA revealed cancerous tissue in 4 and suspicious tissue in one patient. All 5 patients were operated and papillary carcinoma was found in 4, and follicular carcinoma in one patient, respectively. In all other patients cytology/histology revealed benign tissue. A significant correlation between cytology/histology and ultrasound measurement was found only for RTE ($r = 0.32$; $P < 0.05$). When using PD pattern 3&4 for the diagnosis of malignant nodules sensitivity, specificity, PPV, and NPV were 80, 47, 17, and 94%, respectively. When using elastography score 3&4 for the diagnosis of malignant nodules sensitivity, specificity, PPV, and NPV were 80, 75, 31, and 96%, respectively. And when using PD and RTE criteria together sensitivity, specificity, PPV, and NPV increased to 80, 83, 29, and 97%, respectively. The only malignant nodules which both methods missed, was the follicular carcinoma. Sensitivity and NPV for the diagnosis of papillary carcinoma was 100%. No specific CEUS pattern could be identified to differentiate between benign and malignant nodules.
Discussion
RTE can be used with high NPV in the work-up of thyroid nodules to exclude papillary thyroid cancer. However, follicular carcinoma remains a challenging problem. CEUS does not improve the characterization of thyroid nodules.

P60

The value of TSH-assisted 18F-FDG PET in initial stage M0 thyroid carcinoma with suspicion of residual or recurrent disease

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Purpose
Persistence of well-differentiated thyroid carcinoma most commonly involves cervical lymph nodes. The purposes of the study were to evaluate the ability of FDG-PET/CT imaging to localize residual disease in initial stage M0 thyroid carcinoma thyroid cancer patients and to compare FDG-PET/CT to neck US.
Methods
FDG-PET/CT and neck US results of 93 patients were retrospectively analysed. All FDG-PET/CT were performed during thyrotropin stimulation (34 patients after THW and 59 patients after rhTSH).
Results
About 37/93 patients had a FDG uptake and 29/37 of the FDG avid lesions underwent re-operation. FDG-PET/CT findings were true-positive in 22 patients: cervical disease only ($n = 17$), cervico-mediastinal disease ($n = 3$), pulmonary metastases ($n = 2$). The sensitivity, PPV, specificity, NPV and accuracy of FDG-PET/CT were 35, 59.5, 50, 26.8 and 39.7%, respectively. All patients with persistent Tg and aggressive papillary thyroid carcinoma (PTC) variants had FDG foci. Moreover, FDG-PET uptake provided more frequently complementary information compared to neck US in aggressive PTC variants than in other PTC histotypes ($P < 0.0001$). FDG-PET/CT was also more useful in patients with rhTSH-Tg > 5 or hypo-Tg > 10 ng/ml than those with lower TSH-stimulated Tg values ($P < 0.049$). In this group, FDG-PET provided additional value to neck US in 83.3% of reoperated patients.
Conclusion
Because FDG-PET has a low sensitivity in initial stage M0 thyroid carcinoma, it should not been performed in all patients with residual Tg. However, if only patients with high TSH-stimulated Tg or aggressive PTC variants are considered, FDG-PET provides additional information to neck US in most patients with positive FDG-PET.

P61

Influence of pentoxifylline on peripheral blood mononuclear cells proliferation and apoptosis in Graves' ophthalmopathy

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Pentoxifylline (PTX), a phosphodiesterase inhibitor, has a positive therapeutic effect in some autoimmune diseases due to immunomodulatory action.
The aim of the study was to assess the influence of PTX on apoptosis and peripheral blood mononuclear cells (PBMC) proliferation in patients with Graves' ophthalmopathy (GO).
Twenty-four patients with GO and 32 healthy controls were investigated. GO patients were divided into two groups: I – 14 patients treated only with methimazole, II – 10 patients treated with methimazole and PTX. PTX retard dosage form was prescribed in a dose of 600 mg once daily/6 weeks. We assessed the proliferation of PBMC stimulated with phytohemagglutinin (PHA) 0.5 μg/ml, 10.0 μg/ml, PMA, PWM. Both spontaneous and PHA-induced three-day apoptosis was evaluated by flow cytometric analysis. There was a significant difference of stimulated apoptosis between GO patients and controls before treatment ($P < 0.0001$). Following 6 weeks of therapy with pentoxifylline, the apoptosis value significantly increased among patients group II (3.54 ± 1.16 vs $21.99 \pm 7.42\%$, $P < 0.05$). There was no significant difference in the levels of apoptosis in group I after treatment (4.23 ± 1.21 vs $8.6 \pm 4.12\%$, $P > 0.05$). Results of proliferation assay being expressed as stimulation index (SI). Mean SI for patients with GO prior to therapy was significantly higher then controls (PHA 0.5 μg/ml, $P < 0.01$; PHA 10.0 μg/ml, $P < 0.05$). After treatment SI decreased in both group of patients. However, significant differences observed in group II only (PHA 0.5 μg/ml 22.34 ± 4.82 vs 7.12 ± 2.6, $P < 0.05$; PHA 10.0 μg/ml 98.83 ± 10.28 vs 53.57 ± 4.31, $P < 0.001$; PWM 39.04 ± 6.33 vs 24.68 ± 3.96, $P < 0.05$). In conclusion, we have demonstrated significant increasing of apoptosis and profound inhibitory effect of PTX on mitogen-induced proliferation of PBMC in GO patients. This results showed the potential of PTX in the management of GO.

P62

Soluble CD40 and its ligand CD154 in patients with Graves' orbitopathy during combined therapy with corticosteroids and teleradiotherapy

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It was shown recently that orbital fibroblasts express intensively CD40 and its ligation stimulates proinflammatory cytokines, glicosoaminoglycans and PGE_2 production. CD40/CD154 interaction in the pathogenesis of Graves' orbitopathy (GO) is suggested an important pathway of T cells induced fibroblast activation and proliferation.
Aim
To assess the role of CD40/CD154 interaction in GO pathogenesis and to estimate usefulness of soluble CD40 (sCD40) and CD154 (sCD154) measurements as markers of GO activity.
Material and methods
Fifty-one individuals in 4 groups: 1/15 euthyroid patients with clinical symptoms of GO who underwent corticosteroid therapy consisting of intravenous infusions of methylprednisolone (MP) and subsequent treatment with oral prednisone (P) and teleradiotherapy (TR); 2/14 patients with hyperthyroid Graves' disease (GDtox); 3/22 patients with GD in euthyreosis treated with methimazol (euGD); 4/10 healthy volunteers age and sex-matched to group 1–3. The serum

samples were collected 24 h before MP, 24 h after MP, after TR and at the end of therapy. Serum CD40, CD154 and TPOab were determined by ELISA and TSHRab by RIA.
Results
Serum concentrations of CD40 (in pg/ml) and CD154 (in ng/ml) were increased in GO patients: 84.9 (74.7–93.9) and 4.0 (2.5–7.3) respectively in comparison to controls ($P<0.001$ and $P<0.05$ respectively). Serum CD154 in GO group was elevated as compared to both hyperthyroid and euthyroid GD without clinical ophthalmopathy ($P<0.001$ both). The sCD40/sCD154 quotient was significantly elevated during in nonrespondent GO patients after MP ($P<0.05$) and at the end of the study ($P<0.01$).
Summary
Our data suggest an important role of CD40/CD154 interaction in the pathogenesis of autoimmune process leading to inflammatory infiltration in Graves' orbitopathy, however usefulness of sCD40 and sCD154 measurements in prediction of effects of GO treatment and its monitoring needs further investigations.

P63

Analysis of the genetic markers: ANLN, BIRC5, UBE2C, IRAK1, ZMYCD11, CENPA in needle aspiration cytology (FNA) of thyroid and their used as targets for molecular-diagnosis and prognostic value

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Thyroid carcinoma is the most common endocrine malignancy. However, the absence of prognostic markers for identified well-differentiated tumors versus undifferentiated/anaplastic or poorly differentiated enhances of the progression of recurrent forms with an unfavorable prognosis. In order to identify potential markers for thyroid cancer prognosis prediction, we analyzed by cDNA micro-array the gene expression profile of tumors of the thyroid, with different degrees of malignancy, allowed identifying 23 genes can predicted a worse prognosis in the patients. Fine needle aspiration cytology (FNAC) is a well-established technique for pre-operative investigation of thyroid nodule(s). This technique is an efficient method of differentiating benign versus malignant thyroid nodules; however, the molecular techniques are not routinely applied in FNAC and their implement can be used for improved more efficiency the diagnosis.
Aim
The purpose of this study was to evaluate if the molecular analysis using residual samples of FNAC, is feasible and could be employed for molecular preoperative studies in the future. Moreover, if the expression of genes considered like as specific molecular signature, are useful as a target for molecular-diagnosis routine using FNAC.
Methods
The expression of ANLN, BIRC5, UBE2C, IRAK1, ZMYND11, CENPA mRNA in 22 thyroid benign tumors and 24 papillar carcinoma (PTC) and 5 normal thyroid tissues by means of the cDNA analysis through the qRT-PCR technology in the Abi7000 platform. The relative expression levels were determined by Comparative CT Method using normal thyroid samples as a tissue calibration control expression.
Results
RNA was successfully isolated in 50% (11/22) of thyroid benign tumors tested, and 79.1% (19/24) in PTC. The expression of the genes: ANLN, ZMYND-11, IRAK-1 from PTC, showed an increase of the 3.4-fold, 21.7-fold and 963.5-fold mRNA levels respectively compared to the control tissue with statistically significant. Moreover, in samples from benign tumors their increase expressions were lower: 2.9-fold (ANLN), 8.9-fold (ZMYND-11) and 468.7-fold (RAK-1), when the results were compared with the PTC. The analyses of the genes UBE2C, BIRC5 and CENPA, showed also a significally increase in FNAC from PTC samples, 118.2-fold, 16.3-fold and 63.1-fold respectively compared with the control. However, they were lower when we compare these results with the benign tumors: 591.2-fold (UBE2C), 56.5-fold (BIRC5) and 319.9-fold (CENPA).
Summary
FNAC can be used to evaluate gene expression by qRT-PCR technology, without modifying the routine procedure. The expression of the genes ANLN, ZMYND-11, IRAK-1 can be useful in the suspicious samples. However, the expression of the genes UBE2C, BIRC5 AND CENPA increases cannot useful to differentiate between benign hyperplasia versus PTC. Finally, these results suggest the usefulness of the quantitative measurement of ANLN, ZMYND-11, IRAK1 mRNA in molecular-based diagnosis of thyroid PTC. Further analysis with undifferentiated/anaplastic or poorly differentiated tumors would be of interest.

P64

Association of G1201/T exon8 and A2257/C exon 12 polymorphisms with anti-TPO titter

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Objective
The aim of this study was the assessment of association of two single nucleotide polymorphism (SNP) of thyroid peroxidase (TPO) gene with anti TPO antibody titer for the first time.
Materials and methods
In this case–control study, peripheral blood samples of 428 individuals, 178 men (29–76 y) and 250 women (21–76 y) from unrelated families were genotyped (G1201T of exon 8 and A2257C of exon 12) by PCR amplification and RLFP method. The sera anti TPO antibody titter were determined in all subjects.
Results
One hundred and seventy-eight (125 women and 53 men) individuals had positive TPO test (41.6%) and 250 (125 women and 125 men) negative (58.4%). G1201/T polymorphism was observed in 116 individuals (65.1%) with positive TPO test and 160 controls (64%). A2257/C polymorphism was observed in 110 individuals with positive TPO test (61.8%) and 30 controls (12%). There was no association between G1201/T exon8 of TPO gene and TPO auto antibodies but an association between A2257/C exon 12 and the auto antibodies was found ($P<0.05$).
Conclusion
For the first time our result suggests the association between A2257/C exon 12 of TPO gene and antiTPO antibodies rising.
Keywords: TPO, SNPs, polymorphism.

P65

Prevalence of growth hormone deficiency in autoimmune hypothyroidism

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Background
Autoimmune hypophysitis can result in growth hormone deficiency (GHD). Although autoimmune hypophysitis is uncommon, it is associated with other autoimmune endocrine diseases like autoimmune hypothyroidism (AIH). Recent studies suggest a high prevalence (5%) of GHD in AIH, which could contribute to the reduced quality of life frequently observed in patients with AIH despite adequate treatment with thyroxine.
Objective
To establish the prevalence of growth hormone deficiency in patients with AIH.
Patients
We included patients with AIH (TPO-Ab $\geq$ 100 kU/l), who were adequately treated with thyroid hormone suppletion (TSH 0.2–5.0 mU/l). Exclusion criteria were prior I^{131} treatment, thyroid surgery, or a history of hypothalamic or pituitary disease. Patients were recruited via our outpatient clinics and via patient self-help organizations. Eight hundred and thirty-seven patients applied for the study.
Research design and methods
We measured TSH, FT4, TPO-Ab and IGF-I. If the IGF-I concentration was $<$10th percentile of age specific reference values, a GHRH/GHRP-6 test was done. GHD was defined as a growth hormone peak after GHRH/GHRP-6 below the 2.5th percentile according to age specific reference values.
Results
In total 837 patients applied for the study, 515 (476 female, 39 male) were included. Three hundred and twenty-two were not included (157 because TPO-Ab $<$ 100 kU/l, 165 had TSH $<$ 0.2 or $>$ 5.0 mU/l).The IGF-I concentration was $<$10th percentile in 49 of 515 patients. These 49 underwent a GHRH/GHRP-6 test. Two had a growth hormone peak $<$2.5th percentile.
Conclusion
The prevalence of GHD in Dutch patients with AIH is 0.4% (2 out of 515 patients).

P66

Differences in expression pattern of all-trans retinoic acid and retinoid X nuclear receptor subtypes in papillary thyroid carcinoma: a comparison with anaplastic thyroid carcinoma

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Retinoid receptors (RARs) upon a proper ligand binding act as all-trans retinoic acid-inducible transcription factors interacting as heterodimers with retinoid X receptors (RXRs). Predominantly, novel synthetic retinoid analogues acting through RARs as redifferentiation agents would be of great value in treating patients with advanced thyroid cancer.
The objective of this study was to investigate all-*trans* retinoic acid/9-*cis* retinoic acid nuclear receptor subtypes (RARalpha, RARbeta, RARgamma, RXRalpha, RXRbeta, RXRgamma) expression pattern in papillary thyroid tumour tissue of patients in order to compare it with that of anaplastic thyroid carcinoma and the intact thyroid tissue of the corresponding patients. The expression of the retinoid/rexinoid nuclear receptor subtypes has been analyzed by the RT-PCR technique.
The data has shown that papillary thyroid carcinoma of investigated patients expressed all subtypes of RARs and RXRs when compared to intact thyroid tissues of the corresponding patients that were lacking to express RXRgamma. In papillary thyroid carcinoma, expression of RXRgamma was enhanced in comparison with that of RXRalpha or RXRbeta. Expression of RXRgamma in the patient with anaplastic carcinoma was found to be lower than that of in patients with papillary carcinoma.
In conclusion, this type of diagnostic approaches enlisted into the diagnostic algorithm of patients before their possible treatment with retinoic acids or retinoid analogues might thus enhance therapeutical potentialities and bring positive results in the treatment of thyroid cancer.
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Rapid preparation of patients with hyperthyroidism for thyroidectomy

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Thyroidectomy is an alternative treatment in the therapy of hyperthyroidism in patients who are non-compliant, drug-resistant or have various side effects to the antithyroid drugs. Preoperative preparation of hyperthyroid patients is extremely important to avoid per operative complications due to severe thyrotoxicosis. We investigated the effects of lugol solution usage with or without thionamides in the rapid preparation of thyroid surgery retrospectively. Twenty-two patients with Basedow-Graves disease, 19 patients with toxic multinodulary goiter and 3 patients with toxic adenoma were enrolled into the study. Mean ages of patients were 46.6 ± 14.7 years and mean duration of hyperthyroidism was 38.2 ± 59.3 months. The indications of surgical treatment were as follows: unresponsiveness to medical treatment ($n=19$), pancytopenia ($n=9$), hepatotoxicity ($n=6$) allergic reactions ($n=3$) and noncompliance ($n=7$) with antithyroid drugs. To restore euthyroidism before surgery, 27 patients treated with lugol solution whereas 17 patients treated with lugol solution and thionamides. Mean dose of lugol solution was daily 27.7 ± 5.5 drops and the mean usage of lugol solution was 9.7 ± 2.3 days. Beta-blocking agents were used in 31 patients. After lugol treatment serum free T4 concentration decreased from 2.5 ± 1.6 to 1.37 ± 0.71 ng/dl (normal range = 0.7–1.48 ng/dl) while serum free T3 concentrations decreased from 10.0 ± 7.3 to 3.9 ± 3.7 pg/ml (normal range = 1.71–3.71 pg/ml). Percentage changes of serum free T4 and free T3 levels were not different in patients treated with lugol solution alone as compared with patients treated lugol solution and thionamides. All patients were clinically in euthyroid status before surgery. Uneventful total and subtotal thyroidectomy performed in 37 patients while hemithyroidectomy was performed in 7 patients.
In conclusion, lugol treatment with and without antithyroid drugs is safe and effective choice in rapid preparation of patients with hyperthyroidism to thyroidectomy when surgery cannot be delayed.

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Treatment of patients with Graves' orbitopathy (GO) with rituximab: effects on humoral immunity

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Rituximab (RTX) is a monoclonal antibody which binds CD20 antigen and induces B cell depletion. It is not known if its therapeutic effect in autoimmune diseases is mediated by modifications of the humoral immune response, namely the antibody production. Aim of the present study was to evaluate the effect of RTX on serum TSH-receptor antibodies, both binding (TBII) and stimulating (TSAb) and on serum antibodies against three orbital antigens, calsequestrin, XIII collagen and the flavoprotein subunit of succinate dehydrogenase (FP-SDH). Nine patients, 7 with active GO and 2 with only lid signs have been treated with two infusions of RTX at two week-interval with a follow-up of 50 weeks. At each visit patients were assessed by measuring peripheral blood lymphocytes count, thyroid function, TBII and TSAb and the antibodies anti-orbit. The ophthalmological evaluation aimed at defining the disease activity (CAS) and severity (NOSPECS). TSAb serum activity was tested in a CHO-TSHR stable cell line. Cells were incubated with 5% serum in hypotonic medium and cAMP accumulation was determined by RIA. The anti-orbit antibodies were measured by ELISA. We did not observe significant reduction of TRAb in relation to peripheral B cell depletion ($P=$NS) and to the clinical activity of GO ($P=$NS); TRAb had a slight significant negative correlation with time ($P<0.01$) due to the attainment of euthyroidism in all patients at the end of follow-up. Serum TSAb did not change after RTX therapy and significantly correlated with serum TRAb concentrations ($P<0.0001$). Finally, no significant changes of serum anti-orbit antibodies were observed at each time of the follow-up ($P=$NS). In conclusion, the effect of RTX in GO does not seem to be mediated by changes of anti-thyroid and anti-orbit antibody production. It is reasonable to hypothesize that the effect of RTX is mediated through the pathway of B cell antigen presentation.

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Diagnostic ability of computed tomography to assess Graves ophthalmopathy

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Objective
CT findings indicating that a patient is at risk for developing optic neuropathy are worth- while observations.
Aim
The aim of our study was to determine the utility of CT imaging in identifying patients at risk for optic neuropathy and to compare quantifiable nonvolumetric CT data from a large series of orbits with Graves ophthalmopathy.
Method
A total of 226 patients (452 orbits), 175 women (350 orbits) and 51 men (102 orbits) with Graves ophthalmopathy were enrolled. These patients were referred to National Institute of Endocrinology C. I. Parhon between 2002 and 2008. All subjects were scanned in both axial and direct coronal planes with a high-resolution CT scanner and normal criteria from literature were used. Maximum diameters of orbital muscles, muscle diameter index, muscle enlargement index, optic nerve sheath retrobulbar and waist, maximum superior ophthalmic vein diameter and proptosis have been calculated for all patients. The patients were subgrouped into those with optic nerve involvement and those without optic nerve involvement based on CT findings.
Results
The mean age was 48 years, no statistical differences between women and men. The most prevalent pattern of muscle involvement was enlargement of a solitary muscle, the superior muscle group (60 patients). Optic nerve involvement was seen in 142 patients, with mean diameter of the retrobulbar optic nerve sheath at axial CT increased. The muscle diameter index was statistical correlated with optic nerve involvement ($P<0.01$). Mean superior ophthalmic vein diameter and proptosis were significantly increased ($P=0.0082$ and 0.001)) in the subgroup with optic nerve involvement.
Conclusions
One hundred and forty-two patients were seen with optic nerve involvement as an increase in the retrobulbar portion of optic nerve sheath. The optic nerve

involvement subgroup was characterized most often by solitary muscle involvement (superior muscle group). The muscle diameter index was statistical correlated with optic nerve involvement. Superior ophthalmic vein diameter and proptosis significantly increased in the group with optic nerve involvement and may indicate that a patient is at risk for developing optic neuropathy.involvement and may indicate that a patient is at risk for developing optic neuropathy.
Muscle diameter index was statistical correlated with optic nerve involvement. Superior ophthalmic vein diameter and proptosis significantly increased in the group with optic nerve involvement and may indicate that a patient is at risk for developing optic neuropathy.

P70

Recombinant-human TSH (rhTSH) testing in patients with history of thyroid microcarcinomas

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Papillary thyroid microcarcinoma (mPTC) is being diagnosed increasingly frequently. Multifocality and nodal involvement are sometimes reported on diagnosis. Management ranges from observation to total thyroidectomy (Tx) followed by radioiodine (RAI) ablation. The role of rhTSH testing in mPTC has not been fully investigated. Torlontano *et al.* (2006) recently observed that rhTSH-stimulated Tg levels mainly depend on normal tissue remnant. Aim of this study was to further evaluate the role of rhTSH testing in mPTC. From a cohort of 52 subjects with mPTC (52.4±15.8 years, 44 females; average follow-up 4.6 years, range 1–26 years) 69% underwent total Tx and 39% Tx plus RAI; 24 subjects were also evaluated by standard rhTSH testing. Tg levels were measured from days 0 to 9 and 0.9 mg of rhTSH was given on days 1 and 2. Ablative RAI had been performed in 18 of the 24 subjects. Tg levels were <1 ng/ml after rhTSH administration in 89% of these RAI-treated patients and in 50% of those not treated with RAI. In 1 subject treated with total Tx plus RAI and in 3 treated only with Tx, increased Tg levels after rhTSH were interpreted as a consequence of a remnant of normal thyroid tissue, as revealed by neck sonography, and length of time from diagnosis. In only 1 patent were Tg-stimulated levels 12 months after RAI regarded as probably due to persistence of thyroid disease. In conclusion, our experience shows that undetectable Tg levels can be observed long after Tx, even in 50% of mPTC not ablated with RAI. RAI ablation increases the rate of patients in whom a disease-free condition can be recognized early. RhTSH testing is also useful in non-ablated patients without evidence of disease on neck sonography, in whom undetectable Tg levels may indicate a disease-free condition sooner than clinical follow-up.

P71

Comparison of the analgesic efficacy of lidocaine/prilocaine (EMLA) cream and needle-free delivery of lidocaine during the fine-needle aspiration biopsy of thyroid nodules

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Objective
Efficacy of eutectic mixture of local anesthetics (EMLA) cream and the needle-free injection of local anesthesia for reducing the pain associated with fine-needle aspiration biopsy (FNAB) of thyroid nodules was previously reported. However, direct comparison of the analgesic efficacy for both methods has not been established yet. The aim of this study was to compare the analgesic efficacy of EMLA cream and needle-free injection of lidocaine for FNAB-associated pain.
Subjects and methods
One hundred and thirty-eight patients having their first ultrasonography-guided thyroid nodule biopsy were allocated to receive either EMLA cream ($n=68$) or needle-free injection of lidocaine ($n=70$) 1 h and a few minutes, respectively, before FNAB of thyroid nodules. Patients rated pain associated with the procedure according to a 100-mm visual analog scale (VAS), an 11-point numeric rating scale (NRS), and a 4-category verbal rating scale (VRS).
Results
When the EMLA group was compared with the lidocaine group, there were no significant differences with respect to age, sex, thyroid volume, nodule size, or nodule site. Significant differences were noted in the pain ratings of those 2 groups according to all 3 pain scales. When the effectiveness of EMLA was compared with that of needle-free injection of lidocaine, the mean VAS score was 23.4±20.5 vs 12.7±15.5 mm ($P=0.001$) and the mean NRS score was 2.8±2.1 vs 1.6±1.7 points ($P<0.001$). The absolute numbers according to VRS score in each group was also significantly different ($P=0.001$).
Conclusion
Needle-free injection of lidocaine provides more effective and faster analgesia than EMLA cream application during the FNAB.

P72

The role of anti-DNA antibodies in pathogenesis of Hashimoto's thyroiditis

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Introduction/objective
The exact mechanism of autoimmune thyroid destruction in patients with Hashimoto's thyroiditis (HT) remains unclear. Recently it has been found that the HT patients demonstrate increased levels of anti-DNA antibodies, which may be directed against various nuclear structures and may be involved in process of apoptosis. In this study, we examined the potential association between anti-DNA antibodies levels, thyroid function and activity of autoimmune process in patients with HT.
Patients and methods
The study included 183 females with mean age of 35.4±14.6 years who had various clinical and morphological types of HT, 40 patients with euthyroid endemic goiter (EEG) and 30 healthy donors equal by sex and age. The diagnosis was confirmed according to the standard criteria. The concentrations of free thyroid hormones, TSH, antibodies to thyroglobulin (TG), thyroid peroxidase (TPO) and to double-stranded DNA (dsDNA) were measured by ELISA.
Results
All patients with HT showed a significant elevation ($P<0.05$) of anti-dsDNA antibodies in comparison to donors and patients with EEG. Sensitivity of anti-dsDNA antibodies detection in HT diagnostics was 94.5%. Serum anti-dsDNA antibodies in HT patients showed a significant positive non-linear correlation both with anti-TG antibodies ($r=0.79$, $P<0.001$) and anti-TPO antibodies ($r=0.99$, $P<0.0001$). Investigation of serum anti-dsDNA antibodies in HT patients with different thyroid function demonstrated a significant positive linear correlation between this antibodies and TSH levels ($r=0.87$, $P<0.001$).
Conclusions
In patients with HT concentrations of anti-dsDNA antibodies in serum are positively associated with anti-TG and anti-TPO antibodies levels and TSH. These results suppose possibility that anti-dsDNA antibodies may have a role in pathogenesis of autoimmune thyroid destruction and hypofunction.

P73

Effects of 900 MHz electromagnetic fields emitted from cellular phone on T_3, T_4 and cortisol hormones of Syrian Hamsters (*Mesocricetus auratus*)

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In this study, the effects of exposure to a 900 MHz electromagnetic field (EMF) on serum cortisol and triiodothronine–thyroxin (T_3–T_4) hormones levels of adult male Syrian Hamster were evaluated. Seventy two hamsters were used in three independent groups, 24 of which were control (without stress and EMF), 24 of which were exposed to 900 MHz EMF for 10 days and 24 of which were exposed to 900 MHz EMF for 50 days. The exposures were performed 1 h/d to 900 MHz EMF emitted from cellular phone. The concentration of cortisol and T_3–T_4 hormones in the hamster serum was measured by using an immunoradiometric assay (IRMA) method. Results showed cortisol values at the 900 MHz EMF group for 50 days higher than the other groups ($P<0.01$).Concentration of T_3 in the control group higher than the other groups ($P<0.01$) and Concentration of T_4 in the 900 MHz EMF group for 50 days higher than the other groups ($P<0.01$). These results indicate that 900 MHz EMF emitted by cellular telephones in long term exposure increased serum cortisol and T_4 levels and decreased T_3 level in Syrian Hamster, and it can destroy endocrine system generally.

P74

The effect of overt and subclinical hypothyroidism on the development of nondipper blood pressure pattern

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'Nondippers' are individuals with absence of anticipated nocturnal decrease in blood pressure (BP) and increased incidence of target organ damage. The pathogenesis of nondipper hypertension is not clear at present. We aimed to investigate the effect of overt and subclinical hypothyroidism on the development of nondipper blood pressure pattern via 24-hour ambulatory blood pressure monitoring. One hundred and nine normotensive patients with overt and subclinical hypothyroidism were evaluated and 95 of these patients without reverse dipping and masked hypertension were included in the study. Seventy-five out of 83 normotensive and euthyroid individuals were included in the control group. Median serum TSH levels were 7.61 and 1.59 in patient and control groups, respectively. The number of dipper individuals according to systolic (SBP), diastolic (DBP) and mean arterial pessure (MAP) measurements were 28/95 (29.5%), 55/95 (57.9%) and 38/95 (40%) in the patient group and 43/75 (57.3%), 61/75 (81.3%) and 54/75 (72%) in the control group, respectively. The differences between groups were significant for all 3 parameters ($P<0.001$). When patients with overt hypothyroidism and subclinical hypothyroidism were individually compared with control group, the differences were still significant for dipping pattern in SBP,DBP and MAP measurements. Spearman's test was used to analyze the correlations between nondipper pattern and serum TSH, fT3, fT4 levels, smoking status, BMI, age and sex; the only significance was a negative correlation between TSH and dipping in SBP, DBP and MAP. Consequently, despite the fact that how hypothyroidism affects nondipper BP pattern is not known, this pattern is more frequent in patients with hypothyroidism. It has an increased frequency even in patients with subclinical hypothyroidism. When the adverse effects of nondipper BP profile is taken into consideration, the necessity of thyroid hormone replacement therapy in patients with subclincal hypothyroidism becomes more clear.

P75

Influence of thiamazole, lithium carbonate or prednisone administration on the efficacy of radioiodine treatment (131I) in hyperthyroid patients

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Introduction
Effects of selected drugs on the efficacy of (^{131}I) radioiodine therapy were examined.
Material and methods
The study involved 200 hyperthyroid patients, treated with radioactive iodine. They were divided into five (5) groups (40 persons in each). In group I – beside ^{131}I, patients were additionally administered antithyroid drugs, in group II – ^{131}I and lithium carbonate, group III – ^{131}I only (the assumed absorbed dose – 150–200 Gy, the same as in groups I and II, controls), in group IV – ^{131}I and corticosteroids and in group V – ^{131}I only (250–350 Gy, the same as in group IV, controls). Therapeutic results were evaluated after 6 months on the basis of clinical and hormonal status. The evaluation included also effects of the initial hormonal status on the outcome of ^{131}I therapy in groups II and IV (versus respective controls, groups III and V); such an analysis was not performed in group I because all the patients in that group were initially hyperthyroid. The results of treatment were assigned into 2 classes in each of the study groups: effective therapy – euthyroidism or hypothyroidism; ineffective therapy – persistent hyperthyroidism.
Results
In 145 patients (72.5%), the therapy with ^{131}I was effective (group I – 55.0%, group II – 72.5%, group III – 75.5%, group IV – 87.5%, group V – 70.0%). In 55 patients (27.5%), the therapy with ^{131}I turned out ineffective.
Conclusions
The application of thiamazole during peritherapeutic period in patients, treated with ^{131}I, reduced the effectiveness of radioiodine, while lithium carbonate had no effect on the therapy outcome. Prednisone increased the effectiveness of the therapy with radioiodine. Normalisation of the initial concentration of TSH was favourable for the ^{131}I therapeutic outcome only when the assumed absorbed doses of 150–200 Gy were applied.

P76

BRAF^{V600E} mutation and timp-1 hyper-expression in classical variants of papillary thyroid carcinoma (PTC)

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BRAF^{V600E} mutation is considered useful in recognizing thyroid cancer aggressiveness or poor prognosis particularly in certain variants of papillary thyroid cancinoma (PTC). A recent meta-analysis identified 12 cancer-versus-non cancer gene candidate as markers of thyroid cancer; among these TIMP-1 (tissue inhibitors of metalloproteinases) was found consistently up-regulated. Our aim was to evaluate BRAF^{V600E} mutation and TIMP-1 expression in 14 PTC classical variants (CV) in comparison to 14 PTC other variants (2 tall-cell, 8 follicular, 4 sclerosant variants; OV). BRAF^{V600E} mutation was detected in 11 (78.6%) CV-PTC and in 3 (21.4%) OV-PTC. Using qRT-PCR TIMP-1 was found significantly hyper-expressed in CV-PTC harbouring BRAF^{V600E} mutation (median 14.3 (interquartile range: 8.2–40)) in comparison to respective normal tissues (1.2 (1–2.2); $P=0.004$). A significant TIMP-1 hyper-expression was confirmed in all 14 BRAF-mutated PTC (median 22.9 (9.2–89.3)) with respect to 14 wild type PTC (median 6.3 (2–13.8); $P=0.024$).
The proof-of-principle was assessed *in vitro* using BCPAP cell line, which harbours BRAF^{V600E} mutation, and was found to hyper-express TIMP-1. When BCPAP cells were transiently transfected with target-specific BRAF-siRNA (MU-A) TIMP-1 was significantly down-regulated. Our data prove that BRAF^{V600E} mutation is strongly associated with TIMP-1 up-regulation in CV-PTC, suggesting their potential invasiveness through the well-known TIMP-1 anti-apoptotic activity.

P77

Analysis of sonic hedgehog gene in patients with thyroid hemiagenesis: preliminary report

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Thyroid hemiagenesis (TH) is a rare inborn anomaly presenting as failure of the development of one thyroid lobe. Recent research on the molecular background underlying thyroid dysgenesis have mainly focused on patients with congenital hypothyroidism; in contrast, subjects presenting TH were only sporadically involved. Changes in transcription factor genes, including *TTF1*, *TTF2* and *PAX8*, which play an important role in thyroid embryogenesis, have been postulated. However, causative mutations that correlate with the phenotype of TH were found in only a few cases. The mechanism which governs the process of symmetric bilobation of the thyroid is still unknown. In addition, whether the same factors are responsible for development of TH and other forms of thyroid dysgenesis is still to be elucidated.
Recent experimental study has identified a novel role of Sonic Hedgehog protein (SHH) in indirect control of thyroid development. In *shh* knock-out mice, the thyroid develops as unilobate structure, while the process of differentiation in thyrocytes is undisturbed. We assume that dysfunction of human ortholog *SHH* could have similar phenotypical consequences and determine TH. To date, screening of *SHH* gene in patients with TH has not yet been performed. The aim of this study is to search for *SHH* gene mutations in a large cohort of subjects diagnosed with TH.
The studied group consists of 40 patients presenting TH, including 4 familial cases. The condition was diagnosed during medically indicated or performed as screening examination, thyroid ultrasonography. Complete unilateral absence of functional thyroid tissue was confirmed by thyroid scintiscan. Three exons of *SHH* gene were amplified using standard PCR conditions and were subsequently subjected to bidirectional sequencing. The preliminary results of the SHH analysis in 5 out of 40 patients did not reveal any abnormalities in coding sequence. Therefore a screening of the entire, representative cohort of patients needs to be conducted to provide more comprehensive knowledge in regards to the key factors in pathogenesis of TH in humans.

P78

Value of ultrasound elastography of the thyroid gland in differentiating malignant nodules

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Background and objective
One of the key features of thyroid gland cancer evaluated at palpation is the degree of firmness: malignant lesions tend to be much harder than benign ones. US elastography is combining the diagnostic advantages of high-frequency US examination and the accuracy of thyroid cancer diagnosis based on the lesion's stiffness. The aim of our prospective study was to evaluate the elastographic appearance of thyroid gland tumours and to explore the sensitivity and specificity of US elastography for differential diagnosis of thyroid cancer, with histopathologic analysis as a reference standard.
Materials and methods
A total of 34 patients (2 male and 33 females; 48.8 ± 13.71 years) were included in the study, presenting one or several suspicious thyroid nodules diagnosed by sonography. Local Ethical Committee approval has been obtained concerning the design of the study. Elastography was performed by the same examiner with the same settings of the machine. The nodules were classified in five classes of tissue stiffness (class 1 for soft nodules, class 2 and 3 for intermediate inhomogeneous stiffness and class 4 and 5 for hard, homogenous nodules) similar to the classical score established for the breast nodules. All the patients were operated and the results of elastography were compared with pathological results.
Results
The 34 patients had 99 thyroid nodules that were investigated. 65 nodules were soft in elastography (score 1–3) and 34 were hard (score 4–5). At pathological exam all the 65 soft nodules were benign and from the 34 hard nodules 17 were benign and 17 malignant. In 4 patients multiple malignant nodules were found.
Conclusion
Elastography showed a sensitivity of 100% and a specificity of 79% in diagnosing malignant nodules. With a positive predictive value (PPV) of 50% and a negative predictive value (NPV) of 100% it seems more valuable in excluding malignancy than in affirming it.

P79

Autoimmune thyroiditis: ultrasound phenotypes in 1500 patients

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Introduction
Autoimmune thyroiditis is a disease where the immune system attacks and destroys the thyroid gland. If the pathological exam lacks, the serum antithyroid antibodies are used to diagnose it. Also a useful tool is represented by the anterior cervical ultrasound.
Aim
Our aim was to study the ultrasound phenotype in patients diagnosed with Hashimoto's thyroiditis (HT+).
Material and methods
The patients were investigated by anamnesis, serum analysis as thyroid stimulating hormone (TSH), antithyreoperoxidase antibodies (ATPO) and anterior cervical ultrasound. In order to perform the statistical analyses based on echographic aspects, 7 phenotypes were described. They refer to the echogenity, thyroid nodules and homogeneity. The patterns are 0 – no thyroid presented at the moment of investigation, 2 – hypoechogenic and pseudonodular, 3 – hypoechogenic and homogenous, 4 – hypoechogenic and micronodular (nodules <1 cm), 4 – macronodular (nodules >1 cm), 5 – hypoechogenic, inhomogenous and pseudonodular, 6 – anechogenic micronodular, 7 – diffuse hyperchogenic (normal).
Results
We studied 1500 patients. The sex ratio was 1483 women and 17 men. The HT+ group included 755 patients with levels of serum ATPO >34 IU/ml. The control group (HT-) included 745 patients with levels of ATPO <34 IU/ml. The mean age was 50.71 years in the first group and 55.19 years in the second group. For each pattern, the sensitivity, the sensibility and the positive predictive value were the following: pattern 0–0.46%, 99.36%, 50%, pattern 1–57.58, 93.07, 92.07%, pattern 2–13.41, 88.06, 61.09%, pattern 3–5.14, 87.29, 36.13%, pattern 4–8.17, 60.46, 22.42%, pattern 5–11.2, 93.97, 72.19%, pattern 6–0.64, 95.25, 15.91%, and pattern 7–3.4, 82.54, 21.39%.
Conclusion
Based on 93% specificity of the hypoechogenic pseudonodular phenotype, this pattern is highly suggestive for HT. The lowest chance of HT, based on our data, is if macronodules are described (specificity-60%). The hyperechogenic pattern with a PPV of 21% implies the fact that rarely HT is presented in a normal echographic thyroid.

P80

Association between BMI and serum TSH in euthyroid subjects: the Tehran thyroid study

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Introduction
It is evident that hypothyroid patients have higher BMI than euthyroids. Until recently, much attention has been focused on finding whether minor abnormalities of thyroid function or differences in thyroid status of euthyroid subjects are related to body weight. Controversies, however, exist regarding the role of TSH in weight changes of euthyroid subjects.
Objective
The aim of this study, the Tehran Thyroid Study, was to determine any possible relationship between thyroid function tests and BMI in euthyroid subjects.
Material and methods
From the cross sectional phase of the Tehran Lipid Glucose Study (TLGS), a population based study of 15 005 participants, 1107 euthyroid subjects (506 male and 601 female, mean age of 37 ± 8 years) with normal serum TSH (0.4–3.5 mU/l), aged over 20 years, were randomly selected. Multiple linear regression analysis was used to investigate the role of TSH in BMI changes, after adjustment for confounding factors.
Results
After adjustment of age, sex and physical activity, no significant relationship was found between serum TSH concentration and BMI ($r=0.7$, $P=0.1$); results were the same in men and women. Subjects were divided through categories of TSH (low 0.4–0.9 mU/l, middle 1–1.9 mU/l and upper 2–3.5 mU/l tertiles) and the median BMI was found to be similar in all three groups (26.5, 26.9, 27.3 kg/m^2 respectively).
Conclusion
No association was found between thyroid status and BMI in euthyroid subjects.

P81

Peroxisome proliferator-activated receptor-γ (PPARγ) expression in parathyroid adenomas in primary hyperparathyroidism

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Background
Primary hyperparathyroidism (pHPT) is an important endocrinologic cause of metabolic bone disorder in human. The regulatory mechanism of the cells of parathyroid gland proliferation is not exactly known. Peroxisome proliferator-activated receptor-γ (PPARγ) is a member of nuclear receptor superfamily. PPARγ is expressed in adipose tissue at a high level. It plays a role on number of disorders such as adipose tissue differantiation, insulin sensivity, lipid metabolism, osteoporosis, arteriosclerosis, cancer, inflammation, antiangiogenesis, and cell differentiation.
Methods
This study was carried out to evaluate PPARγ expression with immunohistochemical staining in parathyroid adenomas in pHPT. Twenty surgically removed parathyroid adenomas diagnosed with the biochemical and imaging methods preoperatively in the patients with pHPT and 10 normal parathyroid tissue samples which were obtained from the archives of the Pathology Department were included in the study. The samples were incubated in mouse monoclonal antibody against PPAR gamma.
Results
Two (10%) of 20 adenomas with pHPT had +++, 14 (70%) had +, 4 (20%) had − (negative) staining. However, 7 (70%) of 10 normal parathyroid gland samples had +++, 1 (10%) had ++, and 2 (20%) had − (negative) staining. There was a significant difference between parathyroid adenomas and normal parathyroid tissues ($P<0.001$).

Conclusions
PPARγ expression was insufficient in pHPT. We concluded that PPARγ expression deficiency in parathyroid adenomas may explain the pathogenesis of the development of adenomas, insulin resistance and glucose intolerance in the patients with pHPT.

P82

Somatostatin receptor 2 expression in cold thyroid nodules exceeds that of hot thyroid nodules, papillary thyroid carcinoma and Graves' disease

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Objective
The specificity and cellular origin of the SSRS findings in CN, HN, PC and GD is currently unclear and partially contradicts the well defined action of somatostatin on thyroid cell signaling. Therefore, we systematically evaluated SSTR2 expression in benign cold (CN) and hot thyroid nodules (HN), papillary carcinomas (PCs) and Graves' disease (GD) in comparison with intraindividual control surrounding tissues (ST) by means of immunohistochemistry.
Design and methods
Tissue sections from 29 HN, 22 CN, 19 PC and their surrounding tissues and 8 GD thyroids were immunostained for SSTR2. with an affinity-purified rabbit polyclonal antibody against SSTR2 (Bio Trend, Cologne, Germany) in a final dilution of 1:1000. Membranous SSTR2 staining was quantitated by evaluating 10 high power fields (HPF) systematically distributed along the largest diameter of the tissue section.
Results
The area covered by thyroid epithelial cells in 10 HPF expressed as median in mm^2 was 0.53 for CN, 0.44 for HN, 1.5 for PC, 1.3 for GD and 0.3 for the surrounding tissues. The percentage of SSTR2 positive thyroid epithelial cells/area covered by thyroid epithelial cells in 10 HPF expressed as % was 16.6% for CN, 2.0% for HN, 3.7% for PC and 3.9% for GD and 2.4% for the ST of all groups.
Conclusions
Our study shows that SSTR2 can be immunohistochemically demonstrated in normal, hyperplastic and neoplastic thyroid cells. In addition to the immunocompetent cells infiltrating the thyroid gland in Graves' disease also the thyroid epithelial cells in Graves' disease express SSTR2 receptors. The repeated SSRS detection in PC is mostly related to SSTR2 expression on thyroid epithelial cells and not dependent on their lymphocytic infiltration. The highest density of SSTR2 receptors was detected in CN. This has to be considered when using SSRS for the diagnosis and localisation of radioiodine negative thyroid cancer.

P83

The role of repeat fine-needle aspiration biopsy (FNAB) in the management of thyroid nodule

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Introduction
In 2004, the multidisciplinary thyroid nodule committee of our centre adopted the cytological classification from the British Thyroid Association for reporting fine-needle aspiration biopsy (FNAB) results and agreed about its proper management: Thy1, inadequate sample, repeat FNAB. Thy2, benign; follow up, repeat FNAB if nodule growth; Thy3 indeterminate or follicular lesions; surgery or repeat FNAB at 6 months if low clinical, sonographic or cytologyc suspicious; Thy4, suspicious and Thy5, malignant; surgery.
Aim
To assess the role of repeating FNAB in the evaluation of thyroid nodules initially classified as benign (Thy2) or indeterminate (Thy3).
Results
We reviewed a cohort of 149 patients: 108 classified as benign nodule (Thy2) and 41 as follicular lesion (Thy3) over a 5 years period (2004–08). Repeat FNAB under ultrasound guidance was performed in all patients. Surgical pathology results were available in 44 patients.
Among 108 Thy2-patients, 93 continue as Thy2 (86%): 23 (25%) have undergone surgery: 21 adenomatosus nodules and 2 follicular adenomas. Fifteen patients (14%) change to Thy3: 8 have had surgical excision: 1 adenomatosus nodule with a papillary carcinoma focus, 1 follicular adenoma and 6 adenomatosus nodules. Among 41 Thy3-patients, 30 change to Thy2 (73%): 3 have been operated, all without neoplasia. Eleven patients (27%) maintain the Thy3 score or change to Thy4: All have been managed with surgery except one patient who refused: 2 follicular adenomas (20%), 2 papillary carcinomas (20%), and 6 non-neoplastic results (60%).
Conclusions
Repeat FNAB in thyroid nodules diagnosed as thy3, could avoid surgery in 70% of cases if there is no clinical or sonographic suspicious. This approach could delay surgery in less than 5% of patients with malignancy. Repeat FNAB for growting thyroid nodules, when the initial diagnosis is thy2, is not useful and only rare cases of adenomatosus nodules with carcinoma focus, could be missed.

P84

Clinicopathologic features of incidental and nonincidental papillary thyroid microcarcinoma

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Objective
Most of the papillary thyroid microcarcinomas (PTMC) are incidentally discovered in pathological examination after the surgery of benign thyroid disorders. The purpose of the present study was to evaluate clinicopathologic features of incidental and nonincidental PTMC.
Material and methods
We evaluated 56 patients with PTMC between 2003 and 2008 at the Division of Endocrinology of the Numune Training and Research Hospital. We analyzed the tumor size, multicentricity, capsular and vascular invasion, lymph node metastases, extrathyroid extension and distant metastases.
Results
Fifty-two of 56 patients were women and 4 were men. Mean age of the patients was 47.5 ± 11.4 years and mean follow-up period was two years. Patients with incidental PTMC ($n=25$) had been operated on for Graves' disease in two patients (7.2%), toxic multinodular goiter in one patients (3.6%), multinodular goiter in 22 patients (78.5%) and parathyroid adenom + nodular goitre in three patients (10.7%). There were no differences in the tumor size, multicentricity, capsular and vascular invasion, lymph node metastases, extrathyroid extension and distant metastases between those with incidental PTMC and those with nonincidental PTMC ($P=0.179$, $P=0.451$, $P=1.00$, $P=1.00$, $P=0.275$, $P=1.00$ respectively). There was no extrathyroideal metastases and distant metastases. Nine patients had cervical lymph node metastases at the time of diagnosis (16.1%). Among these patients 3 were incidental, 7 patient's tumor size were ≥ 5 mm, 5 patient's tumor were multicentric and bilateral and 2 patient had capsul invasion.
Conclusion
Our results suggest that there was no significant differences in clinical and histopathological characteristics between incidental and nonincidental PTMC. The presence of cervical lymph node metastases at the time of diagnosis were more common in patients with tumor size ≥ 5 mm.

P85

Is there any beneficial effect of L-thyroxine replacement theraphy on cardiovascular risk factors in patients with subclinical hypothyroidism?

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The relationship between increased risk of cardiovascular disease (CVD) and atherosclerosis in subclinical hypothyroidism (sHT) have been demonstrated in several studies. This relation was attributed to dyslipidemia which is common in sHT. Apo A1, apo B100, Lp (a), hsC-reactive protein (hsCRP), fibrinogen and total homocysteinemia (tHcy) are the main non-traditional cardiovascular

risk factors. In addition, paraoxon 1 (PON 1) activity is an enzyme responsible for the anti-oxidant effect of HDL cholesterol. We aimed to investigate the effects of L-thyroxine (L-T4) treatment in women with sHT on the anthropometric and hemodynamic properties, lipid parameters, hsCRP, tHcy, fibrinogen and PON 1 activity. We enrolled 27 women with mild sHT referred to our out-patients' clinics. All patients underwent TRH stimulation test. Subsequently patients were randomized into two groups. Twelve patients were received 100 μg/day levothyroxine (LT4) and 15 patients were observed without treatment for four months. LT4 dose adjustment was made to maintain TSH level between 0.5 and 2 μIU/ml in every month. We did not find any significant difference in anthropometric properties, lipid parameters, hsCRP, tHcy, fibrinogen and PON 1 activity between two groups at the end of the study. In conclusion, we could not find any evidence that levothyroxine treatment has beneficial effects on lipid parameters and non-traditional CVD risk factors in patients with mild sHT.

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A comprehensive surgical approach of persistent cervical papillary thyroid carcinoma based on initial surgery and modern preoperative imaging modalities

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Context
Reoperative surgery is the most efficient treatment of cervical persistent thyroid carcinoma. The extent of the surgery should be guided by the preoperative imaging findings, the primary surgery and the patient's prognosis. However, there is no consensus concerning the optimal surgical strategy.
Objective
Primary objective was to evaluate morbidity of a reoperative surgery. Secondary objectives were to evaluate performances of preoperative modalities, clinical and biochemical outcomes.
Methods
Thirty-two consecutive patients were operated on, by the same experienced surgeon, for cervical persistent disease. Surgical approach was based on imaging findings and initial surgery. Patients with initial adequate surgery were treated with focalized approach. By contrast, in patients with inadequate initial surgery, surgical re-dissection was performed. Follow-up included laryngeal examination, calcium measurement, assessment of TSH stimulated-Tg levels (after THW or rhTSH) and imaging procedures.
Results
Among 32 patients, mean age ranged from 13 to 76 years. All tumors corresponded to papillary carcinomas with aggressive histotypes in 41%. Initial pTNM stages were pT3-T4 and/or N1 in 81% of patients.
Preoperative stimulated-Tg was positive in 87% of patients ($n=30$). Palpation was positive in only 21.8% of patients. Sensitivity, specificity, PPV and NPV of combination of neck US and FDG-PET scan were 95.8, 96.2, 82.1 and 99.2%, respectively.
Most of patients (11/12) with persistent disease in the central compartment have been previously operated with an 'inadequate central LN dissection'. In patients with 'inadequate lateral LN dissection', residual LN were distributed throughout the both lateral compartments. By contrast, only superior and inferior groups of the ipsilateral lateral compartment were concerned in patients who were previously treated with an 'adequate lateral LN dissection'. Morbidity rate related to reoperation was low (6%) and transitory. At the end of follow up, 53% reached remission criteria, 20% had low TSH-stimuated Tg levels, Tg <5 ng/ml under rhTSH or Tg <10 ng/ml under THW) with negative imaging study and 27% had high residual Tg values.
Conclusion
Surgical strategy for persistent/recurrent disease based on imaging study and compartment oriented approach can be performed with low morbidity and acceptable efficacy rates.

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Long-term exogenous subclinical hyperthyroidism is not associated with decrased bone mineral density in men with differentiated thyroid carcinoma

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Objectives
To determine the effect on bone mineral density (BMD) of the sub clinical hyperthyroidism (SH) due to TSH-suppressive treatment with levothyroxine after thyroidectomy in men with differentiated thyroid carcinoma (DTC).
Materials and methods
Cross-sectional and retrospective study in 32 men (56 ± 14 years) treated with levothyroxine for a minimum of 5 years, with TSH concentrations <0.05 mUI/ml and normal T3 levels in all determinations performed every 3–6 months during the follow-up. The control group included 32 men matched for age and body mass index. Exclusion criteria: patients under treatment or diseases that could interfere with the BMD.
Determinations
TSH, T4L, T3, calcium, alkaline phosphatase, PTH, vitamin D, testosterone, urinary calcium excretion in 24h and urine N-terminal telopeptides of type I collagen. BMD was measured by DEXA (proximal femur, distal radium and lumbar spine). Calcium intake, physical activity, toxic habits and history of bone fractures were collected using a questionnaire.
Results
Duration of levothyroxine treatment: 15 ± 5 years. Dose: 2.6 ± 0.7 mcg/kg per 24 h. There were no significant differences in anthropometric data, physical activity, unhealthy habits or calcium intake between patients and controls. TSH concentration was lower in patients compared to controls (0.11 ± 0.24 vs 2.15 ± 1.12 MCR/IU per ml, respectively, $P<0.01$), and FT4 values higher (1.87 ± 0.39 vs 1.17 ± 0.15 ng/dl, respectively, $P<0.01$).
No significant differences were found between patients and controls in serum calcium, alkaline phosphatase, PTH, Vitamin D and testosterone, 24 h urinary calcium excretion and urinary N-telopeptides. There was no difference in BMD (Table) and neither patient nor control had a history of bone fracture.

g/cm^2	Patients	Controls	*P* value
Femoral neck	0.948 ± 0.128	0.996 ± 0.154	0.2
Distal radium	0.628 ± 0.137	0.697 ± 0.688	0.06
Lumbar spine	1.253 ± 0.156	1.240 ± 0.173	0.7

Conclusions
Long-term suppressive levothyroxine treatment for DCT was not associated with decreased BMD or increased risk of fracture in men.

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What is the outcome of combined therapies in amiodarone-induced thyrotoxicosis?

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Objective
To assess the outcome of medical and ablative therapies in amiodarone-induced thyrotoxicosis (AIT).
Subjects and methods
Seventy patients (32 M/38 F, aged 59.7 ± 1.4 years) with AIT (24-type 1, 20-type 2 and 26-mixed forms) were followed-up 13.4 ± 2.3 months. TSH was measured by immunoradiometric assay, TT_3 and TT_4 by chemiluminescence.
Results
Antithyroid drugs (ATD) were used as single therapy in 44 patients and combined with glucocorticoids in 22 patients. Glucocorticoids were used in monotherapy in 3 patients. Mean duration of treatment was 9.1 ± 1 months for ATD and 2.1 ± 0.2 months for glucocorticoids. Radioiodine was administered in 9 patients and thyroidectomy was performed in 4 patients. Amiodarone was withdrawn in 66 patients (94.3%). TSH normalized in 5.7 ± 0.5 months, and T_3 and T_4 in 3.8 ± 0.4 months under ATD and/or glucocorticoids.
Thyrotoxicosis control was noticed in 17/24 patients with type 1 AIT: after 8.1 ± 0.5 months of ATD ($n=7$), after ATD+radioiodine ($n=6$) and after ATD+thyroidectomy ($n=4$). Seven patients received ATD for less than 3 months. Thyrotoxicosis control was noticed in 15/20 patients with type 2 AIT: after ATD and/or glucocorticoids ($n=14$, mean period 5.3 ± 1.1 months) or spontaneously after amiodarone withdrawal ($n=1$, subclinical AIT); 5 patients received ATD+glucocorticoids for less than 3 months. Thyrotoxicosis control was noticed in 10/26 patients with mixed type AIT, treated 11.8 ± 3.4 months; 3 patients developed hypothyroidism after radioiodine; 13 patients are still on therapy.

Medical treatment duration was significantly longer in type 1 and mixed type AIT as compared with type 2 AIT, $P=0.04$, t test. Remission rate was similar in type 1 after medical and ablative therapy (17/24 patients, 70.8%) and in type 2 AIT after ATD and/or corticotherapy (15/20 patients, 75%), $P=0.7$, χ^2 test. One patient died despite FT_4 normalization on ATD + glucocorticoids.
Conclusions
Combined medical and ablative therapies were effective in most patients with AIT, decreasing mortality rate.

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Our experience in visualization of non-radioiodine-avid differentiated thyroid carcinoma (NRADTC)

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Introduction
Lack of radioiodine uptake in differentiated thyroid carcinoma (DTC) is a big diagnostic and therapeutic problem. This sign is associated with worse prognosis. In NRADTC patients with elevated thyreoglobulin levels with no evidence of disease in radioiodine scintigraphy, scintigraphy with the somatostatin analog labeled with ^{99m}Tc seems to be an alternative imaging method.
Aim
Assessment of scintigraphy with the somatostatin analog labelled with technetium – ^{99m}Tc-HYNIC-TATE in visualization of NRADTC.
Materials and method
Ten patients with metastatic NRADTC (6 with papillary thyroid carcinoma (PTC) and 4 with follicular thyroid carcinoma (FTC) underwent neck, chest and upper abdomen scintigraphy with ^{99m}Tc-HYNIC-TATE produced by OBRI POLATOM Świerk/Poland.
Results
Pathological uptake of ^{99m}Tc-HYNIC-TATE were found in metastatic lesions located in neck, mediastinum, lung and scapula in seven patients with DTC (6 PTC and 3 FTC). In two patient with PTC and metastatic lesions in neck, lung, mediastinum and mesentericum lymph nodes we found pathological uptake of ^{99m}Tc-HYNIC-TATE only in neck and mediastinum (lung's lesions was lower then 1 cm in CT, mesentericum lymph nodes metastases we found only in ^{18}F-FDG PET/CT). In one patient with FTC we found pathological uptake in neck's lymph nodes, mediastinum, lung and scapula but we didn't find two metastatic lesions in the ribs (diagnosed in skeletal scintigraphy with ^{99m}Tc-MDP). In one patient with local recurrence of FTC (in CT) there wasn't observed any pathological uptake of radiopreparation.
Conclusion
Non-radioiodine-avid differentiated thyroid carcinoma can be visualizated with ^{99m}Tc-HYNIC-TATE scintigraphy. This method can be useful for qualification to surgery and/or further receptor radionuclide therapy.

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Long-term follow-up of antithyroid peroxidase antibodies in patients with Hashimoto's thyroiditis

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Background
Although a number of studies show that the serum levels of antithyroid peroxidase antibodies (TPO-Ab) in patients with Hashimoto's thyroiditis decline during levothyroxine treatment, only a few provide quantitative data. The objective of the present study was to provide this information.
Methods
This was a retrospective study of TPO-Ab concentrations in 44 women and 4 men (median age 45.5 years; range 17–76 years) with Hashimoto's thyroiditis as defined by the following criteria: elevated plasma TPO-Ab and typical hypoechogenicity of the thyroid in high-resolution sonography at first presentation or during follow-up. The decrease in percentage of the TPO-ab level during follow-up was calculated.
Results
At the study entry 31 had elevated TSH and L-thyroxine treatment was started. Ten became hypothyroid in follow-up, and then L-thyroxine treatment was started. The remaining 7 patient followed without treatment (median 4 years). The 41 patients who started L-thyroxine treatment were analysed in quartiles based on duration of L-thyroxine treatment. In the first quartile, the median decrease in TPO-ab level was 57.8% (follow-up $\leq$ 1.5 years). The median decrease in TPO-ab level was similar in all groups receiving L-thyroxine (in the second quartile 54.7%, follow-up duration was 1.5–3 years; in the third quartile 57.4%, follow-up duration 3–7 years; in the last quartile was 55.9%, follow-up >7 years). The median decrease in TPO-ab level was 33.7% in Hashimoto thyroditis group without treatment. Although the number of patients without treatment was small, yet it was statistically significant ($P=0.02$).
Conclusions
TPO-ab immediately decreases following the start of L-thyroxine treatment for Hashimoto thyroiditis, and the decrease remain stable during long-term follow-up.

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Evaluation of chronic urticaria in patients with autoimmune thyroid disease

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Background
Chronic urticaria (CU) is defined as recurrent episodes of hives with or without angioedema of at least 6 weeks' duration; in almost 40% of the cases an underlying autoimmune process is implicated. Increased prevalence of autoimmune thyroid disease (ATD) has been reported in patients with CU, however the clinical significance of this finding remains controversial. Moreover, data concerning the prevalence of CU in patients with ATD are few.
Objective
To evaluate the presence of CU in patients with newly diagnosed thyroid disease in order to assess possible association of ATD with CU.
Patients and methods
Thirty seven patients (28 women (14 pre- & 14 postmenopausal), 9 men) aged 19–78 years (50.7 ± 17.6) underwent a clinical examination, a biochemical evaluation (routine biochemistry, thyroid function tests, anti-TPO and anti-Tg levels, total IgE levels, CRP), skin prick tests (SPTs) in 13 common inhalant allergens and a thyroid ultrasound. No patient was receiving any medication affecting thyroid function at the time of the study. CU diagnosis was based on documented history of typical lesions while the presence of atopy was assessed by medical history of atopic diseases (allergic rhinitis, asthma) and/or positive SPTs.
Results
ATD was diagnosed in 25/37 (67.6%) patients: Hashimoto thyroiditis in 24 (18 euthyroid, 6 with subclinical hypothyroidism) and history of Graves' disease in 1 euthyroid patient with nodular goiter. The remaining 12/37 patients (32.4%) had non-autoimmune thyroid disease (non-ATD). CU was diagnosed in 8/25 (32.0%) of patients with ATD and in 2/12 (16.7%) patients with non-ATD and atopy was assessed in 12/25 (48.0%) and in 3/12 (25.0%) respectively. Although a tendency for higher prevalence of CU and atopy was observed in ATD compared to non-ATD patients, this difference did not reach statistical significance.
Conclusion
These findings suggest that CU affects a large proportion of patients with ATD. It is uncertain whether these diseases share common autoimmune mechanisms, especially in atopic individuals, or their concurrence is accidental. Further investigation is needed in large series of patients with ATD and CU, especially in those with the autoimmune form of CU.

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Autoimmune thyroiditis, Graves' disease and cardiovascular risk factors

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Aims
To examine whether treated autoimmune thyroiditis (AIT) and Graves' disease (GD) are associated with increased cardiovascular risk factors.

Patients and methods
We analysed the levels of total cholesterol (TC), HDL, LDL, triglycerides (TG), ApoB, ApoA1, Lp[a], homocysteine, CRP, folic acid and vitamin B12 in 50 patients with GD and in 130 patients with AIT, after normalization of thyroid function. Patients with GD were treated with propylthiouracil until they normalized TSH, FT3 and FT4 levels. Patients with AIT were treated with levothyroxine, in order to normalize FT3, FT4 and TSH levels. Statistical analyses were made with *t*-Student and with Pearson's correlation test. A two-tailed $P<0.05$ was considered significant.
Results
The ApoB levels were significantly higher in patients with AIT than in patients with GD (102.00 ± 21.78 vs 94.73 ± 24.64 mg/dl; $P<0.05$). The TG levels were also significantly higher in patients with AIT than in patients with GD (0.12 ± 0.77 vs 0.11 ± 0.56 g/l; $P<0.05$). We found that patients with AIT had significantly higher levels of CRP (0.50 ± 0.60 vs 0.23 ± 0.16 mg/dl; $P<0.05$), anti-TPO (805.56 ± 587.14 vs 403.18 ± 495.18 UI/ml; $P<0.05$), and anti-Tg (127.53 ± 116.43 vs 74.64 ± 69.68 UI/ml; $P<0.05$). In patients with AIT there were positive correlations between TSH and CT ($r=0.83$, $P<0.01$), LDL ($r=0.75$, $P<0.01$), TG ($r=0.76$, $P<0.01$), and ApoB ($r=0.56$, $P<0.01$). In patients with GD, HDL negatively correlated with FT3 ($r=-0.37$, $P<0.05$), and there were significant correlations between TG and TRAb (antibody against receptor for TSH), ($r=0.37$, $P<0.05$).
Conclusions
Even in the euthyroid range, TSH was positively associated with total cholesterol, LDL, Apo B and TG, in this group of patients with autoimmune thyroid disease. These findings suggest a pro-atherogenic pattern associated to the low grade of chronic inflammation in euthyroid patients with autoimmune thyroid disease.

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Current iodine status in Turkey

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Surveys for the assessment of the iodine status, carried out between 1997 and 1999 in Turkey showed that the country was severe to moderately iodine deficient. (National median UIC 36 μg/l, goiter prevalence % 31.8). Therefore, a national IDD control program had been implemented and mandatory salt iodization were applied by July 1999 with 50–70 mg/kg KI or 25–40 mg/kg KIO_3 to the household salt.
Objectives
To evaluate the current iodine status by using urinary iodine concentrations (UIC) and household salt iodine content, nation wide.
Methods
A school-based, survey was conducted in 2007 by using multistage 'proportionate to population size' (PPS) cluster sampling method. The study population composed of 900, 6–12 years old school-age children (SAC) from different centrums, towns, villages. Urine samples, and salt samples that were used for cooking at home were collected. UIC were analyzed using 'the Sandell-Kolthoff reaction'. Iodine content of the salt samples were measured by using iodometric titration method.
Results
Median UIC was 107 μg/l. Severe ID was found in 7.2%, moderate and mild ID in 20.6% and 19.2%, of the SAC respectively. UIC were sufficient in 50%, and excessive (>500 μg/l) in 3%. 662 (73.5%) of the 900 salt samples were iodized. Five hundred and eight samples (56.5%) contained adequately iodized salt (iodine content >15 ppm).
Conclusions
Moderate to severe ID still exists in 27.8% of the Turkish population, which is much better than 1997 and 2002 surveys (i.e. 58, 38.9% respectively). We also conducted a follow-up monitoring study for 30 cities, in 2007, and found that ID have been eliminated in 20. In combination with that data, the present study shows that ID has been eliminated in most of the urban population, however is still an important problem in rural areas, which should be the target of the future program.

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Results of the 2007 follow-up monitoring survey for iodine status in Turkey

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Objectives
Assessment and monitoring which are two major components of a sustainable programme to eliminate IDD is being performed in Turkey since 1997. The initial survey, for the assessment of the iodine status, carried out between 1997 and 1999, showed that the country was severe to moderately iodine deficient (national median UIC: 36 μg/l, goiter prevalence % 31.8). Therefore, a national IDD control program had been implemented and mandatory salt iodization were applied by July 1999 with 50–70 mg/kg KI or 25–40 mg/kg KIO_3 to the household salt. The second survey was performed in 2002 in 30 cities. Over 7000 UIC of school-aged children (SAC) were determined, and the result demonstrated an obvious improvement (median UI 53 μg/l). In 2007, with the follow-up monitoring purposes, we conducted this survey in the same areas as previous surveys and studied UIC concentrations of 2280 SAC living in urban areas were examined. The overall median UIC was 130 μg/l. Median UI was ≧100 μg/l in 20 of 30 cities surveyed. In 8 areas (Burdur, Hatay, Kahramanmaraş, Kayseri, Bayburt, Çorum, Erzurum and Van) median UIC was between 50 and 100 μg/l, and in 2 (Bitlis and Diyarbakır) it was between 20 and 50 μg/l. Median UIC did not exceed 200 μg/l in any of the areas studied.
Conclusion
Eight years following the mandatory iodization of salt in Turkey, iodine status has reached to optimal levels in about two third of the cities studied, and ID has been eliminated in most of the urban areas. However another study reported in the meeting, showed that the situation is different in rural areas and thus needed further attention for elimination of IDD.
UIC did not exceed 200 μg/l in any of the areas studied.

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Evaluation of metabolic and endocrine complications in β-Thalassemia major: cross sectional study of 65 patients

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Introduction
Blood transfusion is critical for survival in Thalassemia major. Hypertransfusion therapy increased the frequency of complications due to iron overload. The aim of this study was evaluation of endocrine disturbances in patients with thalassemia major older than 10 years old.
Materials and methods
Fifty six patients with thalassemia major greater than 10 years enrolled. Patients have been examined to determine their pubertal status and standard deviation score of height for evaluation of short stature. For evaluation of glucose tolerance, fasting blood glucose and oral glucose tolerance test were performed. Evidence for diabetes mellitus was American Diabetes Association and World Health Organization criteria. Serum level of calcium, phosphorous, thyroid stimulating hormone, free thyroxin, luteinizing hormone, and follicular stimulating hormone, estradiol in girls and testosterone in boys were measured.
Results
Fifty-six patients with thalassemia were evaluated (mean age15.62 ± 4.44 years). In this study prevalence of diabetes mellitus was 5 patients (8.9%), impaired fasting glucose was 16 patients (28.6%) and impaired glucose tolerance test was 4 patients (7.1%). Short stature ($SDS\leq-2$) was seen in 25 patients (70%) of boys and 14 patients (73%) of girls. Impaired puberty occurred in 40 patients (71%) of our patient. Hypocalcaemia and primary overt hypothyroidism were present in 23 patients (41%) and 9 patients (16%) respectively.
Conclusion
Despite therapy with Desferal in the management of beta-thalassemia the risk of secondary endocrine dysfunction remains high. Hypogonadism is one of the most frequent endocrine complications. Endocrine evaluation in patients with thalassemia major must be carried out regularly.

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When antithyroid drugs must be started in patients with hyperemesis gravidarum?

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Hyperemesis gravidarum is characterized by prolonged, severe nausea and vomiting in early pregnancy that. It occurs in about 1.5% of pregnancies and is more common in Asian women than in white women. Many patients do not need anti thyroid drugs, except in patients with severe nausea and vomiting and thyroid dysfunction after 18–20 weeks of pregnancy.
Material and methods
One hundred and thirty-five patients with hyperemesis gravidarum whom admitted to Ob- Gyn hospital were selected. After excluding criteria, 103 patients underwent investigations including thyroid function test and β-hCG.
Results
Thirty-five women were found abnormal thyroid function test with FT_4I 4.74 ± 0.54 and in another group (68 women) was 2.9 ± 0.39 ($P < 0.0001$). B-hCG in first group was $59\,406 \pm 14\,899$ miu/ml and in second group was 6750 ± 3476 miu/ml ($P < 0.0001$). In 5 patients PTU started due to severe sign and symptoms of hyperthyroidism. Thyroid function test rechecked for all of 35 patients after 4 weeks routine therapy for hyperemesis gravidarum. Thyroid function test was normalized in 11 patients with hyperemesis graridarum but was abnormal in 22 patients so PTU was started and anti-TPO anti-body was measured. Thyroid function test was done for all of them monthly and PTU adjusted with the thyroid function test. Means of the therapy was 2.76 months and 60.63 mg/d for Anti-TPO negative and 5.33 months and 170 mg/d for anti-TPO positive patients.
Conclusion
In our study, thyroid dysfunction in hyperemesis gravidarum was 35% and, 20% of patients needed anti-thyroid therapy. Routine assessment of thyroid function is necessary for women with hyperemesis gravidarum especially in patients with clinical features of hyperthyroidism. We must consider PTU in hyperemesis gravidarum with severe weight loss, vomiting and biochemical hyperthyridism. We reported a female predominance among offspring of mothers with hyperemesis gravidarum.

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Frequency of metabolic syndrome in hypothyroid patients

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Objective
One of the common features of hypothyroidism is weight gain or failure to lose weight. Also bradycardia and mild hypertension can be seen. Impact of thyroid hormone deficiency on glucose and insulin metabolism is not fully understood. Thyroid hormones play role in lipid synthesis, metabolism and mobilization. Metabolic syndrome is a state which most features of hypothyroidism can be seen. Our aim is to investigate the frequency of metabolic syndrome in hypothyroid patients.
Methods
Overt hypothyroid 100 patients, subclinical hypothyroid 100 patients and 200 healthy controls enrolled in this study. NCEP-ATP III criteria was used for metabolic syndrome diagnosis.
Results
Body mass index was similar between the groups. Waist circumference was lower in the control group according to hypothyroid patients ($P = 0.0001$). HOMA insulin resistance was higher in the hypothyroid group according to controls ($P = 0.008$) and subclinical hypothyroid group ($P = 0.014$). Metabolic syndrome prevalence was 44% in the hypothyroid group, 35% in the subclinical hypothyroid group and 33% in the control group. ($P = 0.016$ for hypothyroid group versus controls and $P = 0.002$ for hypothyroid group versus subclinical hypothyroid group). Waist circumference was higher in hypothyroid metabolic syndrome patients according to subclinical hypothyroid group and controls ($P = 0.001$). Blood glucose, lipid parameters and blood pressure were similar between the groups.
Conclusions
Metabolic syndrome is increased in patients with hypothyroidism therefore hypothyroidism should be considered in newly diagnosed metabolic syndrome patients.

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Lipid oxidation, antioxidants and paraoxonase enzyme activity in subclinical thyrotoxicosis

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Patients with thyroid dysfunction are more susceptible to oxidative stress, and may show enhanced LDL-C oxidation. The purpose of this study was to evaluate serum paraoxonase activity and antioxidants in patients with endogenous subclinical hyperthyroidism.
Forty-one subclinical hyperthyroid patients, 30 women and 11 men, aged 47 ± 13 years, and 40 age and sex matched healthy controls were studied. Serum paraoxonase activity, lipid, lipoprotein, oxidized-LDL, Total antioxidant capacity (TAC), vitamin A, E and β-carotene levels were measured in fasting samples.
In subclinical hyperthyroid patients, significantly lower serum paraoxonase activity (53 ± 26 vs 77 ± 35 iu/ml, $P < 0.001$), oxidized-LDL (50 ± 12 vs 65 ± 25 mg/dl, $P < 0.01$), TAC (1.6 ± 0.2 vs 1.9 ± 0.3 μ/l, $P < 0.001$), PON/HDL (0.96 ± 0.65 vs 2.1 ± 1.1, $P < 0.001$), vitamin A, β-carotene and uric acid were found. The results show significant changes of lipid oxidation and antioxidant levels in subclinical thyrotoxicosis. In addition, the significant reduction in serum paraoxonase activity observed in these patients may predispose lipids to oxidation.

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The prevalence and the significance of nodular thyroid disease (NTD) in thyroid autoimmune disease (TAD)

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The aim of this study was to analyze retrospectively (2003–2007) the coexistence of NTD with TAD (Graves' and Hashimoto's diseases) in an iodine-replete area. The first study group included 381 cases with Graves' hyperthyroidism, not previously treated with surgery or radioiodine therapy. The second group comprised 213 patients with hyperplasic Hashimoto's disease and heterogeneous functionality.
NTD was diagnosed by: clinical examination, thyroid ultrasonography (performed at the time of diagnosis and repeated annually) and fine-needle aspiration (FNA – repeated after 2 years).
About 7.34% of Graves' patients presented NTD at the time of first examination. FNA performed in the nodules showed the following types of smears: 23 benign, 3 indeterminate and one non-diagnostic. The morphopatological diagnosis in patients with indeterminate cytological aspect showed 2 follicular carcinomas. Among other 6 operated cases (compressive signs), 2 presented papillary hyperplasia and 4 colloid goiters, one associated with a micropapillary carcinoma. Among the cases with goitrous chronic autoimmune thyroiditis (CAT), 7.98% presented NTD.
The cytological examination revealed the following types of smears: 6 benign (5 of them with specific aspect of CAT), 5 indeterminate and 6 malignant. The morphopathological diagnosis in cases with indeterminate smears was as following: follicular adenomas with CAT (4 cases) and an oxyphilic adenoma with a papillary microcarcinoma and CAT.
The histological analysis showed in malignant smears 6 papillary carcinomas (2 classical forms, 1 multicentric form, 3 papillary carcinomas, follicular variant, all associated with CAT).
Clinical and mainly ultrasonographical evidence of NTD was frequently observed among patients with TAD.
The ultrasonography and the cyto-morphological examination showed in TAD a large variety of nodular lesions, with a relative high incidence of malignancy.
NTD associated with TAD poses sometimes difficult diagnostic problems, imposing in correctly selected cases an aggressive therapeutic approach.

P100

Correlation of sonographic findings with thyroid function and autoimmune activity in patients with vitiligo

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Introduction
Vitiligo is an acquired depigmenting disorder due to destruction of melanocytes. Most authorities believe that the most important pathogenetic mechanism of the disease is autoimmunity. Other autoimmune disorders such as thyroid autoimmune diseases occur commonly in association with vitiligo. This study was designed to determine the role of thyroid ultrasonography as a noninvasive and cost effective diagnostic method for early detection of thyroid disorders in patients with vitiligo.
Methods
Fifty patients with vitiligo were evaluated in this case–control study. Control group (35 cases) were matched with case group respecting confounding factors such as age and sex. Comprehensive dermatological examinations, thyroid autoimmune and function tests and thyroid ultrasonography were performed for all cases (in both case and control groups). Sonographer was blind to dermatologic and endocrinologic findings of cases. Mean thyroid volumes, echopattern and texture of thyroid in sonographic studies and mean levels of thyroid autoimmune and function tests were compared between case and control groups. $P<0.05$ was considered statistically significant.
Results
Although mean total thyroid volume and mean left and right lobes volumes were not significantly different between cases and controls, but texture and echopattern of thyroid gland as well as mean serum Anti-TPO levels were significantly different between two groups ($P<0.05$). Serum anti-TPO levels had significant correlation with thyroid volume changes.
Conclusion
Thyroid ultrasonography, as an accurate and noninvasive diagnostic method, can be used to study morphology, size and parenchyma of thyroid gland in patients suspected to have autoimmune thyroid diseases.

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Thyroid node pathology: correlation between cytology, histology and radiology using a new cytological classification

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Introduction
A modification of the cytological classification from the 'British Thyroid Association – Royal College of Physicians' has been adapted for reporting fine-needle-puncture (FNP) cytology results since 2004 (Thy score). Five diagnostic categories have been agreed by our multidisciplinary committee: Thy1, inadequate sample; Thy2, benign; Thy3 indeterminate; Thy4, suspicious; Thy5, malignant.
Aim
Evaluate the correlation between cytology reports (Thy score), definitive histology and radiology findings. The new score specially aims to discriminate the former unspecific category of 'follicular lesion' reports into 2 new categories (TIhy3 and Thy4) for more accurately selecting those cases advisable for surgical treatment (follicular adenomas and differentiated carcinoma).
Methods
All FPN cases undertaken between January 2004 and February 2007 later on surgically treated have been included. Correlation and discordances between Thy score and definitive histology has been studied. Available ultrasound information is also evaluated for the presence of suspicious criteria.
Results
Surgical treatment was undertaken in 132 patients with one o more previous FNP with the following reporting results: Thy1 8.5%, Thy2 42.4%, Thy3 16.6%, Thy4 16.6% and Thy5 15.9%. All cases with Thy2 and Thy5 were congruent with definitive histology reports (benign no neoplastic and malign respectively). Unspecific categories (Thy3 and Thy4) had less congruent results, with a rate of neoplasia (benign or malign but advisable for surgery) of 36.3% and 77.2% respectively. Ultrasound malignity suspicious criteria increased the congruence of Thy4 cases to 90%. Ultrasound findings do not improve congruence in Thy3 cases.
Conclusions
Thyroid nodules Thy scoring system stratifies the risk of malignity and facilitates the communication and understanding between the members of the multi-disciplinary committee when therapeutic decision have to be taken.

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Levothyroxine suppression treatment for benign thyroid nodules alters coagulation

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Objective
Endogenous hyperthyroidism is associated with altered coagulation. The aim of the present study is to investigate the effect of levothyroxine (LT4) suppression treatment for benign thyroid nodules on coagulation system.
Design
Prospective case-control study.
Patients
Thirty consecutive euthyroid pre-menopausal women with nodular goitre disease and 28 healthy controls were included in the study.
Measurements
Plasma fibrinogen, d-dimer, von Willebrand factor (vWF), tissue factor (TF), tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI-1) and tissue factor pathway inhibitor (TFPI) levels were measured at baseline and after LT4 suppression therapy.
Results
Plasma levels of fibrinogen, d-dimer, vWF, TF and PAI-1 increased significantly after treatment with LT4 for one year. Serum FT4 was a significant predictor of increased fibrinogen, vWF and PAI-1 levels, when the data was controlled for age and BMI.
Conclusions
Our results suggest that LT4 suppression therapy for benign thyroid nodules is associated with enhanced coagulation.

P103

The role of large-neddle biopsy (LNB) in thyroid nodules: validation with surgical results in more than 100 cases

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Fine-needle aspiration cytology (FNAC) remains the mainstay in the study of thyroid nodules, with cytologic adequate specimens in almost 85% of cases in expert hands. Apart from the limitation of inadequate samples, there are false negative diagnoses in some cases, particularly in larger (>3 cm) nodules. Considering this, Large-needle biopsy (LNB) could be useful to improve diagnostic precision. We review the pathology reports (PR) of more than 100 patients operated since 2005, in whom LNB had been carried out previously.
Material and methods
We evaluated by LNB 116 thyroid nodules in 114 patients (16 men) aged 14–89 years. They included 64 multinodular, one diffuse and 49 uninodular goitres. LNB were carried out with an automated ultrasound guided spring-loaded device of 18G, and two fragments were obtained for each nodule. LNB were classified as follows: Hyperplasic (HYP), Inflammatory (INF), Follicular tumour (FOL), Hürthle cell tumour (HCT), Papillary carcinoma (PTC), Medullary carcinoma (MTC) and others. Surgical specimens (PR) were classified in the same way and then compared.
Results
We found inadequate tissue results in two LNB, one suspicious necrotic tissue showing necrotic CPT in PR. HYP appears in 53 LNB, coincident with nodular hyperplasia in 46 PR, 3 follicular adenomas, three with thyroiditis and one multicentre microscopic CPT. FOL was diagnosed in 15 nodules, two with follicular carcinoma, 10 with adenoma and three hyperplasic. PR included 30 CPT, five of them incidentals, in nodules different of the selected for LNB. LNB diagnosed 21 out of the other 25, being one the necrotic, other HYP in little fragments, and 2 HCT in LNB. Both CMT and one mucoepidermoid carcinoma were correctly identified by LNB.

Conclusion
Our results indicate that LNB could be an useful technique for the evaluation of nodular disease, particularly with high sample accuracy and diagnostic precision.

P104

Ca 19-9 levels in Hashimoto's thyroiditis

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Introduction
Carbohydrate antigen 19-9 (CA 19-9) is a glycosphingolipid of the Lewis blood group that for years has been proposed as a useful marker for epithelial type gastrointestinal cancers. It is well known that moderately increased concentrations of CA 19-9 can be found in 15–36% of patients with benign conditions such as pancreatic, liver, biliary diseases and benign hydronephrosis. In current study, we aimed to investigate whether there was any tendency CA 19-9 elevation in patients with Hashimoto's thyroiditis.
Patients and method
Seventy one patients with diagnosis of Hashimoto's thyroiditis were included in the study. Patients with malignancy, benign pancreas, liver, lung and biliary diseases, inflammatory bowel diseases, urinary tract infection, hydronephrosis, endometriosis, diabetes mellitus and chronic renal failure were excluded from the study.
Results
In patients with Hashimoto's thyroiditis, mean serum CA 19-9 level was 12.5 ± 10.4 (range, 2.5–55) while it was 11.9 ± 9 (range, 2.5–29.3) and 10.3 ± 8 (range, 2.5–28.9) in patients with Graves' and healthy volunteers respectively. There was no significant difference between Hashimoto's thyroiditis and control groups with regard to the serum CA 19-9 levels.
Conclusion
Although the American Society of Clinical Oncology does not recommend tumor markers like CA 19-9 in screening for malignancies, they may be used for this purpose. In contrast to case reports showing the possible elevation of CA 19-9 in Hashimoto's thyroiditis, we did not detect such a relation. Moreover, there was no clue for the change in CA 19-9 levels in patients with hypo, hyper or euthyroidism.

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No associaton of CTLA-4 gene polymorphism with Graves' disease in Turkish population

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Graves' disease (GD) is an autoimmune and polygenic disorder. The genetic loci conferring susceptibility need to be still defined. Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene has been reported to be associated with GD in various ethnic groups. The aim of the present study was to determine whether CTLA-4 gene was associated with GD in Turkish population. We evaluated the allele distribution of the following loci: CTLA-4 exon 1 (+49 A/G) and promoter (−318 C/T) region. We performed a case control study on 101 patients with GD (79F/22M; mean age; 45.0 ± 14.0 years), and 103 healthy controls (51F/52M; mean age; 38.3 ± 10.5 years). The polymorphisms were analyzed by using polymerase chain reaction fragment length polymorphism (PCR-RFLP). The genotype and alleles of patients and controls were compared using the Pearson X^2 or Fisher's exact test. The distribution of genotype and allele frequencies of the +49A/G and −318 C/T polymorphisms did not differ between patients with GD and healthy subjects statistically There was not a significant relationship between polymorphisms, and thyroid hormone levels before treatment, thyroid autoantibodies, ophtalmopathy, remission and recurrence rates among the patient group. In conclusion, our results do not suggest any significant contribution of common genetic CTLA-4 variants to genetic risk of developing Graves' disease in our population.

P106

The ratio of malignancy in patients who underwent thyroidectomy due to follicular lesion/neoplasia

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Objective
We aimed to evaluate the frequency of malignancy in patients with follicular lesion or follicular neoplasia in cytological examination.
Method
About 29 patients who had follicular neoplasia or follicular lesion in cytological examination after ultrasound guided thyroid fine needle aspiration biopsy (FNAB) were included in the study. Histopathologic results were evaluated after thyroidectomy in all patients.
Results
There were 28 female and one male patient. Ten of the patients had nodular, 18 had multinodular and one had recurrent multinodular goiter preoperatively. One of the nodules was infracentrimetric, 28 were supracentrimetric. Mean age was 42.27 ± 11.06 (21–65). Postoperatively, thyroid malignancy was determined in 12 patients in histopathologic examination. Therefore, the frequency of thyroid malignancy in patients with follicular lesion/neoplasia was found to be 41.4%. All of malign nodules, except one, was supracentrimetric. Six of the patients had nodular and 6 had multinodular goiter. The distribution of thyroid cancer was as follows; 3 follicular thyroid carcinoma, 7 papillary thyroid carcinoma and 2 follicular variant of papillary carcinoma. Three of the histopathologically benign cases had hashimoto's thyroiditis, 2 had hurthle cell adenoma, 2 had follicular adenoma and 10 had nodular hyperplasia.
Conclusion
It is difficult to differentiate follicular and hurthle cell carcinoma from follicular and hurthle cell adenoma cytologically. These nodules are diagnosed as carcinoma if vascular and capsular invasion is seen histopathologically. Studies showed no benefit of frozen sections on differential diagnosis. In the literature the rate of malignancy in follicular neoplasia/lesion is 6–47%. In our study, we found 41.4% malignancy rate in patients who underwent surgery due to follicular lesion or neoplasia. About 75% of the malignant cases were papillary carcinoma. We accept this group of patients as malignant and suggest that total /near total thyroidectomy should be performed.

P107

Concomitant thyroid carcinoma and Hashimoto thyroiditis: effect of thyroiditis on ultrasonographic and histopathologic features of nodules

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Objective
Incidence of concomitant thyroid cancer and Hashimoto thyroiditis (HT) is found to be 0.3–58% in different series. In this study, we aimed to find out HT incidence in thyroid cancer patients in our clinic and we tried to determine ultasonographic and histopathologic features of tumor in these patients.
Method
About 165 patients diagnosed with thyroid carcinoma between 2005 and 2008 were included in the study. Patients with Graves's disease were excluded. Preoperative and postoperative datas were evaluated retrospectively.
Results
Patients were grouped into 2 according to the presence of HT histopathologically. In Group I HT was not detected and 129 patients were in this group. Of these, 112 were female and 17 were female with mean age of 46.28 ± 10.86. In this group, mean antithyroidperoxidase antibody (anti-TPO) was 34.59 ± 69.16 IU/ml (0–35 IU/ml) and mean antithyroglobulin antibody (anti-TG) was 82.67 ± 207.42 IU/ml (0–40 IU/ml). In Group II HT was present with thyroid carcinoma and there were 36 (21.8%) patients in this group. About 34 of these patients were female and 2 were male, mean age was 42.86 ± 12.67. Mean anti-TPO and anti-TG antibody were 272.83 ± 329.59 IU/ml and 442.32 ± 826.5 IU/ml, respectively. Nodule features in ultrasonography were compared in two groups. There was no statistically significant difference between two groups in regard of echogenity, microcalcification, macrocalcification, halo sign and margin irregularity ($P > 0.05$). Additionaly, histopathologically, tumor diameter, presence of capsule invasion and vascular invasion, multifocality were similar

in two groups ($P>0.05$). Nonetheless, extrathyroidal invasion was found to be more in HT patients ($P=0.023$). Also, thyroid autoantibodies were significiantly higher in this group ($P<0.001$).
Conclusion
In this study we found HT in 21% of thyroid carcinoma patients. Besides, we concluded that presence of HT has no effect on ultrasonographic appearence of nodules. However, histopathologically in patients with HT, extrathyroidal invasion was more common. This result suggests that tumors may behave more aggressive in the presence of HT.

P108

Quality of life changes, clinical outcomes and radiation protection issues in thyroid cancer patients undergoing radioiodine remnant ablation with recombinant human thyrotropin: a randomized controlled study

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Background
Recombinant human TSH (rhTSH) has become the modality of choice for radioiodine remnant ablation of residual thyroid cancer tissue in low-risk patients.
Methods
The FACIT-F was administered from the early postoperative period to 9 months. Socio-demographic parameters, anxiety and depression scales were also evaluated. At 24 h, 48 h and d6 post-therapy, dose rate were measured. Using a simplified model, radiation exposure to public was estimated in both groups. At 9 months, patients underwent an rhTSH stimulation test, diagnostic 131-Iodine whole body scan (dxWBS) and neck ultrasonography.
Results
About 74 patients were enrolled in the study. There was a significant decrease in QoL from baseline (t0) to t1 (RRA period) in the hypothyroid group with significant differences in FACIT-F TOI ($P<10$-3), FACT-G total score ($P=0.005$) and FACIT-F total score ($P=0.003$). By contrast, QoL was preserved in the rhTSH group. In the mulivariate analysis, FACIT-TOI changes were only affected by the modality of TSH stimulation performed for RRA. No difference in ablation success was observed between rhTSH and hypothyroidism groups, 91.7% and 97.1% respectively. A higher rate of persistant thyroid remnants was observed in the rhTSH arm, although in most cases uptake was $<0.1\%$ and of no clinical significance. At 48 h, dose rate were lower in the rhTSH-group. Radiation exposure to public is also reduced in the rhTSH arm.
Conclusions
rhTSH preserves QoL of patients undergoing RRA with similar rates of ablation success compared to hypothyrodism. The use of rhTSH decreases the duration of hospitalization and is in line with the current legislation.

P109

Incidental and nonincidental papillary thyroid microcarcinomas in the material of Endocrinology Clinic and Institute of Pathology, Targu Mures

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Objective
To study thyroid microcarcinomas in surgical samples obtained by thyroid-ectomy.
Material and methods
We studied 311 patients thyroidectomized for different thyroid diseases in 2007, histology being made in the Institute of Pathology Tg.Mures.
Results
Surgery was made for uni- and multinodular goiter in 278, for Graves-disease in 28, and for other forms of hyperthyro-i-dism in 5 cases. Thyroid cancer was diagnosed in 65 cases (20.9%), 53 being papillary carcinomas. Almost half (31 subjects – 47.7%) of these well-diffe-rentiated forms were microcarcinomas, and the main part of them (21 cases – 67.7%) were detected incidentally by histology. From the total of 21 incidental papillary thyroid microcarcinomas (PTMCs) 4 (19%) harbored potential aggressive behaviour (capsular invasion, multifocality, lymph node micrometastases, Whartin-like variant). From the 10 non-incidental PTMCs 8 were unifocal and 2 multi-focal, but these 2 multifocal PTMCs were diagnosed before surgery as unifocal forms, and other foci of 1–2 mm were detected histologically in the contralateral lobe only after total thyroidectomy. Half of the 10 PTMCs diagnosed noninci-den–tally by histology had potential aggressive behaviour. From all incidentalomas 17 were resolved by total thyroidectomy ($\pm$lymph node dissection) and 4 by hemithyroidectomy and isthmectomy (all 4 unifocal, but 1 with extrathyroidal extension).
Conclusions
Microcarcinomas presented 47.7% out of papillary thyroid cancers. Most of them were detected incidentally (67.7%). About 1/3 of all PTMCs (9 out of 31 cases, 29%) showed signs that suggest an aggressive evolution: 3 multifocal growth (1 Whartin-like variant), 2 extra-thyroi-dal extension, 2 regional lymph node micro-metastases, 2 trabecular histological variant. From the 21 incidentalomas 4 (19%), and out of the 10 nonincidental microcarcinomas 5 (50%) had aggressive behaviour.

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Erythrocyte membrane cholesterol concentration in patients with hyperthyroidism

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Background
Cholesterol is a major component of the cell membrane. It plays an important role in its physiology affecting vital properties, such as membrane fluidity, cation transport, cell receptors, osmotic resistance etc. Abnormal conditions that change serum cholesterol concentration (SC) can also alter erythrocyte membrane cholesterol concentration (EMCC) possibly resulting in differentiation of several membrane functions.
Aim
To investigate whether changes in SC, usually observed in hyperthyroidism, affect EMCC.
Patients and methods
About 35 healthy controls (24 male, 11 female, age: 39.46 ± 10.86) and 23 patients with hyperthyroidism (3 men, 20 women, age: 36.00 ± 8.19) were studied. SC, EMCC, triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) were measured in all.
Results
Serum T_3 did not differ between the two groups (1.22 ± 0.30 versus 1.10 ± 0.24). In the patients as compared to the controls T4 was significantly ($P<0.001$) higher (11.51 ± 0.59 versus 8.22 ± 1.70) and TSH significantly ($P<0.001$) lower (0.12 ± 0.06 versus 2.59 ± 1.14). SC was significantly lower ($P<0.001$) in the patients (151.21 ± 29.27) than in the controls (216.49 ± 21.28). EMCC was also significantly lower ($P<0.001$) in the patients (36.08 ± 9.95) than in the controls (145.37 ± 17.06). The ratio of SC/EMCC was significantly ($P<0.001$) higher in the patients (4.68 ± 2.23 versus 1.51 ± 0.23). In the patients with hyperthyroidism there was a significant negative correlation of SC to T_4 ($P=0.023$) and a positive one of EMCC to TSH ($P=0.031$).
Conclusions
Hyperthyroidism seems to decrease both SC and EMCC in a degree related to its severity. However the decrease of EMCC is much greater, thus resulting in a considerable depletion of erythrocyte membrane from its structural component. The possible consequences of this depletion to cell physiology have to be investigated.

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Association of thyroid function tests with thyroid malignancy

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Objective
Role of thyroid stimulating hormone (TSH) in thyroid oncogenesis is not clear. There are few trials about relationship of TSH, thyroid hormones and autoantibodies with malignancy. We aimed to investigate thyroid function tests and malignancy in patients evaluated in thyroid disease council and decided to be managed with thyroidectomy.

Method
About 272 patients were included in the study. Hyperthyroid and hypothyroid patients, patients on L-thyroxine and antithyroid treatment and those with previous thyroidectomy were excluded. Thyroid function tests (TSH, free T3, free T4, anti-TPO, anti-TG) were recorded before thyroidectomy. Patients were grouped in 2 according to histopathologic results, benign and malignant; and grouped in 3 according to TSH levels, group 1:TSH $<$ 0.9 μIU/ml, group 2: TSH: 0.9–1.49 μIU/ml, group 3: TSH $\geq$ 1.5 μIU/ml.
Results
There were 224 female and 48 male patients and mean age was 44.4 $\pm$ 11.7 (18–75). Histopathologically, there were 174 benign and 98 malign reports.
TSH levels and malignancy was found to be correlated significiantly ($P < 0.001$). Although there was no difference between group 1 and 2, difference between 2 and 3 was significant ($P < 0.001$, OR:2.87). Malignancy was higher in patients with TSH $\geq$ 1.5 μIU/ml. Median free T3 was 3.55 pg/ml (1.4–5.24) in patients with benign pathology whereas it was 3.35 pg/ml (1.8–4.79) in patients with malign pathology. There was statistically significant difference ($P = 0.006$, OR:0.61). Anti-TPO was positive in 17.7% of benign group and 31.1 in malign group. Again, these results were statistically significant ($P = 0.014$, OR:2.10). Antithyroglobulin was positive in 19.9% and 32.2% of benign and malign patients, respectively ($P = 0.028$, OR:1.92). Multiple regression analysis also showed TSH and free T3 effect on malignancy.
Conclusion
Our results showed that, in euthyroid patients, presence of autoantibody, low free T3 even in normal ranges and TSH levels above 1.5 μIU/ml are all related to malignancy

P112

Preoperative thyroid problems in a cardiovascular hospital
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Changes in thyroid hormone function may have deleterious effects during cardiovascular surgery. Thus meticulous endocrinology consultation is demanded preoperatively. Thyroid dysfunction may present as subclinical or overt hypothyroidism, subclinical or overt hyperthyroidism, euthyroid sick syndrome, nodular goitre or minor elevations in thyroid hormone levels with normal TSH. The aim of the study was to determine retrospectively the prevelance of thyroid dysfunction in patients undergoing cardiac surgery.
Data from the endocrinology consultations for thyroid problems preoperatively for patients operated between the dates of 1'st of June 2008 to the 15'th of November 2008 at Kartal Kosuyolu Heart Education and Research Hospital were examined. Patients were operated for coronary bypass surgery, cardiac valvular surgery or peripheral bypass surgery. Total number of cardiovascular surgery was determined from the hospital database.
Of the 1615 patients operated 106 (6.5%) patients were consulted. Twenty patients (18.8%) had overt, 37 patients (34.8%) had subclinical hyperthyroidism, 22 patients (20.7%) had overt, 8 patients (7.6%) had subclinical hypothyroidism, 11 patients (10.5%) had minor elevations in free T4 levels, 3 patients (2.8%) had nodular goitre and were euthyroid and 5 patients (4.8%) had euthyroid sick syndrome. Among the patients with overt hyperthyroidism, 11 patients had diffuse uptake on thyroid scan, 6 had uptake suggestive of toxic nodules and 3 had no uptake. All patients had undergone angiography in a period of one week to 2 months prior to consultation.
In conclusion among thyroid disorders for which patients are consulted prior to cardiovascular surgery, subclinical followed by overt hyperthyroidism are more common than the other disorders. This may possibly be due to previous contrast patients are recieveing during angiography.

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The changes of the IL-2,IL-4,IL-12, TNF-α and IFN-γ levels with L-thyroxine treatment in patients with Hashimoto's throiditis
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Background
Hashimoto's thyroiditis is a chronic autoimmune thyroiditis. It is the most common cause of primary hypothyroidism in adolescent period, via autoimmune thyroid tissue destruction and affecting 2% of the population. In this study we want to investigate the role of the cytokines such as IL-2, IL-12, TNF-α and IFN-γ in the pathogenesis of the disease and the changes of cytokine levels with the L-thyroxine treatment.
Methods
About 65 female patients, aged 18–73 years with Hashimoto's thyroiditis referred to Celal Bayar University Medical Faculty Endocrinology polyclinic were included in this study. After a 10–12 weeks L-thyroxin therapy period, all of the patients were turn into euthyroidic state. There was a statistically significant decrease in the levels of TSH ($P < 0.0001$) and increase in the levels of FT4 ($P < 0.0001$) at the same time. Also, the levels of anti-Tg ($P < 0.01$) and anti-TPO ($P < 0.001$) were significantly lower than pre-trreatment period. After the L-thyroxine treatment, a statistically significant decrease was shown ($P < 0.001$) for the IL-12 levels. But decreasing of the IFN-γ levels was not statistically significant ($P = 0.276$). On the other hand, no changes were determined of the IL-2 and IL-4 levels.
Conclusion
In our study which took a 10–12 week treatment (therapy) period, although there was a statistically significant decrease in serum IL-12 level, the statistically insignificant decrease in IFN-γ level can be interpreted as the inflammatory process in Th 1 type was stopped or slowed down.

P114

Analysis of demographic and clinical factors, affecting the outcome of radioiodine therapy in patients with hyperthyroidism
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Introduction
The influence of demographic and clinical factors on the outcome of ^{131}I therapy in hyperthyroid patients has been examined, based on a retrospective evaluation of results obtained in patients, submitted to ^{131}I treatment. The goal of the study was an analysis of factors, including the age and sex of patients, disease duration time, as well the hormonal status before ^{131}I application, which could have influenced the effects of therapy with radioiodine ^{131}I.
Patients, materials and methods
The study involved five hundred (500) randomly selected patients with hyperthyroidism, treated with ^{131}I radioiodine. The following three (3) groups were defined: Group 1 – patients with multinodular goitre – $n = 200$; Group 2 – patients with a single autonomous nodule of the thyroid – $n = 100$; Group 3 – patients with Graves' disease – $n = 200$. The local ethics committee approved the study.
Results and conclusions
The obtained results indicate that the efficacy of therapy with ^{131}I, applied in patients with multinodular goitre, single thyroid nodule and Graves' disease, does not depend on either patient sex or patient age. The length of antithyroid treatment before ^{131}I therapy onset does not appear to have any effect on the therapy outcome, while the baseline TSH concentration seems to be significant only in case of Graves' disease.

P115

Thyroid function and volume disorders correlates with IQ in mental retard children (interim report)
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Background and objectives
Goitre is still one of endemic health problems in Gorgan city after one decade of universal salt iodization in Iran. Hypothyroism have different complications in children that between them, developmental disorders of CNS are so important. This study proposed to determine that prevalence of thyroid function and volume disorders and its correlation with IQ in the mental retard (MR) children.
Material and methods
This cross-sectional study was carried out on 120 mental retard students of tow rehabilitation center of Gorgan city, north of Iran. We exclude the cerebral palsy and major metabolic disease suffering patients from this study. Thyroid volume was measured by an ultra-sonography (US) specialist. IQ was evaluated by standard questionnaire.
Results
Mean age of children was 11.7 years. Goitre prevalence in physical examination was 42% but it was 84% in US evaluation. Mean concentration of TSH and T4 in all cases was 3.9 and 5.7 respectively. TSH concentration had a reverse linear correlation with IQ but T4 concentration was opposite this ($P<0.05$). About 34 cases (28.3%) had TSH concentration upper than normal range. About 45 cases had low IQ score and 42 was moderate and 33 had high IQ score.
Conclusion
We found that serum TSH and thyroid volume have had a reverse correlation with IQ in MR children. Thyroid enlargement and hypothyroidism is more prevalent in mental retard children than others. So we should make some decisions to screen and cure thyroid disorders in this high risk population. We consider to evaluate the iodine intake status and thyroid autoimmunity in this population for future investigation.

P116

Application of flow cytometry for evaluation of the phenotype of lymphocytes, present in the thyroid glands of patients with lymphoma in extrathyroid localisation

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Introduction
Cytological diagnostics has got an established position in thyroid diseases. In the recent years, attempts have been undertaken to use the biological material, left in needles after cytological preparation is ready, to obtain additional diagnostic data.
Goal of study
The goal of the study was evaluation of the possibility to use the results of fine-needle aspiration biopsy (FNAB) for confirmation of either the presence or the absence of lymphomatous cells in patients with thyroid disease and with coexisting lymphoma in extrathyroid localisation.
Methods
The evaluation was performed in two (2) patients with non-Hodgkin lymphoma of low malignancy and with coexisting thyroid disease. The first patient was with diagnosed, chronic, autoimmunological thyroiditis with status of compensated hypothyroidism (anti-TPO >600.00 IU/ml, anti-Tg – 401.60 IU/ml). The other patient had a non-toxic, nodular goitre. FNAB of the thyroid gland was performed in either of the patients. Following smear preparation, the aspiration needles were flushed with PBS solution. Then, an analysis of the lymphocyte phenotype was done by means of flow cytofluorometry, using a panel of commercially available monoclonal antibodies.
Results
In both studied cases, the material, obtained in FNAB, was diagnostically sufficient and appropriate for the evaluation to be accomplished. No features of clonal lymphocyte proliferation were found in result of the analysis. Regarding the cytodiagnostic categories of thyroid diseases, the result of FNAB in the patient with Hashimoto's disease was non diagnostic, while in the patient with nodular goitre, it did confirm the clinical diagnosis.
Conclusions
The presence of proliferative cells, identified by flow cytometry, is a simple method to make complete cytological diagnostics in patients with parallel thyroid pathologies and diseases of the haematopoietic system.
It can be used to extend the diagnostics of focal changes in the thyroid gland, especially in patients with chronic thyroiditis.

P117

Subjective and objective sleep evaluation in patients with hypo- and hyper-thyroidism

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Hyper- and hypo-thyroidism are considered as clinical conditions of sleep alterations. At present, however, these clinical reports have never been confirmed by studies providing a structured description of subjective and objective sleep quantity and quality. To this aim, we enrolled 15 patients with naïve overt hyperthyroidism (HYPER), 9 with naïve overt primary hypothyroidism (HYPO) and 15 healthy age-, sex- and BMI-matched control subjects (CS). Clinical conditions or drug therapies known to affect sleep per se were considered as exclusion criteria. In all the subjects sleep quantity and quality were evaluated by: 1) self-reporting questionnaires; 2) wrist actigraphy (Actiwatch, Mini Mitter Co., Inc.; Bend, OR, USA) on three consecutive days in free living conditions. The self-reporting questionnaires revealed a reduction in sleep time and an increase in sleep latency in HYPO versus CS ($P<0.01$) without alterations in the perceived sleep quality. The actigraphic study, however, did not show differences between HYPO and CS in terms of actual sleep time, actual sleep percentage, assumed sleep, sleep latency, sleep efficiency, fragmentation index and moving time percentage. Unexpectedly, both the questionnaires and the actigraphy failed to reveal differences in sleep quality and quantity between HYPER and CS. Notable, however, in the whole cohort of subjects, positive correlations between fT4 levels and fragmentation index ($R+0.542$, $P<0.01$) and moving time percentage ($R+0.545$, $P<0.01$) and a negative correlation between fT4 and actual sleep percentage ($R-0.560$, $P<0.01$) were found. In conclusion, our preliminary data show that hypothyroidism, but not hyperthyroidism, seems to be associated with some subjective impairment of sleep quantity that, however, is not confirmed by the actigraphic evaluation. The existence of positive correlations between fT4 and some actigraphic parameters still supports some influence of thyroid function on sleep, that, however, seems not to be clinically detectable.

P118

Demographic, clinical, laboratory, ultrasonographic and cytological features of patients with Hashimoto's thyroiditis: results of a university hospital of 769 patients in Turkey

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Background
We investigated the demographic and clinical features of patients with Hashimoto's thyroiditis who had been diagnosed and treated in Ege University, the main referral center in the Aegean region of Turkey.
Methods
Medical records of patients who had been followed in the endocrinology clinic of Ege University were retrospectively evaluated. Patients who had been diagnosed as having any thyroid disorder were determined. Patients with Hashimoto's thyroiditis were selected among those patients.
Results
Seven hundred and sixty-nine patients fulfilled diagnostic criteria for Hashimoto's thyroiditis (725 females, 44 males; mean age 41.76 ± 12.49 years). 62.7% of patients were between 30–50 years of age. 53.3% of females and 63.6% of males had diffuse enlargement of the thyroid gland. TSH level was above 4.0 IU/l in 25.6% of females, and 27.4% of males. Anti-tyroglobulin antibody was positive in 92% of females, and 93.2% of males. Anti-thyroid peroxydase antibody was positive in 98.4% of females (713 patients), and 100% of males. Thyroid ultrasonography demonstrated single nodule in 52.2% and multiple nodules in 11.3% of female patients; and single nodule 32% and multiple nodules in 20% of male patients. Fine-needle aspirations of the nodules were performed in 207 patients, and none of those biopsies were diagnosed as malignant.
Conclusion
Age and sex distribution and laboratory findings of our patients were comparable to the previous reports. Nodule formation was the most common ultrasonographic finding in our patients, probably due to pseudonodularity. We found no patient with thyroid cancer in our population.

P119

Assessment of the thyroid hormone's profile during pregnancy

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Introduction
In accordance with a rising number of pregnant women with thyroid gland dysfunctions, The Thyroid Gland's Disorders Outpatient Clinic for Pregnant Women has started its activity in our Endocrinology Department in January 2008. Patients with or without thyroid gland dysfunction in history have been under medical care.
During pregnancy thyroid gland is prone to the number of physiological changes, which cause difficulties in the interpretations of thyroid hormones results. Changes in estrogens level and TBG concentration, activity of hCG make TSH level inadequate. Transitory increase of FT4 and FT3 levels in first trimester is observed. However, in second and third trimester mainly FT4 level could be slightly decreased. Increased aTPO level detected during pregnancy has been shown to be associated with 2–4× higher risk of miscarriage.
Aims
(1) Attempt of establishing the referential norms of the FT3, FT4 in each trimester.
(2) Establishing the referential norms of TSH in each trimester.
(3) Morphological changes in thyroid gland in usg examination during pregnancy
(4) Evaluation of the aTPO/hTrab level for post partum thyroid disease risk assessment
Method
Assay of the FT3, FT4, TSH level and aTPO/ hTrab in blood serum (ECL) during 1st, 2nd and 3rd trimester of pregnancy, thyroid USG.
Results
About 173 pregnant women were examined from January 2008 till present day. Mean value of TSH for healthy women was as follows: 0.65 uIU/ml (±0.6), 1.16 uIU/ml(±0.48), 1.35 uIU/ml (±0.51) in 1st 2nd, 3rd trimester respectively. Abnormal result of aTPO have 43 patients (24.7%).
Conclusion
Establishment of reference values for each trimester is fundamental for correct assessment of thyroid function in pregnancy. About 1/4 patients have increased result of aTPO and required further observation. Our researches need to be continued.

P120

Seasonal occurrence of Graves' disease and associated orbitopathy at diagnosis

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A seasonal occurrence of Graves' disease (GD) has been reported in previous studies, with a peak of frequency in the warmer half of the year (Ford 1988, 1991, Westphal 1994) or the period of the year with a higher iodine intake (Phillips 1985); other studies (Facciani 2000) have failed to find a different seasonal occurrence of Graves' orbitopathy (GO). Aim of the present study was to evaluate a possible seasonal difference in the onset of GD and GO in a series of outpatient followed in our Department from April 2002 to October 2008.
About 551 patients with GD and GO were studied retrospectively by analyzing our database. Patients were seen in 1500 consecutive ophthalmological examinations. The mean (±S.E.M.) age at diagnosis was 44.2±0.6 years. Mean (±S.E.M.) time interval between the diagnosis of GO and GD and the onset of symptoms was 5.32±0.5 and 4.61±0.3 months, respectively. We found an increased prevalence of the diagnosis of GO in May ($P < 0.03$) and from August to September ($P < 0.004$ and 0.045) compared to the rest of the year. Similarly, a diagnosis of GD was more frequent in the months of July, August and September ($P < 0.032$, 0.014 and 0.005, respectively). In conclusion, our study shows that although both GD and GO are more frequently occurring during the warmer periods of the years, in accordance to previous studies, GO has a characteristic peak of frequency also in the month of May. These findings suggest perhaps some environmental factors may act as initial triggers of thyroid autoimmunity, similarly to what reported in other autoimmune disease such as type 1 diabetes.

P121

The incidence of thyroid cancer in the North-Eastern Region of Poland: a twelve year follow-up

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Before the introduction of mandatory salt iodination in 1997 the North-Eastern Region of Poland was known to be a moderate iodine deficiency area. It was also exposed to ionizing radiation after the Chernobyl accident in 1986. The aim of the present study was to evaluate the descriptive epidemiological features of incident thyroid cancers diagnosed among the residents of this area between 1996 and 2007. The Regional Cancer Surveillance Program was used to collect data on 834 newly diagnosed thyroid cancers registered during a 12-year period. The average annual incidence of all types of thyroid cancer per 100 000 residents rose from 3.9 in 1996 to 8.8 in 2000 and then decreased slightly to 6.8 in 2006 (mean – 5.8 cases per 100 000 inhabitants). Thyroid cancer was more frequently diagnosed in women (81.9%) than in men. The majority of all cases was diagnosed in the age group of 46–55 years. There were 12 newly diagnosed cancers in children under 15 years of age (4 cases among children born after the Chernobyl disaster). The commonest histological type was papillary carcinoma (73.3%). Follicular type accounted for 11.4%, oxyphilic – 6.4%, medullar – 4.0%, anaplastic – 3.1% and other types – for 1.8% of cases.
Conclusion
The increased incidence of thyroid cancers observed in a 12-year period is most likely explained by the improvement in diagnostic techniques. Iodine deficiency seems to be a less probable factor in view of the predominance of the papillary type of carcinoma.

P122

The TSH receptor antibody levels (TSHRAb) and thyroid function after ^{131}I therapy in patients with Graves' disease – 10 years follow-up

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Aim
The aim of the study was the evaluation of the TSHRAb level changes and the comparison with thyroid function in patients with Graves' disease treated with ^{131}I 10 years ago.
Material and methods
The study has been conducted in 67 patients (57 women, 10 men) aged 27–72 y (mean 48 years) with Graves' disease.
All patients were treated with ^{131}I due to hyperthyroidism confirmed by fT3, fT4 and TSH examination before treatment. Before therapy and after 10 years of follow-up, TSHRAb levels were measured according to radioreceptor method ('TRAK Assay', BRAHMS Diagnostika GmbH). Ten years after ^{131}I therapy TSH levels with IRMA method have been estimated.
Results
The levels of TSHRAb ranged from 1.3–405 U/l (mean 62.66±107.37 U/l) before therapy. In 18 patients (26.9%) the levels were negative, in 5 subjects (7.5%) uncertain, in 44 patients (65.6%) positive. Ten years after ^{131}I therapy, the determined TSH levels ranged between 0.04 and 41.82 mIU/l (mean 5.44±7.7 mIU/l) – 49 subjects with hypothyroidism, 17 euthyroidism, 1 hyperthyroidism. The levels of TSHRAb ranged between 2.2 and 48.4 U/l (mean 7.66±7.74 U/l). In 59 patients (88.1%) the levels were negative, 1 person (1.5%) uncertain (this person was diagnosed as hyperthyreosis), 7 persons (10.4%) positive (6 – hypothyreosis, 1 – euthyreosis). In 2 patients (3%) the increase of the TSHRAb levels has been recorded, in 16 persons (23.9%) the antibody levels did not change, however in 49 patients (73.1%) were decreased.
Conclusions
The obtained results have pointed out the prominent (nine times) and statistically significant ($P < 0.0001$), reduction of TSHRAb levels in a long-term (10 years) follow-up. A long-term ^{131}I therapy induces hypothyroidism requiring permanent systematic substitutive therapy.

P123

The outcome of radioiodine therapy in Graves' hyperthyroidism: thyroid size as prognostic factor

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Aim
The aim of the study was the evaluation of the relationship between thyroid size and the result of radioactive iodine therapy in patients treated due to Graves' hyperthyroidism.
Material and methods
The study group included 150 subjects (127 M and 23 F), aged from 20 to 78 years (mean 48.33 years) at the moment of ^{131}I therapy.
In all patients the thyroid technetium-99m scan and determination of the serum levels of fT_3, fT_4 (FIA 'Delfia' method), TSH (IFMA 'Delfia'method), TSHRAb (radioreceptor method 'TRAK Assay') were performed. Iodine uptake was measured at 24-h, 48-h, then half-life has been determined. The thyroid weight was estimated on the basis of thyroid technetium-99m scan. The therapeutic activity of ^{131}I was calculated according to Marinelli's formula.
After one year follow-up, the thyroid function has been estimated.
Successful therapy was defined as euthyroidism or permanent hypothyroidism.
Results
The thyroid weight in the group of patients before treatment ranged from 54 to 367 g (mean 72.34 $\pm$ 47.24 g). After one year, euthyrosis was observed in 47 patients (31.35%), hypothyroidism in 47 parsons (31.35%). In 56 subjects (37.3%) persistent hyperthyroidism have been diagnosed. Among patients with successful therapy, the thyroid mass ranged between 5.4 and 367 g (mean 61.97 $\pm$ 45.5 g), but in ineffective therapy group ranged between 8.2 and 196.0 g (89.59 $\pm$ 45.36 g).
The difference of thyroid mass was statistically significant ($P=0.0004$).
Conclusions
The obtained results confirm, that ^{131}I therapy is effective method of hyperthyroidism treatment, but patients of the ineffective therapy group presented larger goiter.

P124

Prognostic role of sub-clinical hypothyroidism in chronic heart failure outpatients

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The aim of this study was to evaluate the prognostic role of subclinical hypothyroidism in patients with chronic heart failure (CHF). We evaluated 338 consecutive outpatients (260 male; age 64 $\pm$ 13) with stable CHF (NYHA class 2.3 $\pm$ 0.6) receiving conventional therapy (ACE inhibitors and/or ARBs 93%, Beta-blockers 88%, Digitalis 26%, Diuretics 85%, Spironolactone 54%, Amiodarone 32%). The patients underwent a physical examination, electrocardiography and echocardiography. Blood samples were drawn to assess renal function, and Na+, haemoglobin, NT-proBNPs, fT3, fT4 and TSH levels.
Results
TSH levels >5.5 mIU/l were found in 34 patients (10%): none of these had low fT4 levels even though they show fT3 and fT4 values lower than subjects with normal TSH values. The patients with sub-clinical hypothyroidism were older, more frequently affected by diabetes and atrial fibrillation, and often treated with amiodarone; they had higher mean NYHA class, worse renal function, and lower mean arterial pressure. During the follow-up (mean 15 $\pm$ 8 months; median 16 months), the progression of heart failure led to the hospitalization of 79 patients, of whom 18 died after hospitalization and six underwent transplantation. One patient experienced sudden death, and three died of non-cardiac causes. At univariate analysis, progression was significantly associated with age, diabetes, NYHA class, mean arterial pressure, heart rate, atrial fibrillation, LVEF, LVEDD, MR, GFRc, hemoglobin, natremia, NT-proBNP, the absence of ACE inhibitor/ARB therapy, and the absence of beta-blocker therapy. Furthermore, univariate regression analysis showed that TSH ($P<0.0001$), fT3 ($P<0.0001$), fT4 ($P=0.016$) and fT3/fT4 ($P<0.0001$) were associated with heart failure progression but multivariate analysis showed that only TSH considered as a continuous variable ($P=0.001$) as well as subclinical hypothyroidism (TSH$>$ 5.5 mUI/l; $P=0.014$) remained significantly associated with the events as did mean arterial pressure ($P=0.003$), NYHA class ($P<0.001$), heart rate ($P<0.0001$), natremia ($P=0.041$), and NT-proBNP (<0.0001).
Conclusions
In CHF patients TSH levels even slightly above normal range are independently associated with a greater likelihood of heart failure progression. Routinely monitoring of TSH could be useful to identify high risk patients and to improve their prognosis through levothyroxine administration.

P125

Cognitive functions and concentrations of thyroid hormones and thyrotropin in hyperthyroidism in the course of Graves' disease

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Cognitive dysfunctions, observed in the course of thyroid diseases (hyper- and hypothyroidism), have – in the recent years – been the subject of interest for many research teams. Even mild disorders of thyroid functionality are associated with hormone concentration changes which affect the general mood and cognitive functions. The efficiency of cognitive functions, which allow the man's adaptation to environment al conditions, is determined by the activities of particular brain areas, while normal concentration of thyroid hormones is important to maintain proper brain functionality.
The goal of the reported study was an evaluation of the relationship between the efficiency of cognitive functions and concentrations of thyroid hormones (fT_3 and fT_4) and thyrotropin (TSH).
Fifty (50) patients with Graves' disease (39 female and 11 male) were qualified into the study, the mean age: 41.1 $\pm$ 10.7 years. Graves' disease was confirmed in laboratory tests by increased concentrations of anti-TSH-R antibodies. The control group comprised 31 healthy volunteers (23 female and 8 male), the mean age: 40 $\pm$ 10.3 years. The study group and the control group were matched with regards to their sex and age. Serum concentrations of TSH, fT_3 and fT_4 were measured in both groups.
The following neuropsychological tests were applied for assessment of cognitive functions: the Trail Making Test A&B (TMT A&B), Stroop Colour-Word Interference Test, the Verbal Fluency Test (FAS), the N-back Test and the Wisconsin Card Sorting Test (WCST).
The results, obtained in the group of patients with Graves' disease, do not indicate any significant relationships between the concentrations of thyroid hormones and TSH and the efficiency of studied cognitive functions. Only in case of the WCST test, were significant correlations noted with the concentrations of the evaluated hormones, what provides some evidence for the higher sensitivity of working memory and executive functions to disorders resulting from hormonal variations.

P126

Antitumor effects of aminobisphosphonates on anaplastic thyroid carcinoma cell lines

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Anaplastic thyropid carcinoma (ATC) is one of the most aggressive endocrine tumors with morphological features of undifferentiated neoplasm. Patients with ATC have a poor prognosis with a mean survival time of 2–6 months. Surgery, radiotherapy and chemotherapy do not improve survival rate. Bisphosphonates, analogs of endogenous pyrophosphates in which a carbon atom replaces the central oxygen atom, are successful agents for the prevention and treatment of postmenopausal osteoporosis and also an emerging class of drugs mostly used in the palliative care of cancer patients. Our aim was to investigate the *in vitro* activity of different bisphosphonates – clodronate, pamidronate, alendronate and zoledronic acid -, in four human anaplastic thyroid cell lines. For this purpose, we studied KAT-18, SW1736, 8505C and C643 cell lines by MTT assay and flow citometry (propidium iodide) after addition of different bisphosphonates ranging from 0 to 100 μM. A cell growth reduction in all anaplastic cell lines treated with pamidronate, alendronate and zoledronic acid at different concentrations was observed. Reduction of viability was founding cytometric analysis (10.6%, 18.8% and 42.51% respectively). Apoptosis was also assessed by DNA laddering. Our preliminary data confirm that the bisphosphonates can induce apoptosis in anapalstic thyroid carcinoma.

P127

Chronic autoimmune thyroiditis morpho-functional and immunological aspects

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The study group consists of 159 cases of chronic autoimmune thyroiditis (CAT) (age $=40.48\pm15.22$ years; F/M=156/3) distributed in 3 groups after the morpho-functional criteria: CAT with goiter – 48.43% cases (F/M=77/0); atrophic CAT – 33.33% cases (F/M=52/1); asymptomatic CAT – 18.24% cases (F/M=27/2). The thyroid ultrasonography allowed the thyroid volume measurement and the evaluation of the parenchyma echogenicity. Most of the cases presented a moderate (39.62% cases) or a marked hypoecogenicity (55.35% cases). The assessment of the thyroid functional parameters (hormonal values) revealed: in the goitrous CAT: euthyroidism (20.78% cases), subclinical hypothyroidism (33.76% cases), clinical hypothyroidism (41.56% cases), thyrotoxicosis (3.90% patients); in the atrophic CAT: subclinical hypothyroidism (33.96% cases), clinical hypothyroidism (66.04% patients); in the asymptomatic CAT: euthyroidism (100% cases). It was observed: significant high serum levels of the thyroid hormones ($P<0.001$) in patients with euthyroidism versus subclinical hypothyroidism; significant high serum TSH levels ($P<0.001$) in patients with clinical hypothyroidism versus subclinical hypothyroidism; significant high thyroid volumes ($P<0.05$) in patients with subclinical hypothyroidism versus clinical hypothyroidism.
The assessment of the humoral autoimmune parameters revealed significant high titres of the antithyroidperoxidase antibodies in the patients with goitrous CAT versus asymptomatic CAT ($P<0.05$). The patients with goitrous CAT presented, also, significant high titres of the antithyroglobulin antibodies versus those with atrophic CAT ($P<0.05$) and asymptomatic CAT ($P<0.05$). In patients with clinical hypothyroidism, the titres of the antithyroidperoxidase antibodies were significant superior ($P<0.05$) versus the patients with CAT with euthyroidism.

P128

Coexistence of hyperparathyroidism and non-medullary thyroid carcinoma

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Background – objective
Medullary thyroid carcinoma and hyperparathyroidism coexistence is well described in the literature. On the other hand, data regarding the coexistence of non-medullary thyroid cancer and hyperparathyroidism are scarce. The aim of this study was to evaluate the occurrence of such coexistence.
Methods
This is a retrospective study of all patients with primary or secondary hyperparathyroidism who underwent parathyroidectomy in our endocrine surgery unit from 2003 to 2006. Thyroid surgery was additionally performed in those cases that preoperative or intraoperative findings were suspicious of thyroid cancer.
Results
Sixty consecutive patients (38 female: 63.3%) were included in our study. Mean age of the patients was 56.3 ± 7.4 years (range: 26–80 years). Twenty-nine patients (48.3%) had primary and 31 (51.7) secondary hyperparathyroidism. Total thyroidectomy was performed in 15 cases (25%). Thyroid cancer was found in 7 cases (11.6% of the total study group and 46.6% of the patients with thyroidectomy); in all these cases the final histopathology report was consistent with primary papillary thyroid cancer. One patient with thyroid carcinoma had secondary hyperparathyroidism (3.2%) and 6 primary disease (20.7%). This difference was found to be statistically significant ($P=0.04$).
Conclusions
Non-medullary thyroid cancer may be identified in a substantial proportion of patients with hyperparathyroidism that preoperative or intraoperative findings suggest thyroid disease. In our study, there was a significant coexistence of papillary thyroid carcinoma and primary hyperparathyroidism. The surgeon should, therefore, not overlook the thyroid gland when medullary carcinoma is excluded and focus merely on the evident parathyroid disease in such a setting.

P129

The effect of radioiodine therapy in patients with non-toxic goitre

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There is no consensus regarding the optimum treatment of benign non-toxic goitre. Randomised studies have shown that levothyroxine has poor evidence of efficacy and is inferior to radioiodine therapy regarding goitre reduction.
The aim of our study was to assess the efficacy of radioiodine therapy (RIT) to reduce thyroid volume with minimal risk of hypothyroidism in patients with non-toxic nodular goitre.
Material and methods
During the last 7 years we treated 150 patients, aged 22–76 years; 88% female and 12% male; initial RAIU after 24 h was ranged between 22 and 44%, and thyroid volume ranged between 44 and 170 ml. Qualifications of these patients were based on normal levels of serum TSH, fT3, and fT4, and characteristic appearance on thyroid scans and ultrasound. Some of the patients complained of compressive symptoms (65 patients). Malignant changes were excluded in all nodules by fine needle aspiration biopsy. The therapeutic radioactivity was calculated by the use of Marinelli's formula and ranged between 400 and 800 MBq. The absorbed dose (Gy) ranged between 180 and 300, and was proportional to thyroid volume. Follow up control was done every 6 weeks.
Results
After 12 months of radioiodine therapy a mean thyroid volume reduction of 46% was achieved in all the patients, euthyroidism persist in 93% of patients, and hypothyroidism develop in eleven patients (7%). All patients were highly satisfied; the compressive symptoms relieved and exercise tolerance improved.
Conclusions
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) especially in patients with special professions (singer, teacher) or in patients who wish a non-invasive treatment modality. The reduction of thyroid volume with low percent of hypothyroidism, were due to accurate measurement of administered activity, relatively high effective half-life, and well-organised follow up.

P130

A case of complete deficiency of total thyroxine-binding globulin (TBG) associated with Graves' disease

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Thyroxine-binding globulin (TBG), the major transport protein for thyroid hormone in circulation, is synthesized in the liver. Complete TBG deficiency was first reported in 1964, and in 1991 a single nucleotide deletion was found in the first base of the codon for amino acid 352 of the common-type TBG molecule. This mutation causes a frameshift in translation and premature termination. Most people with abnormal TBG concentrations are euthyroid. Cases of Graves' disease with periodic paralysis and complete deficiency of TBG have rarely been reported.
We here describe a 28-year-old male with total thyroxine-binding globulin (TBG) deficiency associated with Graves' disease. He experienced symptoms of periodic paralysis for several days before admission. His thyroid function showed low TSH (<0.01 uIU/ml) and elevated free T4 level (3.86 ng/dl), but total T3 concentration was normal (100 ng/dl). Other test results are as follows: total T4; 4.0 ug/dl, TSH binding inhibitory immunoglobulin (TBII); 51.1%, and TBG level <1.0 ug/ml.
Allele specific PCR and DNA sequencing of the patient revealed a single nucleotide deletion was found in the first base of the codon for amino acid 352 of the TBG. We treated him with methimazole and followed.

P131

Regression of pulmonary arterial hypertension after treatment of hyperthyroidism

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Several pathological processes contribute to the development and progression of pulmonary arterial hypertension (PAH), which is a disorder with high morbidity and mortality rates. However, although cardiac manifestations are common in hyperthyroidism (HT), they have been seldom described in association with HT. Thus, the objective of this study was to evaluate echocardiographic parameters in patients with Graves Disease (GD) during uncontrolled hyperthyroidism and after its reversion with radioactive iodine therapy. We evaluate prospectively six patients with GD, (41.0 ± 14.6 years), of whom four were female. The PAH was defined using Systolic Pulmonary Arterial Pressure (SPAP) ≥ 30 mmHg. The SPAP was determined by measuring the average of the regurgitation flow through the tricuspid valve (Bernoulli's equation). Tricuspid insufficiency (TI) was classified as mild, moderate and severe. In the initial evaluation these six patients had suppressed TSH, raised free T_4 (5.4 ± 0.9 ng/dl) and raised T_3 (431.8 ± 137.0 ng/dl). In the initial evaluation all the patients had raised SPAP (61.0 ± 9.7 mmHg), 16.6% (1/6) had severe TI, 66.6% (4/6) moderate TI and 16.6% (1/6) mild TI, and five patients had severe manifestations of right cardiac insufficiency. After the normalization of thyroid function they all presented a reversal of the PAH (PSAP 30.83 ± 3.06 mmHg) and mild TI. There was no correlation between the free T_4 and SPAP during HT ($r = 0.00621$; $P < 0.05$). This data suggests an association between hyperthyroidism and PAH. The observation of this abnormality in patients with hyperthyroidism as well as its regression after euthyroidism reestablishment demonstrate the importance of systematic echocardiographic evaluation of hyperthyroid patients, especially of those who present right cardiac insufficiency.

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Thyroid function, serum lipids and insulin resistance in patients with autoimmune thyroiditis

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Objective
The aim of this study was to examine the hypothesis that thyroid function, in euthyroid subjects with autoimmune thyroiditis (AIT), is associated with insulin resistance, serum lipid concentrations, and other cardiovascular (CV) risk factors. Subjects and Methods: We recorded thyroid function tests, BMI, insulin resistance markers comprising the Homeostasis Model Assessment for insulin resistance (HOMA-IR), the Quantitative Insulin Sensitivity Check Index (QUICKI), HISI (Hepatic Insulin Sensitivity Index), WBISI (Whole-Body Insulin Sensitivity Index), IGI (Insulinogenic Index) and the levels of total cholesterol (TC), HDL, LDL-cholesterol, triglycerides (TG), apolipoprotein B (ApoB), ApoA1, lipoprotein(a) (Lp[a]), homocysteine, CRP (C-reactive protein), folic acid and vitamin B12 levels, in 250 patients with AIT. A 75-g OGTT was performed in the morning (before 11 AM), and blood samples were obtained every 30 min for 120 min for measurements of plasma glucose, insulin, and C-peptide. Statistical analysis was performed with ANOVA and Pearson's correlations test. Results are expressed as means S.D. or percentages. A two-tailed P value < 0.05 was considered significant.
Results
There were significant positive correlations between TSH and serum total cholesterol ($r = 0.382$, $P = 0.01$), LDL ($R = 0.384$; $P = 0.01$), TG ($R = 0.278$; $P = 0.01$), and ApoB ($R = 0.341$; $P = 0.01$). BMI was positively associated with FT4 ($R = 0.274$; $P = 0.01$) and negatively associated with HDL ($R = -0.279$; $P = 0.01$) and Apo A1 ($R = -0.299$; $P = 0.01$). There were significant negative correlations between CRP and HDL($R = -0.269$; $P = 0.01$) and a significant positive correlation between CRP and TG ($R = 0.567$; $P = 0.01$), and homocysteine ($R = 0.234$; $P = 0.05$). There were significant positive correlations between IGI and TG ($R = 0.264$; $P = 0.01$) and TSH ($R = 0.217$; $P = 0.05$), and between WBISI and HDL-C ($R = 0.203$; $P = 0.05$).
Conclusion
Thyroid function and lipid levels are associated even in subjects classified as being euthyroid, thereby extending the established relation between (sub)clinical hypothyroidism and hyperlipidemia in the normal range. These findings are consistent with an increased cardiovascular risk in subjects with low normal thyroid function.

P133

Association of HTLV-I with autoimmune thyroiditis in patients with myelopathy/tropical spastic paraparesis and in HTLV-I carriers in Mashhad, North East of Iran

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Objectives
There are some reports about association of autoimmune thyroid diseases with human T cell leukemia virus type I (HTLV-I) infection. The objective of this study was to estimate the seroprevalence rates of anti-thyroid antibodies in HTLV-I carriers and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients in Mashhad, north east of Iran, to determine any association between HTLV-I infection and Hashimoto's thyroiditis (HT).
Materials and methods
About 46 HTLV-I infected patients (24 patients with HAM/TSP and 22 asymptomatic carriers) and 40 HTLV-I seronegative healthy individuals were screened for the presence of thyoid autoantibodies. The diagnosis of HT was based on the presence of positive thyroid autoantibodies (Anti thyroid peroxidase and/or Anti thyroglobulin) and at least one of two additional criteria (hypothyroidism and/or goiter). Analysis of data was done using Fisher-Exact test by statistical software SPSS version 13.0. A P value below 0.05 was considered statistically significant.
Results
Positivity for thyroid autoantibodies was found in 14 (63.6%) of 22 asymptomatic carriers, 6 (25%) of 24 patients with HAM/TSP and 3 (7.5%) of 40 HTLV-I seronegative healthy individuals. HT found in 45.4% of asymptomatic carriers, 25% of HAM/TSP patients and 7.5% of seronegative healthy individuals.
Conclusion
This study demonstrates a high prevalence of HT in the HAM/TSP patients and the HTLV-I carriers in Mashhad. Our findings suggest an association between HYLV-I infection and HT in our region.

P134

Influence of thyrometabolic state on distribution of subpopulations and fenotypes of dendritic cells in peripheral blood of the patients with chronic thyroiditis and patients monitored because of differentiated thyroid cancer

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Considering, the pivotal role of DC in formation and development of autoimmunological processes, the investigation of influence of of thyrometabolic status on maturation and function of subtypes of human peripheral blood DC, seems to be reasonable and of particular interest. THE AIM of the present study was a complex analysis of the dendritic cell subsets and fenotypes in patients with chronic thyroiditis (ChT) as well as the patients monitored for differentiated thyroid cancer (DTC).
Patients and methods
Blood samples were collected from patients suffering from ChT before and after treatment with L-T_4 ($N = 18$). Moreover, to investigate the influence of thyroid

hormones, blood samples for ex vivo analysis were collected from thyroidectomised (because of differentiated thyroid carcinoma) patients ($n=21$) at two time points: (i) after withdrawal of L-T4 treatment group before treatment, and (ii) during 2 months of L-T4 administration in order to suppress TSH concentration group after treatment. FACS analysis of expression of selected molecules on the blood dendritic cells was performed. Furthermore, the investigation of the DC cell culture was performed in the conditions of deficiency and excess of T3.
Results
We found that the percentage of pDCs and mDC in peripheral blood was dependent on thyrometabolic state and that in patients with ChT this regulation was partially impaired. Additionally, we observed lower expression of CD86 on myeloid DCs in hypothyroid ChT patients as compared to thyroidectomised patients. Interestingly this difference was attenuated by L-T_4 treatment. The effect of thyroid hormones on surface expression of co-stimulatory molecules was then confirmed *in vitro* in experiments with freshly sorted human DCs.
Conclusions
Results of our study indicate that thyroid hormones influence the biology of peripheral blood DCs. This regulatory effect was furthermore affected by chronic thyroiditis. This observation might be of great importance for understanding of immune disorders of endocrine system.

P135

Evaluation of cyclins a and b1 expression in classical and nonclassical variants of papillary thyroid carcinoma

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The loss of regulatory control of the cell, leading to unrestrained cell proliferation, is a hallmark of cancer. In a number of cancers, over expression of cyclin A and cyclin B1 proteins has been reported and in some instances the level of expression correlated well with the grades of malignancy. In the present study, we analyzed, by immunohistochemistry, the expression of cyclins A and B1, proteins enable passing G(2)-restriction point, in different histological variants of papillary thyroid carcinomas (PTC). We investigated immunostaining patterns in 40 tissue specimens of PTC for cyclin A and B1 which were divided in 3 groups: 20 classical PTC, 9 follicular variant of papillary thyroid carcinoma (FVPTC) and 11 other that FVPTC nonclassical PTC. Nuclear and/or cytological immunostaining detected in $\geq 5\%$ of tumor cells was considered the cut-off for both cyclins. We observed a significant differences in expression of cyclin A and B1 between the groups. The highest expression was observed in group of other that FVPTC nonclassical PTC, the lowest expression was observed in group of classical PTC. The results of the study may potentially explain more aggressive character of other that follicular, nonclassical, variants of PTC. Evaluation of cyclins A and B1 in various thyroid lesions may be helpful in diagnostically doubtful cases. However, we cannot yet consider cyclins as a prognostic markers because the group of nonclassical PTC is to small.

P136

Alterations in TSH and thyroid hormones following mobile phone use

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Background
In recent years, the widespread use of mobile phones has lead to a public debate about possible detrimental effects on human health. In spite of years of research, there is still a great controversy regarding the possibility of induction of any significant physiological effects in humans by microwave radiations emitted by mobile phones. This study is an attempt to investigate the effects of electromagnetic fields induced by GSM mobile phones on the TSH and thyroid hormones in humans.
Materials and methods
Seventy seven healthy university students participated in this study. The levels of T3, T4 and TSH were measured by using appropriate ELISA kits (Human, Germany).
Results
The average levels of T3, T4 and TSH in the students who moderately used mobile phones were 1.25 ± 0.27 ng/ml, 7.76 ± 1.73 μg/dl and 4.25 ± 2.12 μu/l respectively. These levels in the students who severely used mobile phones were 1.18 ± 0.30, 7.75 ± 1.14 and 3.75 ± 2.05 respectively. In non-users, these levels were 1.15 ± 0.27, 8.42 ± 2.72 and 2.70 ± 1.75, respectively. The difference among the levels of TSH in these 3 groups was statistically significant ($P<0.05$).
Conclusion
Based on our findings; a higher than normal TSH level, low mean T4 and normal T3 concentration in mobile users, it seems that minor degrees of thyroid dysfunction with a compensatory rise in TSH may occur following excessive use of mobile phones. It may be concluded that possible deleterious effects of mobile microwaves on hypothalamic–pituitary–thyroid axis affects the levels of these hormones.

P137

Prevalence of thyroid dysfunction in the elderly women of Iran

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Objectives
The present study aimed to investigate the prevalence of thyroid dysfunction in the elderly women of Tabriz city, the largest city in North West Iran.
Design
Cross-sectional study.
Setting and participants
By using the records of the local household registry, a sample of 1150 subjects was drawn by simple random sampling. After the exclusion of nonresponse subjects, 1000 subjects aged between 60 and 89 years (mean 64.5 ± 5.4) were included in our survey.
Measurements
Tests of thyroid function including TSH concentration in all subjects; and free T4 concentration, free T3 concentration and anti-microsomal antibodies in those with abnormal TSH were conducted.
Results
Seventy-three (7.3%) participants had high (>4.5 mU/l), and 54 (5.4%) had low (<0.3 mU/l) TSH levels. The overall prevalence of thyroid dysfunction in the sample was 12.7%. Of the 73 participants with high TSH levels, 15 (20.5%) had overt hypothyroidism, and of the 54 participants with low TSH levels, 12 (22.2%) had overt hyperthyroidism. Only 1 participant (1.85%) had T3 toxicosis. High titers of anti-microsomal antibodies were found in 60.6% of those with high TSH levels.
Conclusion
The prevalence of abnormal biochemical thyroid function reported here is substantial and confirms previous reports in other populations. Individual symptoms were not very sensitive, but patients who report multiple thyroid symptoms warrant serum thyroid testing.
We found that the prevalence of thyroid dysfunction is high in elderly female population of East Azerbaijan. The results provide baseline information to settle public health plans and to track the changes.

P138

Experimental method of post-surgery hypothyroidism treatment

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Growth of morbidity with thyroid nodes and increase of thyroidectomy in Ukraine up to 4000–5000 annually forces to find more efficient methods of correction of post-surgery hypothyroidism as the most frequent complication after thyroid surgery. In 10–20% of cases reach adequate euthyroidism by exogenous L-thyroxin is impossible.
Methods
Experiments were performed on A (control) and B (experimental) groups of dogs (20). All were made thyroidectomy to model hypothyroidism. Removed thyroid was cut into plates and undergone for cryopreservation (-196 °C). In the group B thyroid tissue was exposed to processing by oxygen under pressure 3 atm in pressure chamber before freezing and before transplantation. In both groups on the 20th day after surgery we made thyroid autotransplantation. All the dogs before surgery, on the 40th and 60th day after thyroid removal determined clinical signs and hormones level (TSH, T_4).

Results
By the end of the 2nd week after thyroidectomy all the dogs had the symptoms of hypothyroidism: weight loss, decrease of fatty tissue thickness, decrease of T_4, and increase of TSH.
Dynamics of hormonal status of animals

	Before surgery		On the 40th day		On the 60th day	
	A	B	A	B	A	B
TSH, mUn\l	0.5±0.05	0.5±0.05	0.65±0.05	0.58±0.02	0.8±0.03	0.65±0.03
T_4, nmol\l	33.6±0.04	33.6±0.04	29.6±0.04	31.6±0.02	26.4±0.04	28.6±0.04

Conclusions
(1) We improved the method of thyroid autotransplantation and treatment of post-surgery hypothyroidism in experiment.
(2) Our method of thyroid tissue cryopreservation under hyperoxygenation is simple, oxygenated thyroid graft functions more efficiently: T_4 on the 40–60th day exceeds the control group for 8%, TSH is averagely less for 12%.
This method of hypothyroidism treatment enables to restore physiological hormonal status of experimental animals.

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Physical and psychological well-being in adults with thyroid abnormalities

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Background
Patients with thyroid abnormalities often also suffer with anxiety and depression. Our objectives in this study was to investigate prospectively the effect of thyroid dysfunction per se on quality of life and levels of depression and anxiety.
Methods
A total of 102 patients who were referred to thyroid clinic were enrolled in the study in consecutive order. Enrollment criteria comprised patient aged 20–60 with no major life events, previous history of depression, anxiety, or any other significant co-morbidities. Patients were actively excluded if they had postpartum depression, previous history of overdoses or were on antidepressant or previous psychiatric history. These comprised 32 patients with hyperthyroidism, 34 patients that were euthyroid,and 36 patients that were hypothyroid.
Results
Patients with hypothyroidism or hyperthyroidism were more likely to experience a poor quality of life than patients that were euthyroid. Hypothyroidism or hyperthyroidism were more likely to be associated with depression or anxiety than euthyroid patients. Anxiety, depression and quality of life were evaluated using Hamilton Anxiety rating scale and shortform 36.
Conclusion
Treatment of hypothyroidism or hyperthyroidism and return to euthyroid status is accompanied by improvement in quality of life and psychological symptoms.
Larger controlled randomised studies are required in future that asses anxiety,depression and quality of life in thyroid patients and follow through treatment so that they can act as their own control.

P140

Anemia frequency and etiology in primary hypothyroidism

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Objective
Thyroid hormones directly or indirectly, through erythropoietin, stimulate growth of erythroid colonies. Anemia is often the first sign of hypothyroidism. Hypothyroidism can lead to a wide variety (%20–60) of anemic disorders. Numerous mechanisms are involved in the pathogenesis of these anemias which can be microcytic, macrocytic and normocytic. Microcytic anemia is usually ascribed to malabsorption of iron and loss of iron by menorrhagia. Macrocytic anemia is caused by malabsorption of vitamin B12, folic acid, as seen in pernicious anemia and in inadequate nutrition. Normocytic anemia is characterized by reticulopenia, hypoplasia of erythroid lineage, decreased level of erythropoietin, mainly regular erythrocyte survival. We designed this study to investigate the anemia frequency and if present, etiology of anemia in hypothyroid patients.
Research design and methods
Overt hypothyroid 100 patients, subclinical hypothyroid 100 patients and 200 healthy controls enrolled in this study.
Results
Anemia prevalence was 43% in the hypothyroid group, 39% in the subclinical hypothyroid group and 26% in the control group which was statistically significant ($P=0.003$ and $P=0.021$ respectively related to controls). There was no difference between the hypothyroid groups in terms of anemia. Vit B12, Fe, folic acid were similar between the groups.
Conclusions
According to our findings, chronic disease anemia is the most common type of anemia in hypothyroid patients similarly with the literature. Suspicion of hypothyroidism should be considered in every case of anemia with uncertain etiology.

P141

Thyrotropin suppression by metformin in a cohort of patients with differentiated thyroid cancer in follow-up

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Background
It has been reported that metformin might modify thyroid hormone economy. This farmacological tool appears very useful in patients with differentiated thyroid cancer usually receiving high Levo-tiroxine (L-T4) doses to suppress thyrotropin (TSH). In those patients that after five years of follow-up showed no persistence of disease, it's useful to abolish the iatrogenic hyperthyroid condition.
Objective
To evaluate metformin efficacy to suppress TSH.
Methods
From a population of patients with differentiated thyroid cancer in follow-up we selected a cohort of 30 long-standing subjects (mean age: 48.1±9.52; F/M: 24/6) in which oral glucose tolerance test documented an insulin-resistance syndrome. Patients were in L-T4 substitutive/suppressive therapy at the dose of 2.2–3 mcg/pro kg body weight. BMI, TSH, FT4 were measured at baseline, after two and four months. At baseline L-T4 therapy was reduced to 2 mcg/pro kg body weight, aimed to reduce subclinical hyperthyroidism, and after two months metformin 500 mg tid was introduced.
Results
At the study start patients showed undosable TSH (0.22±0.20); in this stage L-T4 therapy was reduced to 2 mcg/pro kg body weight. Two months after reduction of oral L-T4 therapy TSH levels were in the normal range (0.96±0.62) showing statistical difference from baseline ($P=0.003$). After four months TSH levels were suppressed (0.21±0.29) showing significant difference from the value obtained after two months of L-T4 reduction ($P=0.03$) but not from baseline ($P=$ns). There was no change in FT4.
Conclusions
Initiation of treatment with metformin caused suppression of TSH to subnormal levels without clinical symptoms of hyperthyroidism in any patients. No other potential causes of TSH suppression, including medication changes or interference in the TSH assay, could be identified. Thus, metformin administration is associated with a significant fall in TSH useful to avoid iatrogenic subclinical hyperthyroidism.

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Human thyroid tissue HCO_3^--ATPase in norm and pathology

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Scientific literature contains data on HCO_3^--activated and Mg^{2+}-stimulated ATPase detected in various tissues of vertebrate animals, specifically in the pancreas mucus, liver, kidneys, erythrocytes, diaphragm and various structures in the brain. It has been discovered that maximum enzymatic activity is characteristic to secretory tissues.
The nature and function of this ferment has not been fully discovered, although several assumptions do exist. One of them maintains that HCO_3^--ATPase is an active participant in the regulation of intracellular pH.
This research has aimed at the study of human thyroid gland HCO_3^--ATPase and changes in its activity under various pathologies (gland adenoma, carcinoma and diffusive-toxic goiter).
In order to achieve the goals set before the research we have studied distribution of enzymatic activity in the mitochondrial, nuclear fractions and those taken from endoplasmic reticulum and plasma membranes from healthy and affected human thyroid tissues, for which a relevant permit was pre-obtained from the Ministry of Health. The received data have shown an especially high enzymatic activity in the mitochondrial and plasma membrane fractions. Alongside we have observed radical changes in HCO_3^--ATPase activity under various gland pathologies, such as adenoma, carcinoma and diffusive-toxic goiter.
In order to establish the nature of HCO_3^--ATPase activity changes we have studied kinetic properties of the enzyme, such as V_{max} and K_m in healthy and sick gland tissues and determined kinetic parameters of the enzyme.
Based on the available data we have assumed that formation of various thyroid pathologies is accompanied by changes in HCO_3^--ATPase activity, which could possibly lead to the gland-related diseases.

P143

Clinical and epidemiological characteristics of thyroid hemiagenesis: ultrasound screening in patients with thyroid disease and normal population

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Objective
Thyroid hemiagenesis is a rare form of thyroid dysgenesis, in which one thyroid lobe fails to develop. The true prevalence of this rare abnormality is about 0.05–0.2% in normal population. We aimed to determine prevalence of thyroid hemiagenesis in patients with various thyroid disorders and a normal population in a mild to moderate iodine-deficient area.
Subjects and methods
The clinical and thyroid ultrasonography records of 4.833 patients who presented with various thyroid disorders were reviewed. In addition, ultrasonographic data of two large surveys carried out for the community screening of iodine status of children ($n=4.772$) and thyroid disorders of adult subjects ($n=2.935$) were analyzed.
Results
In patients with thyroid disorders, we found 12 cases with thyroid hemiagenesis (0.25%). Thyroid hemiagenesis was due to the agenesis of the left lobe in all cases. The underlying thyroid diseases were Hashimoto's thyroiditis ($n=4$), euthyroid multinodular goiter ($n=4$), and toxic adenoma ($n=1$). Three subjects have no underlying thyroid disease. In ultrasonography screening of normal population, altogether, the absence of the left lobe was detected in only two cases, indicating a true prevalence of thyroid hemiagenesis of 0.025%. None of the reviewed patients had thyroid dysfunction.
Conclusion
Our community-based data on thyroid hemiagenesis is in accordance with previous studies in terms of prevalence and male-to-female ratio.

P144

Targeted high-risk case finding of thyroid dysfunction in an Iranian pregnant population

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Introduction
To evaluate efficacy of universal screening versus high risk group screening for thyroid dysfunction in pregnant women.
Method
From February to July 2008, prospective study was performed on 608 pregnant women in Bandar abbas, Iran. All of pregnant women were tested for T4, T3, T3RU, FTI, TPO Ab in the first pre natal visit. Then, were assigned into two groups in order to have positive history of thyroid dysfunction or first degree family history and other autoimmune disorders in high risk group or not in low risk group. The rate of thyroid dysfunction (hypo and hyperthyroid) in two these groups compared together.
Results
Of the 608 pregnant women 85.4% were euthyroid, 12.3 hypothyroid (0.5% overt and 11.8% subclinical hypothyroidism) and 2.3% hyperthyroid. 14.7% of hypothyroid women and none of hyperthyroidism reported a positive history of thyroid dysfunction. 17.6%of cases with hypothyroidism and 14.3%of hyperthyroidism had family history of thyroid dysfunction. In this study, only one subject had diabetes mellitus. The correlation between thyroid dysfunction in pregnancy and personal history of thyroid dysfunction in past was significant (P value: 0.00) but not family history of thyroid dysfunction (P value:0.3). 26.7%of hypothyroid women and 14.3% of hyperthyroidism fall in high risk group and 73.3% of hypothyroid and 85.7% of hyper thyroid women were in low risk group.
Conclusion
In this study, more than three quarter of woman with thyroid dysfunction would not be screened because they were in low risk group. It shows Targeted High risk case finding is not enough efficacies for detection thyroid dysfunction during pregnancy.

P145

Assessment of biochemical parameters during Levothyroxine replacement therapy in hypothyroid patients

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Aim of this study was to evaluate biochemical parameters of thyroid gland function, used in evaluation of levothyroxine (L-T4) dose titration during a long time period in hypothyroid patients.
Patients and methods
About 32 hypothyroid women were included in our study. All patients were euthyroid for a long time, treated with levothyroxine replacement therapy, taking an individually titrated daily dosage (50–100 μg). Blood samples were taken from all the patients before therapy (on empty stomach), and two hours after therapy administration. In both blood samples, the following parameters were estimated: TT3, TT4, fT3, fT4 i TSH. Those parameters were measured at once by immunometric assays on ARCHITECT i2000SR. All data were processed by standard statistical analysis.
Results
There is statistical significant increment ($P<0.05$) of fT4 ($X=14.9$ pmol/l; S.D. = 1.9) and TT4 ($X=118$ nmol/l; S.D. = 21.2) values after levothyroxine administration regarding the values measured before therapy (fT4:$X=13.6$ pmol/l; S.D. = 1.7; TT4: $X=107.5$ nmol/l; S.D. = 19.2). There is no statistical difference between values of fT3 and TT3 before and after administration of replacement therapy ($P>0.05$). After therapy administration, there was an estimated decrement of TSH values ($X=2.07$ mIU/l; S.D. = 1.45) regarding the values of TSH before the therapy ($X=2.76$ mIU/l; S.D. = 1.98), but with no statistical difference ($P=0.06$) due to high S.D.
Conclusion
Time interval between levothyroxine administration and blood sampling for fT4, TT4 and TSH measurements should be accounted for order to evaluate the apllicated levothyroxine dose. Due to these results, it is recommended that blood samples should be collected before L-T_4 therapy administration in clinically euthyroid and overtly hypothyroid patients, but when there is a suspicion of

overdose with L-T_4, blood samples should be taken at least two hours after received therapy.

P146

Nondiagnostic fine needle aspiration biopsy results

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Objective
Fine needle aspiration biopsy (FNAB) is a reliable and safe method to distinguish benign and malignant thyroid nodules. FNAB has two major limitations: nondiagnostic and suspicious cytology results. There is uncertainty about clinical approach to the nondiagnostic FNAB in thyroid nodules. Our aim was to evaluate the ratio and reasons of nondiagnostic results, and the ratio of malignancy in these nodules.
Method
About 2082 patients and 3404 nodules in these patients who referred to the thyroid disease outpatient clinic between 2005 and 2008 were analyzed, retrospectively. Nodules with suspicious ultrasonographic images and two nondiagnostic cytology were given to surgery. Nodules reported as nondiagnostic in two cytologies, but without suspicious ultrasonographic images were taken to clinical and ultrasonographic follow-up.
Results
FNAB was performed in 3404 nodules. After the first ultrasonography guided FNAB, the rate of nondiagnostic cytology was 9.3% and a second FNAB was repeated in this group. Cytology was reported as nondiagnostic again in 10.8% of these. According to nodule size, 14.6% of infracentimetric nodules and 7.9% of supracentimetric nodules were nondiagnostic ($P<0.001$). The ratio of nondiagnostic results was 8.9% in solid nodules, 12.3% in mixed nodules and 13.8% in cystic nodules ($P=0.08$). 14 patients with nondiagnostic cytology underwent operation and histopathologically malignancy ratio was found to be 64.3% ($n=9$).
Conclusion
According to our results, the ratio of nondiagnostic cytology results was 9.3%. Nondiagnostic cytology was found to be related to the size of the nodule. In the literature malignancy ratio in nondiagnostic cytology is reported between 9 and 37% in different studies, but ours was 64.3%. The reason for higher malignancy rates in our study may be preference of surgical management not in all patients with nondiagnostic cytology but in patients with clinically and ultrasonographically suspicious nodules. Considering this result, nondiagnostic cytologies might not be of benign cytology and should be evaluated carefully.

P147

Concurrency of primary hyperparathyroidism and thyroid diseases

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Objective
Thyroid diseases are reported to be in 22–70% of primary hyperparathyroidism (PHPT) patients in different studies. Thyroid pathology is detected during neck exploration in some of these patients. In this study we aimed to investigate thyroid pathology in patients operated for PHPT in our clinic.
Method
About 32 PHPT patients were included in the study. Patients were evaluated with thyroid function tests, antithyroglobulin antibody, antithyroid peroxidase antibody, TSH receptor antibody, thyroid ultrasonography (US), and fine needle aspiration biopsy (FNAB). All patients were operated including neck exploration.
Results
Thyroid US before operation yielded thyroid nodule in 21 patients. 17 patients were euthyroid before operation of which 12 had multinodular goiter (MNG), 3 had nodular goiter (NG) and 2 had postoperative recurrent MNG. One of two hyperthyroid patients had toxic MNG whereas the other had toxic diffuse goiter. Chronic thyroiditis and MNG with thyroiditis were responsible from hypothyroidism in 4 and 3 patients, respectively. Thyroid autoantibodies were high in 7 patients. Summing these, preoperative thyroid pathology was found to be in 27(84%) patients. Preoperatively 33 of 54 nodules were aspirated and all were reported as benign. 24 patients had parathyroidectomy with thyroid operation. Among these patients, it was reported that 3 had papillary microcarcinoma(9%), 7 had chronic lymphocytic thyroiditis(21%) and 15 had nodular hyperplasia (47%) histopathologically. PHPT was due to parathyroid adenoma in 31 patients and carcinoma in 1 patient.
Conclusion
In our study, there was concurrent thyroid pathology in 84% of patients operated for PHPT. We like to draw attention to 3 patients (9%) who had incidental thyroid malignancy postoperatively. With minimal invasive surgical approach used much more common in recent years, during parathyroid operations, thyroid pathologies are not evaluated particularly Therefore, patients should have detailed neck US and FNAB when needed before operation and surgical approach should be determined considering thyroid pathologies.

P148

Incidental thyroid carcinoma in patients with thyrotoxicosis

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Objective
Thyroid malignancy detected incidentally in patients who underwent surgery for thyrotoxicosis has been reported at different rates. The aim of this study was to investigate the rate of incidental thyroid carcinoma (ITC) in thyrotoxic patients underwent surgery in our institution.
Methods
The prevalence of ITC was investigated in patients who underwent surgery for Graves' disease (GD), toxic adenoma (TA) or toxic multinodular goiter (TMNG) from 2006 to 2008. Fine-needle aspiration biopsy (FNAB) was done for cytological evaluation in all patients with TMNG when we determined a 'cold' nodule on scintigraphy, and in those with GD and a concomitant solid nodule. Among patients who had side effects of antithyroid drug therapy, or GD with multiple relapses after therapy withdrawal or responsiveness to antithyroid drugs those who refused radioactive iyode treatment and a goiter causing symptoms of compression underwent surgery.
Results
Among 316 thyrotoxic patients (231 women and 85 men; mean age=48.9 years), 54.1% ($n=171$) had TMNG, 13.9% ($n=44$) had TA and 32% ($n=101$) had GD. Thyroid carcinoma was determined in 27 (8.5%) patients. Fifteen (8.8%) of TMNG, 3 (6.8%) of TA and 9 (8.9%) of GD patients had thyroid carcinoma. The incidence of thyroid carcinoma was similar between subjects with GD, TMNG and TA ($P=0.906$). Histologic examination revealed 24 papillary (23 microcarcinoma), 2 follicular, and 1 anaplastic carcinoma. Mean diameters of carcinoma was 0.64 ± 0.29 cm (range, 0.10–1.50 cm). While one patient had multifocal tumor tissue (3.7%) 2 patient had vascular invasion (7.4%) and 3 patient had capsular invasion (11.1%).
Conclusion
Our results also suggest that total thyroidectomi was preferable to subtotal to prevent the need for reoperation for patients with thyrotoxicosis after a detailed preoperative evalution for malignancy including FNAB and cervical lymphadenopathy investigation.

P149

The influence of radioiodine therapy on some parameters of oxidant/antioxidant balance in patients with toxic nodular goitre

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Oxidative stress plays an important role in hyperthyroidism-induced tissue damage.

We aimed to determine whether radioiodine therapy has benefit effect on the oxidant and antioxidant status in patients with toxic nodular goitre.
Material and methods
We studied 40 patients with toxic nodular goitre, (31 female, 9 male), aged 21–65 years. 12 normal adult volunteers (age and sex-matched) were studied as control group. All the patients were in mild hyperthyroidism with serum TSH levels was less than 0.1 mU/l and effective half-life was more than 3 days at the time of treatment. Malignant changes were excluded in all nodules by fine needle aspiration biopsy. In the investigated groups, we evaluate malondialdehyde (MDA) as a marker of oxidative stress, glutathione (GSH) and glutathione peroxidase (GPx) activity as a parameters of antioxidant system before and 6 months after radioiodine therapy. The serum fT4, fT3 and TSH were evaluated before and monthly up to 12 months after RIT. Thyroid ultrasound, and thyroid scan were done after12 months of ^{131}I therapy to assess thyroid volume. The activity dose was calculated by Marinelli's formula and ranged between 280 and 800 MBq. The absorbed dose ranged between 160 and 300 Gy, and was proportional to thyroid volume.
Results
A Significant increase in MDA level with significant decrease in GPx activities and GSH level were observed in these patients before treatment compared to controls subject. Achievement of euthyroidism after 6 months of radioiodine administration resulted in a significant decrease of MDA level, significant increase of GSH level and in GPx activities. Euthyroidism was achieved in 36 patients and hypothyroidism developed in 4 patients. Thyroid volume reduced to about 46% (average).
Conclusions
Our results confirm the imbalance of the antioxidant/oxidant status in patients with toxic nodular goitre. Radioiodine therapy was more effective to improve these balances.

P150

Fever in the debute of the diffuse toxic goiter

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Aim
To pay attention to the so rare symptom of diffuse toxic goiter (DTG) as fever.
Materials and methods
We report about 5 patients (all females, aged 18–38, mean – 31). Before, all patients were examined for fever of unknown origin of long duration. After the others reasons of the febrile body temperature were excluded, DTG was diagnosed. DTG was confirmed by the results of the clinical, hormonal and immunological investigations (T- and B-cell immunity were assessed).
Results
Mediana TSH was 0.09 μU/ml and fT4 26.9 pmol/l. Thyroid volume was exceeded normal (varied from 21 to 35 sm^2, mediana – 22.4 sm^3). The immunological disturbances were revealed: decreased suppressor (6.1%, 15% in control) and helper T-cells (6.1%, 15% in control) count, decreased phagocytosis activity in NST test (9.7%, 79% in control), increased β-cells count (33%, $<$23% in control) and high level of immunoglobulin IgG class (16.4%, 11.5% in control). The TSH-r antibodies titre (Radioassay RAST, Germany) was studied in 3 patients and achieved 36.5%, 56% and 76% respectively (in comparison with $<$13% in controls). Antithyroid therapy (adequate methimazole daily doses) was used. Febrile temperature decreased to normal after euthyroism was achieved.
Conclusion
The hyperthyroidism must be taken into account among the different reasons of fever of unknown origin. That rare clinical symptom mirrored the impaired termoregulation due to increased thermogenesis and due to possible immunological disorders in DTG patients with hyperproduction of the thyroid hormones and may be the consequence of the negative influence of the pyrogenic cytokines (IL1, IL6) on the PgE2 production.

P151

Efficacy RAI in patients with Graves disease using different methods calculation of optimum therapeutic activity I^{131}

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The aim of the study is to compare efficacy of RAI in patients with Graves disease using different methods calculation of optimum therapeutic activity.
Methods
About 60 patients (50 women, 10 men, mean age 44.8 $\pm$ 11.8 years) with Graves decease were treated with RAI. The treatment with ATD was cancel 10 days before RAI to restore the iodine intake by thyroid gland. Therapeutic activity was determent individually 1) with applied the special formula in which the volume of a thyroid gland (V) and capture of diagnostic activity of radioiodine in 24 h (C) was taken into account. 2) with applied absorbed dose (for the formation in thyroid gland absorbed dose 100–150 Gray). Patients who got rated dose broken into two part: Group A – 20 patients who got activity I^{131} less 0.3 mCu/ml (middle dose 10.7 $\pm$ 7.1 mCu) and B – 20 patients who got activity I^{131} more 0.3 mCu per tissue thyroid gland (middle dose – 12.7 $\pm$ 4.7 mCu). Group C – 20 patients who got dose I^{131} taking into account absorbed dose thyroid gland (middle dose – 13.0 $\pm$ 5.7 mCu).
The results: Group A: before RAI the median of TV was 35.3 ml (23.8;56.9); in 12 month – 8.9 (6.3;11.6). Group B: before RAI treatment the median of TV was 30.8 ml (24.1;47.9); in 12 month – 6.1 (2.9;8.9). Group C: before RAI treatment the median of TV was 45.5 ml (35.7;63.5); in 12 month – 14.3 (12.0;16.6).
In 12 month after RAI in group A 7(35%) patients became euthyroid, 7(35%) patients remained thyrotoxic, 6(30%) patient had hypothyroidism. Group B 3(15%) patients became euthyroid, 3(15%) patients remained thyrotoxic, 14(70%) patient had hypothyroidism. Group C 5(25%) patients became euthyroid, 2(10%) patients remained thyrotoxic, 13(65%) patient had hypothyroidism.
Conclusion
It's reasonable to apply the therapeutic dose more then 0.3 mCu per 1 ml thyroid volume to achieve good results.

P152

Comparing the Outcome of radioactive iodine treatment for hyperthyroidism in a Jordanian and British cohorts

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Radioactive iodine (RAI) has been in use for more than 60 years with satisfactory results RAI and safe profile, there has been a different outcome of treatment in different ethnic groups**.** We assessed the demographic features and clinical outcome of a Jordanian and British cohorts of patients treated with RAI.
Methods
Hyperthyroid patients who opted RAI as a primary therapy and those who had relapse after treatment with antithyroid drugs (ATD) or had significant side effects. All patients were advised to stop ATD 3–5 days before RAI dose and special precautions to avoid contact with others according to international standards were fully explained. Thyroid function tests were checked every 12 weeks for the first year after RAI dose. Clinical outcome of euthyroidism, hypothyroidism or persistent hyperthyroidism after at least one year of FU were reported and doses of RAI were compared.
Results
There were 242 patients who received 258 doses of RAI in the Jordanian cohort and 234 in the British cohort.
The demographic features and clinical out come are shown in table 1.
Discussion and conclusion
There was a significant difference in the hypothyroid and euthyroid rates between the two cohorts being better in the British cohort,while the relapse rate was significantly lower in the Jordanian cohort.The interpretation might be in the dose delivered to the Jordanian cohort was higher. More patients in the Jordanian cohort received ATD that might affect the final outcome.

	Jordanian cohort	British cohort	*P* value
Number (fmales)	242(33.5%)	234(20.9%)	–
Grave's disease	132(54.5%)	158(79%)	0.003
Toxic adenoma	81(33.5%)	44(18.8%)	0.0027
Others	29(12%)	27(2.2%)	0.88
Dose of RAI mCi	15.9 $\pm$ 2.5	8.43 $\pm$ 0.23	0.03
Euthyroidism	13.6%	25.8%	0.0015
Hypothyroidism	75%	53.3%	$<$0.0011
Persistent hyperthyroidism	11.2%	20.9%	0.0036
Thyroxin dose for hypothyroidism	114.4 $\pm$ 54.6	101.9 $\pm$ 40.7	0.025
Pat received ATD	203(83.9%)	159(67.9%)	0.0004

P153

Primary hyperparathyroidism and synchronous thyroid disorders: a single center experience

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Background
High association of concomitant thyroid and parathyroid disorders has been reported. The aim of this study was to determine the prevalence and characteristics of thyroid disorders associated with primary hyperparathyroidism (PHPT) in Turkey, a country with mild iodine deficinency.
Material and method
We retrospectively reviewed the records of patients diagnosed with PHPT between 1980 and 2007 at our clinic and analyzed the data related to thyroid.
Results
There were 166 cases available. One hundred and thirty two patients had data regarding thyroid status. (Age 50.8±12.6, F/M; 109/23). One hundred and twelve (67.5%), 1 (0.6%), 7 (4.2%) were euthyroid, hypothyroid and hyperthyroid respectively. Five (3%) and 7 (4.2%) had subclinical hypothyroidism and subclinical hyperthyroidism respectively. Ultrasound was available on 104 of 132 patients. Among these who had autoantibodies and fine needle aspiration biopsy; 36 (34.6%) had multinodular goiter, 12 (11.5%) had solitary nodule, 5 (4.8%) had Graves' Disease, 10 (9.1%) had Hashimoto's Disease, 2 (1.9%) had toxic adenoma and 6 (5.8%) had Plummer's disease. Eight patients (7.7%) had thyroid malignancy. (7 papillary, 1 follicular carcinoma). MEN was not detected. Rest has normal morphology. Among 124 (74.6%) out of 166 patients had thyroid pathology either related to function or morphology.
Conclusion
We report here a 74.6% prevalence of coexisting incidental thyroid disease in PHPT patients. Our results suggest that it is necessary to evaluate thyroid before parathyroid surgery for PHPT particularly in areas with iodine deficiency.

P154

Primary thyroid lymphomas

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Primary lymphomas of the thyroid are uncommon tumours, constituting fewer than 2% of all thyroid malignancies. For this reason their clinical features are not very well known. It seems that most of them arise in patients who have chronic autoimmune thyroiditis.
From our patients with primary thyroid cancer since 1995, we selected those with pathological diagnosis of thyroid lymphoma. Epidemiological data, clinical features and response to treatment were analysed.
We found 7 patients, all of them women, with an age of 66±25.3 years ($X \pm$ S.D.). They were referred as a painless, rapidly enlarging (median 3 months), neck mass (7±2.2 cm). Six out of 7 cases presented lymph node enlargement. Thyroid function was normal in 5 cases, and subclinical hypothyroidism was present in the other two. AntiTPO antibodies were positive in 2 out of 7 cases. The diagnosis of lymphoma was established by FNA in 6 out of 7 patients. Chemotherapy was indicated in all cases except in one patient who was older and had a short life prognosis. Neck radiotherapy was associated in 2 cases. The mean time of follow up was 40.43±55.6 months. Two patients died 3 and 24 months after the diagnosis. These patients were older than the others (87±7.07 versus 57.6± 25.35 years).
In summary, thyroid lymphomas are referred as a rapidly enlarging neck mass. The absence of thyroid autoimmunity does not rule out the diagnosis. A good response to chemotherapy was observed, except in older patients.

P155

Iodine metabolism in hyperthyroidism

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Introduction
Iodine is critical for thyroid morphology and function. On the one hand, iodine is a factor leading and permitting to origin of disturbances of thyroid follicular cells function, on the other hand, it's therapeutic agent.
Aim
The aim of the study was to evaluate iodine metabolism in different forms of hyperthyroidism and to analyze relationship between metabolism and thyroid size and function.
Material and methods
The study group consisted of 300 patients (236F and 37M) aged 20–80 years (mean 50.5). About 150 patients with Grave's disease (GD) and 150 with toxic nodular goiter (TNG). Thyroid technetium-99m scans was performed and serum levels of fT3, fT4 (FIA method), TSH (IFMA method) and TSI (radioreceptor method) were determined. Iodine uptake (RIU) was measured after 24 and 48 h, then effective half-life (EHL-RIU) was determined. In 200 patients PBI was measured after 24 and 48 h, then effective half-life (EHL-PBI) was estimated.
Results
RIU and PBI values are higher and effective half-life is shorter in GD than in THG. In 300 patients, correlation was found between RIU and age, fT3, fT4, TSI, thyroid mass. PBI was related to TSH, fT3, fT4, TSI, thyroid mass. EHL-RIU was related to TSH, fT3, fT4, thyroid mass. EHL-PBI was related to TSH, fT3, fT4 and TSI. In GD, EHL-RIU was related to fT3, fT4 and TSI, PBI and EHL-PBI were related to TSI. In TNG correlation was found between RIU, effective half-live, PBI and fT3, fT4, TSH, thyroid mass.
Conclusions
In GD, RIU is higher and iodine turnover is faster that in TNG. In TNG relationship between RIU and thyroid mass, function can be found. In GD, iodine kinetics are related to thyroid function, immunization level; in TNG to thyroid mass and function. It's necessary to determine form of hyperthyroidism while analyzing results.

P156

Analysis of ghrelin and obestatin levels in children with thyroid disease

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Thyroid disease let to change of weight – in hyperthyroid body mass is reduced, but in hypothyroid it is increased. Recently researches suggest that many new bioactive substances, like ghrelin and obestatin, play a role in regulation of body mass. These closely related hormones have paradoxically different effects-ghrelin increases, but obestatin decreases appetite. The aim of the study was to evaluate ghrelin and obestatin levels in young patients with untreated Graves' disease, subclinical Hashimoto' thyroiditis and in children with struma nodosa in euthyroid clinical state. The study group formed 78 patients suffering from Graves' disease (29 girls and 2 boys; aged from 6 to 21- mean 15.2 yrs) and Hashimoto's thyroiditis (29 girls and 3 boys; aged from 9 to 18- mean 14.5 yrs). The control group consisted of children with struma nodosa (in euthyrosis) – 13 girls and 2 boys; aged from 9 to 18 – mean 14.8 yrs. In all patients were performed ghrelin and obestatin levels – RIA's method (firmy Phoenix Pharmaceuticals, USA). In children and adolescents with hyperthyroid in Graves' disease we found lower levels of ghrelin compared to group of children with struma nodosa and with subclinical hypothyroid in Hashimoto's thyroiditis (123±23 vs 151±36; vs 140±45 pg/ml, $P<0.02$, NS). On the other hand obestatin levels was lower in children with untreated subclinical hypothyroid in Hashimoto's thyroiditis compared to group with struma nodosa or Hashimoto's thyroiditis in euthyroid (203.28±49 vs 222.49±59; 267.24±67 $P<0.03$, $P<0.02$). In group of untreated hyperthyroid in Graves' disease we found relationship between ghrelin and fT3 ($r=-0.36$, $P<0.4$) and fT4 levels ($r=-0.45$, $P<0.01$).
Conclusions
The disturbances in thyroid hormones in thyroid diseases have an essential effect on changes of hormones controlled appetite: ghrelin (in hyperthyroid) and obestatin (in hypothyroid).

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Bronchiectasis as a false-positive on Iodine-131 scintigraphy in thyroid papillary carcinoma- three case reports

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Introduction
After treatment with Iodine-131(I-131) in differentiated thyroid cancer, a diagnostic scintigraphy is performed. We selected three cases in which bronchiectasis appear as a false-positive on the scintigraphy after treatment with I-131.
Case reports
Three women, respectively 62, 64 and 65 years old, being followed in the Endocrinology Department of our Institute, with the diagnosis of papillary carcinoma of the thyroid, were submitted to I-131 therapy, the last one under recombinant thyroid-stimulating hormone and the others under hypothyroidism. In all of them, the scintigraphy showed hyperfixation in the lung, consistent with pulmonary metastases. In the first two, the thyroglobulin value was under 0.2 ng/ml and in the last one the value was 1.6 ng/ml; the antithyroglobulin antibodies were undetectable. Chest computed tomography held later did not define nodules considered suspicious, referring to the presence of bronchiectasic lung changes, overlapping with the images of the scintigraphy.
In all these cases the value of thyroglobulin and the title of antithyroglobulin antibodies remained undetectable, with no evidence of recurrence or persistence of disease.
Discussion
In the cases above, the scintigraphy performed after treatment with I-131 showed abnormal fixation in the lung territory, when clinical, biochemical and imagiologycally there was no evidence of recurrence. The presence of bronchiectasis in overlapping locations supports the hypothesis of false-positive in the scintigraphy, already described in the literature.

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Combined doxorubicin and hyperfracionated radiation therapy of anaplastic thyroid carcinoma: case report

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Introduction
Anaplastic thyroid carcinoma, either by its low frequency, or by its poor prognosis, is still as a therapeutic challenge. One of the options available is the combined chemoradiation therapy, the basis of the following case.
Case report
Male patient, 76 years old, with a history of neck swelling for 4 months. The cervical ultrasound showed a nodule in the right lobe of the thyroid with 7 by 5 cm and the cytology revealed follicular tumor. Submitted to total thyroidectomy, the histopathology was of papillary thyroid carcinoma with anaplastic transformation. Given the fact that the thyroid resection was complete and that the patient had no pulmonary metastases, the patient started chemotherapy with doxorubicin (10 mg/kg) weekly, followed by hyperfracionated radiotherapy, 1.6 Gy, 2 times a day (with a four hour break), 3 times a week, for 6 weeks. By the fifth week of treatment, the patient had already evidence of pharyngoesophagitis and skin erythema, reactions that subsided 4 weeks after the completion of the treatment. At six month follow up, there is no clinical, analytical or imagiological evidence of relapse.
Discussion
In this case, the hyperfracionated radiation resulted in increased survival. In our Institute, this treatment has been used in 4 other patients with complete remission longer than 5 years in 2 cases and inconclusive in other 2 (death by suicide and sudden death after treatment).
Although there are no randomized trials published, the likelihood of partial or even complete remission of the disease is still better than expected with other treatments.

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Three cases wıth thyroıd lymphoma

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Thyroid lymphomas are very rare diseases of the thyroid.
We present three patients with thyroid lymphoma administered our department last year. Two of the patients presented with rapidly enlarging neck mass with pressure symptoms, and the other was diagnosed during the evaluation of a thyroid nodule. Two patients had the Hashimoto's thyroiditis diagnosis. All the patients underwent surgery, since no exact diagnosis could be established with fine feedle aspiration biopsy (US-FNAB). Decompression surgery or total thyroidectomy could not be performed because of hypervascularity of the masses in first two cases. Severe compression signs improved dramatically with the CHOP-R chemotherapy protocol in these patients. Third patient is still being evaluated for staging of lymphoma.
The differential diagnosis of thyroid lymphoma may be problemmatic since most common presentation which is rapidly enlarging neck mass can be confused with anaplastic thyroid cancer and B-symptoms are recorded only 10% of the patients. Relationship between Hashimoto's thyroiditis and lymphomas still remains obscure. Suspicion is the most important step for diagnosing thyroid lymphoma. Ultrasonography, US-FNAB and adjunctive techniques (e.g. cytomorphological immunophenotypic and molecular techniques) appear to have an improved overall diagnostic accuracy. Surgery is not a treatment modality for thyroid lymphoma. Surgery may be performed because of the limitations of ultrasonography and US-FNAB for diagnosis or subclassifications of lymphoma.

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Autoimmune thyroid disease functional evolution: the role of thyroid volume

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Autoimmune Thyroid Disease (ATD) is associated with normal thyroid function (type 1) in most cases with variable incidence of functional evolution toward either hypo (type2, A or B if present or not goiter) or hyperthyroidism (type3).
To study the evolution of thyroid function in a longitudinal study, along a scale of six years, in relationship to thyroid volume, 128 subjects (80 females and 48 males), aged from 28 to 78 years, were considered, with the first diagnosis of ATD put in 2002 on the basis of high thyroid antibodies (TAb) and normal TSH (ATD type 1). They were rechecked after 6 years. TSH and TAb were tested by commercial chemiluminescent assay. Echography was performed with high resolution technology, applying a 7.5 MHz probe, both to calculate thyroid volume (multiplying the three diameters $\times \pi/6$) and to evaluate echostructure considering three grades of hypoecogenicity.After 6 years TSH was stable in normal range in 71.5% of subjects (group 1,G1); sub clinical hypothyroidism (withTSH progression between 4.5 and 10 mUI/ml) was showed in 19% (G2);overt hypothyroidism (TSH $>$ 10) was demonstrated in 9.5% (G3). Only one subject has reached hyperthyroidism stage (TSH $<$ 0.01).
No relationship was demonstrated between TAb levels or thyroid echogenicity and TSH evolution, on the contrary for the thyroid volume.

	Initial volume (ml)	**Volume after 6 years**
G 1	18.5	18.4
G 2	19.2	19.0
G 3	18.7	15.4

Our study seems to demonstrate no role of TAb or thyroid echogenicity in the functional evolution of TDA. Instead a significative reduction of thyroid volume should influence the evolution of the function of TDA to overt hypothyroidism. When the progression is limited to subclinical hypothyroidism no variations of thyroid size were found.

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Predictors of incidental parathyroidectomy during thyroid surgery

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Objective
To identify incidental parathyroidectomy predictors in thyroid surgery.
Methods
All thyroid operations during 4 years were reviewed ($n=1010$). Patients were divided in those with (parathyroidectomy group) and without incidental parathyroidectomy (no-parathyroidectomy group).
Results
Incidental parathyroidectomy occurred in 198 patients (19.6%). The groups were comparable in age, thyroid weight and pathology, hyperthyroidism, operative time, surgeon's experience (high/low volume), operative technique (suture-ligation/Ligasure/Ultracision), surgical procedure (total, near-total, subtotal, hemi-thyroidectomy), modified radical or central neck dissection, reoperation, postoperative calcium, transient and permanent hypocalcemia. Women composed 87.9% of the parathyroidectomy and 70.3% of the no-parathyroidectomy group ($P=0.0001$), and 83.2% of patients with intrathyroidal but 73% of non-intrathyroidal parathyroid glands ($P=0.001$). Female gender was the only predictor in multivariate analysis ($P=0.0001$,OR$=3.28$,95%CI$=2.04$–5.27).
Conclusions
In contrast to all other analyzed parameters, female gender was an independent risk factor of incidental parathyroidectomy in all types of thyroid surgery, probably due to higher likelihood of intrathyroidal parathyroid glands.

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Thyroid surgery with the new harmonic scalpel: a prospective randomized study

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Background-objective
Although the harmonic scalpel has been shown to be safe and effective in thyroid surgery, several surgeons consider the previously available instruments to be large and cumbersome, especially in terms of dissection capabilities. To this context, an innovative technical improvement of the device for thyroid surgery has very recently been implemented and has been made available in 2008. Utilization of this new device, however, has not been evaluated in any study. We hypothesized that this instrument may result in further operative time reduction due to its greater tissue grasping and dissection capability. The aim of this study was to compare the results of total thyroidectomy using the new harmonic scalpel (FOCUS) to that with the previously available device (HARMONIC ACE).
Methods
Prospective randomized study of all total thyroidectomies between February and July 2008. Patients ($n=90$) were randomized into those submitted to total thyroidectomy with FOCUS (group A, $n=45$) and those with HARMONIC ACE (group B, $n=45$).
Results
No significant differences were identified between the two groups in terms of demographics, reoperative thyroid surgery, thyroid gland weight and diameter, pathologic diagnosis, preoperative and postoperative calcium, complications, hospital stay, and final outcome. Mean operative time was significantly shorter in group A than group B (63 ± 7 vs 76 ± 8.5 min, $P=0.009$).
Conclusions
The new harmonic scalpel device is a very useful adjunct to the thyroid surgeon's armamentarium. It is safe, effective and hand-friendly, offering great tissue delicate grasping and dissection capabilities. Utilization of this device significantly reduced operative time compared to the previously available instrument.

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Hashimoto's thyroiditis: the value of antithyreoperoxidase antibodies measurement

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Introduction
Hashimoto's Thyroiditis is a part of the spectrum of thyroid autoimmune diseases. Even the proper diagnosis is obtained by pathological exam; usually the detection of high serum antithyroid antibodies is enough to diagnose the disorder. It also represents the most frequent cause of hypothyroidism in non-iodine deficient areas. Nevertheless the presence of the antithyroid antibodies does not always correlates with thyroid dysfunction.
Aim
Our aim was to study the correlations between the level of plasma antithyreoperoxidase antibodies (ATPO) and the value of thyroid stimulating hormone (TSH), the age of the patient, as well as the presence of a second autoimmune disease (AID).
Material and methods
We studied 1500 patients. They were investigated by anamnesis (age, the presence of an already diagnosed AID). We also performed lab exams (TSH, ATPO).
Results
The sex ratio was 1483 women versus 17 men. The Hashimoto's Thyroiditis (HT+) group included 755 patients with levels of serum ATPO above 34 IU/ml. The control group (HT−) included 745 patients with levels of ATPO below 34 IU/ml. The mean age was 50.71 years in the first group and 55.19 years in the second group. We found no TSH-ATPO correlation ($r=0.16$, slope$=9.65$, $P=$ NS), neither ATPO-age correlation ($r=0.08$, slope$=0$, $P=$NS). In the TH+ group, 118 patients had a second autoimmune disease like vitiligo, anemia, and drug allergies. In the TH− group, there were 78 patients with another AID. The correlation ATPO-AID was statistically significant ($\chi^2=0.879$, $P=0.003$).
Conclusion
Higher levels of ATPO do not necessary associate with anomalies of the thyroid function as shown by serum TSH. Advanced age of the patient does no correlate with higher level of plasma ATPO. The increased values of ATPO as seen in Hashimoto's Thyroiditis showed a higher chance for having a second autoimmune disease.

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Propylthiouracil-induced anti-neutrophil cytoplasmic antibodies positive vasculitis

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Introduction
Graves' disease treatment with antithyroid drugs may be associated to several side effects. Vasculitis development is rare.
Clinical case
The authors present the history of a 41-year-old woman with Graves' disease followed at endocrinology consultation since February 2004. Three years and a half after starting treatment with propilthiouracil (PTU), she developed eritematous and itching stains in the inferior limbs that spontaneously resolved in 3 days. Some weeks later, ecchymosis and a hematic blister appeared in the side face of the right leg. This lesion biopsy revealed 'vascular thrombosis with epidermic necrolysis'. PTU was stopped and oral corticotherapy began. After corticoid withdrawal she developed a necrotic plate with blisters and an inflammatory halo in the right arm, purple plates in the right leg and bilateral malar eritema. Biopsy of the superior right member lesion showed 'vasculitis with leucocitoclasia and thrombosis'. The analytical study was positive for anti-neutrophil cytoplasmic antibodies (ANCA), circulating immune complexes, anticardiolipin antibodies and antithyroid antibodies, and was negative for antinuclear antibodies, anti-dsDNA antibodies, anti-desmossoma antibodies, complement, rheumatoid factor, anti-ENA

antibodies and anti-substance P antibodies. Treatment with PTU was interrupted. She was treated with prednisolone and non-steroidal anti-inflammatory drugs. She underwent total thyroidectomy. We observed progressive resolution of cutaneous lesions and normalization of ANCA levels.
Conclusion
PTU can induce ANCA positive vasculitis. Generally, therapeutic withdrawal leads to sintomatology resolution and drop of ANCA levels.

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New clinical feature in hypothyroidism: paroxysmal supraventricular tachycardia: case report

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Background
The aim of this case report is to underline the possible etiological link between paroxysmal supraventricular tachycardia (PSVT) and hypothyroidism, although supraventricular arrhythmias are ordinary features of hyperthyroidism. We present the case of a patient with repetitive episodes of PSVT whose autoimmune hypothyroidism was diagnosed and thyroxin replacement therapy leaded to remission of arrhythmia.
Methods
A 47 year old women with a long history of smoking, coffee drinking and stressful environment presented two episodes of palpitation, dyspnea, dizziness and anxiety. The electrocardiogram revealed supraventricular tachycardia with 200 beats/minute (PSVT). The possible triggers of arrhythmia were investigated.
Results and discussion
In our patient, hypothyroidism was diagnosed (FT4 = 11.25 pmol/l (normal range: 12–22) and TSH = 26.37 μUI/ml (normal range: 0.27–4.2)) as a result of chronic Hashimoto Thyroiditis (anti TPO > 1/640). Laboratory analysis showed just a slightly increase of cholesterol level, with no electrolyte disturbances. Structural heart disease and systemic vasculitis were investigated and excluded. Thyroxin replacement therapy was started first 25 μg/day, then 50 μg/day. Clinical signs improved substantially and no other episode of PSVT was noted. Although we cannot deny the possible contribution of coffee, smoking and stress in revealing PSVT, the disappearance of the arrhythmia after restoration of euthyroidism suggests that hypothyroidism might serve as a trigger for PTSV.
Conclusions
Prolonged conduction, low voltage, sinus bradycardia and different atrio-ventricular or brunched blocks are classical sings of hypothyroidism, however, our case suggests that PSVT can also be part of the cardio-vascular anomalies during hypothyroidism. The mechanisms involved in the occurrence of tachyarrhythmia in hypothyroidism could be: alteration of myocyte-specific gene expression, interstitial oedema, myofibril swelling with loss of striation, increased arterial stiffness, endothelial dysfunction, premature atherosclerosis, disturbances of the sympathetic-vagal tone with a relative increase in sympathetic tone and autoimmunity.

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Frequency of subclinical hypothyroidism (SH) and autoimmune thyroiditis (AT) in pregnancy

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Background
The frequency of SH and AT in pregnancy is of the 3%, and diagnosis is determinant because of the high risk of complications (postpartum hemorrhage, abruptio placentae, gestational hypertension in the mother and disordered brain development and/or intrauterine growth retardation in the fetus).
Objective
Determine the frequency of SH and AT in 300 pregnant women.
Materials and methodology
About 300 pregnant women were screened. In accordance to gestation time the criterions of exclusion enclosed antecedents of replacement with thyroxine or previous thyroid disease. Blood samples were drawn for TSH, FT4, FT3 and TPO antibodies.
Results
From the 300 women, 120 (40%) were in first trimester; 88 (29.3%) were in second trimester and 92 (30.7%) were in third trimester. Prevalence of SH was found in 25 women (83%) of which 10 were diagnosed in the first trimester, 8 in the second trimester and 7 in the third trimester. All of the patients presented high levels of TSH with normal FT4 and FT3. The TPO antibodies were positive in 8 of the 10 women in the first trimester; 7 of the 8 in the second trimester and in 5 of the 7 in the third trimester. There was no difference in the TSH, FT4, FT3 and TPO antibody levels along of the studied trimesters ($P = 0.21$).
Conclusion
(1) The prevalence of SH and AT can be higher than normally described, in our series it was of the 8.3%.
(2) The high prevalence of SH and AT in different populations can cause high frequency of maternal and fetal complications.

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Evaluation of thyroid disorders in patients with alopecia areata

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Introduction
Alopecia areata is a common disorder presenting with severe hair loss in a specific site. This condition is usually accompanied by autoimmune diseases such as autoimmune thyroiditis, lupus erythematosus, vitiligo, myasthenia gravis, diabetes type II, etc. Considering high incidence of thyroid disease in Iranian population, we decided to evaluate the frequency of thyroidal diseases especially autoimmune thyroiditis in patients with alopecia areata.
Methods
A total of 45 patients with alopecia areata and 35 age and sex matched patients without any evidence of autoimmune disease, as a control group that referring to dermatology clinic of Qaem hospital from October 2006 till July 2008 participated in this study. Questionnaires were filled by data of histories, demographic characteristics, physical examinations and laboratory tests of thyroid gland. Collected data were analyzed statistically using SPSS software.
Results
Incidence of thyroid disorders in patients was higher than control group. ($P = 0.035$) 11.1% of cases showed goiter. Thyroid dysfunction was detected in 13.6% of patients with no significant difference between male and female groups. ($P = 0.13$) Hashimoto thyroiditis was reported in 27.9% of cases which was significantly different between men and women ($P = 0.025$).
Conclusion
Incidence of thyroid disorders in patients with alopecia areata was higher than normal group. This finding confirms the need for thyroid function test screening in alopecia areata patients in our population.

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Unsual onset of subacute thyroiditis

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Subacute thyroiditis (SAT) is a self-limited inflammatory disease of presumed viral etiology, characterized by pain and thriphasic functional thyroid evolution. We report the case of an 54-years-old woman hospitalized in 02.2008 at the Gastroenterology Department for fever, diahreea, significant weight loss (5 kg in one month), with the suspicion of Crohn's disease (CD). Two weeks before she presented a subfebrile episode with bilateral jugular lymphadenopathy, dysphagia, myalgia, treated with antibiotics. High ESR and CRP values confirmed the inflammatory syndrome, but normal irigography and colonoscopy infirmed the suspicion of CD. The adenopathy suggested infectious mononucleosis (IMN) sustained by positive Epstein-Barr antibodies (IgG = 18.1), and high hepatic enzymes. During the hospitalization she presented tachycardia, tremor, thyroid enlargement, and was transferred in the Endocrinological Department. High fT4 (3.6 ng/dl) with inhibited TSH (0.1 mUI/l) confirmed thyrotoxicosis. Thyroid ultrasound (US) revealed inhomogenous hypoechoic pattern with the presence of a 18/16 mm nodule with imprecise limits, and internal Doppler signal. She had moderate sensitivity at thyroid palpation but when FNAB was performed she presented intensive pain. Cytology was suspicious. CT normal 2.2 pg/ml. The persistence of the inflamatory syndrome (ESR 90 mm/h) with the suspicion of IMN determined corticotherapy followed by a spectacular improvement: disparition of fever, diminution of ESR (51 mm/h), amelioration of the US (inhomogenous, hypoechoic zone on the nodule topography, no vascularization). Corticotherapy continued and 2 months later, after a short period of hypothyroidism, thyroid function, ESR (5 mm/h) and hepatic enzymes were normalized, lymph nodes were no more palpable and she had no phisical complaints.

SAT may mimic various thyroid and systemic diseases. Particulary for our case were the mild pain, domination of digestive symptoms and the presence of the adenopathy, which first suggested CD or IMN. The association of signs suggesting thyrotoxicosis impose, in such cases, the investigation of the thryoid function.

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Frequency of thyroid function test abnormalities in an open population in Queretaro, Mexico

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It is important to know the prevalence of common diseases in every country in order to guide us to take decisions regarding public health benefits in screening, in Mexico we do not have information regarding thyroid abnormalities.
Methods
We designed a cross-sectional study in the city of Queretaro (Mexico) in order to know the prevalence of altered thyroid function tests in our population, since we do not have any previous study. We calculated the sample size with the statistical program Epi Info according to the population size and prevalence from previous reports and we obtained a number of 210 individuals.
We went to different public places and factories to invite in a random way the people to participate in the survey; they signed informed consent approved by the local bioethics committee.
We asked questions related to thyroid disease, then we withdrew a blood sample for TSH and free T4.
Results
We included 212 individuals, 119 (56.4%) were female, 92 (43.6%) males. The mean age was 38 + 9.95 years old. We found 3 (1.4%) with TSH > 10 uUI/ml; 10 (4.8%) patients with TSH > 4 and < 10 uUI/ml; 2 (1%) patients with TSH < 0.4 uUI/ml and high free T4; 3 (1.4%) with TSH < 0.4 uUI/ml and normal free T4 level. In total we found 18 (8.6%) patients with abnormalities in the thyroid function tests. According to previous reports our population has a prevalence similar to other populations. It is very important to have this data since we do not have information in order to encourage screening programs in our country. This is a relatively young population and it can be due to the open invitation that we did to the people in public places and at work places. It will be interesting to design a specific survey in older people that we did not find at public places, since they are expected to have higher prevalence.

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Primary cavernous hemangioma of the thyroid gland

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Background
Hemangiomas of the thyroid gland are extremely rare.
Case presentation
We report a case of a 78-year-old euthyroid male patient with a primary cavernous hemangioma of the thyroid gland. The patient presented for evaluation of a symptomatic, slowly growing neck mass. He did not have any previous medical history and, moreover, had no history of trauma, FNA or other neck procedures. Ultrasound scan revealed a multinodular goiter and a hypoechoic nodule of the right thyroid lobe. Total thyroidectomy was performed and the lesion was completely excised. Definite diagnosis was feasible after the histological examination of the surgical specimen.
Conclusions
Cavernous hemangiomas of the thyroid gland are infrequent lesions which may escape diagnosis preoperatively, either due to the complicacy of the examinations needed to differentiate them from other typical thyroid diseases or due to the similar pattern they show in common examinations performed such as the ultrasound scan. An effort should be made to dissect free the thyroid gland without rupture of these lesions in order to ensure a bloodless procedure.

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Heart ischemic disease patients with mild thyroid failure

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It is well known that subclinical hypothyroidism (SH) more often can be revealed in patients with heart ischemic disease (HID). Mild thyroid failure can cause decrease of catabolism at atherogenic lipoproteins, decrease of cardiac output and diastolic disfunction of left ventricle. Results of coronarography (CG) can reliable reflect the severity of coronary atherosclerosis. It seems to us very important to compare the results of CG with TSH level and lipoproteins in heart ischemic disease patients.
Methods
About 863 patients participated in our study. In all patient CG was performed by standart methodology of M. Judkins *et al*. We studied age, gender, body mass index (BMI) of patients, their smoking history, genetic predisposition, treatment with statins. Fasting blood samples were taken for measuring of lipoproteins and TSH level by reagents of third generation.
Results
There were 77.6% of men and 22.4% of women. Middle age was 56.85 ± 0.29 years. To investigate relationship between TSH level and lipoproteins concentration we selected patients without statin therapy. In this group SH was revealed in 10.8% of patients (middle TSH was 7.66 ± 1.11 IU/l). In patients with SH levels of cholesterol and low density lipoproteins were significantly higher than is euthyroid patients (P 0.004 and P 0.001). Multivessel damage of coronary vessels was revealed in 40.2% of patients and correlated with man gender, age, duration of smoking, genetic predisposition, hypertention, hyperlipidemia and TSH level more than 4 IU/l. The truncal damage of left coronary artery was in 38% of patients with SH and in 19.3% of euthyroid patients.

Endocrine tumours and neoplasia

P172

Manifestations of Hyperprolactinoma and its Management by Bromocriptine and Cabergoline

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This is a prospective study analyzing gender differences in the presentation of hyperprolactinemia as well as the efficacy and tolerance to cabergoline and bromocriptine. Thirty-six patients (23 women, 13 men) were recruited and divided into two groups; Group One received bromocriptine and Group Two received cabergoline for three months. The prolactin level was measured before and after treatment in both groups. Galactorrhea and infertility were more common symptoms in women; however, 100% of men with micro or macroprolactinoma had libido disturbances. The prolactin level was higher in men than in women whether they exhibited macro (7640 ± 80 vs. 6230 ± 71 ng/Ml) or microprolactinomas (6167 ± 895 vs. 5998 ± 775 ng/ml). The prolactin level was significantly higher in women with non-tumor hyperprolactinemia (3390 ± 164 vs. 1279 ± 53, $P = 0.038$). The mean serum prolactin level was significantly decreased in both groups whether they received bromocriptine or cabergoline (5790 ± 370 vs. 2725 ± 124 ng/ml; $P = 0.001$). The prolactin reduction was more prominent in the cabergoline group whether in men or women, than in the bromocriptine group at the end of the three months of treatment (5791 ± 723 ± vs. 1725 ± 318 ng/ml; $P = 0.001$).

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Von-Hippel-Lindau disease: clinical report

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Background
Von-Hippel-Lindau disease (VHL) is a rare (1/36.000 newborns), autosomal, dominant inherited tumour syndrome. A germline mutation in VHL tumour suppressor gene predisposes carriers to tumours in multiple organs. In the presence of positive family history, it can be diagnosed clinically in a patient with at least one typical VHL tumour.

Clinical report
In December 2007, a 34 years-old women presented with palpitations and tachycardia, but normal blood pressure. She had a previous history of surgeries, performed in another hospital: left adrenalectomy due to pheochromocytoma, spinal and cerebellar hemangioblastomas. Her mother and three aunts had VHL disease. Biochemical study revealed: urinary metanephrines 120.75 μg/24 h (25–312), vanillymandelic acid 11.44 mg/24 h (<15), ACTH 15 pg/ml (8am) (normal: 9–52) and 9.6 pg/ml (11pm), plasmatic cortisol 24 μg/dl (8am) (normal: 5–25) and 9.7 μg/dl (11pm). I^{123}-Metaiodobenzylguanidine scanning: '…right adrenal pheochromocytoma'. Normal ophthalmologic evaluation. Preoperative medical management was performed with a daily dose of phenoxybenzamine 20 mg, amlodipine 10 mg and propanolol 10 mg. A right adrenalectomy was performed. Histology confirmed the diagnosis. Six months after surgery, an abdominal CT revealed a '…solid pancreatic mass with 4.5×3.5 cm…' Laboratorial study: plasmatic insulin 10 μUI/ml (<30), C-peptide 2.4 ng/ml (1.0–7.6) and chromogranin A 60 ng/ml (19–98). A distal pancreatectomy was performed without complications (pathology: '…Well differentiated, intrapancreatic, endocrine tumour.'). Two months later, a somatostatin receptor imaging with octreotide didn't show fixation. At this moment, the patient maintains normal blood pressure, under treatment with hydrocortisone (20+5+5 mg) and fludrocortisone (0.05mg), daily. In the last evaluation (September 2008), she had normal plasmatic ionogram, ACTH<5 pg/ml, plasmatic cortisol 40 μg/dl and urinary free cortisol 108 μg/24 h (10–80).
Conclusions
The authors present this case due to its rarity and point out the need of follow-up during the entire patients' life due to the possible development of other tumours. Finally, the importance of family vigilance and genetic study, which allows a precocious diagnosis and treatment.

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Assessment of interferon α-2a in pharmaceutical formulations by liquid chromatography methods

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The recombinant human interferon α-2a (rhIFN α-2a) is a cytokine with antiviral, antiproliferative and immunomodulatory properties, indicated for the treatment of hepatitis B and C and leukemias. The rhIFN α-2a consists of a 165–166 amino acids with molecular mass of 19.5 kDa. The aim of this work was to develop and validate the reversed-phase (RP-LC) and size-exclusion (SE-LC) liquid chromatography methods for the physico-chemical characterization of rhIFNα-2a in pharmaceutical formulations. The RP-LC method was carried out on a Jupiter C_4 column (250×4.6 mm I.D.) with detection at 214 nm. The mobile phase A consisted of water with 0.1% TFA, and the mobile phase B was acetonitrile with 0.1% TFA. The SE-LC method was performed on a BioSep-SEC-S 2000 column (300×7.8 mm I.D.), using the mobile phase with 0.001 M monobasic potassium phosphate, 0.008 M dibasic sodium phosphate and 0.2 M sodium chloride buffer pH 7.4, with detection at 214 nm. The procedure was applied for the potency evaluation of rhIFN α-2a, dimers and higher molecular mass substances. The separation by the RP-LC method was obtained with the retention time of 32.6 min and the method was linear in the range of 0.5–50 MIU/ml (r^2=0.9999). The SE-LC method yielded results with quantitation limit of 0.5 MIU/ml and detection limit of 0.19 MIU/ml. Eight batches of pharmaceutical formulations were analyzed in parallel by RP-LC and SE-LC methods giving results respectively, within 91.53–100.75%, with sulphoxides and deamidates content (<0.78%), and within 91.53–104.56% of the stated potency, with dimers and aggregates lower than 0.21%. The results were correlated to the anti-proliferative cell-based assay demonstrating the validity of the methods which can contribute to improve the quality control of rhIFN α-2a in pharmaceutical formulations, and to assure the therapeutic efficacy and safety of the biotherapeutic.

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Pregnancy and childbirth in active acromegaly patient treated with long acting somatostatin analog

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In 24 years old woman in March 2007 was diagnosed acromegaly (somatoprolactinoma) an active phase, hyperprolactinemia. Manifestations: rugged features, amenorrhea, galactorrhea, fasting GH – 144 ng/ml (*N*<10 ng/ml), IGF-1 – 586 ng/ml (*N* 48–450 ng/ml), PRL – 6726 mU/l (*N* 40–530 mU/l). According to pituitary MRT a tumor volume was 14.4 cm^3, with supra- and infrasella growth. She was operated in May 2007: transnal transsphenoidal subtotal removal of the pituitary adenoma. After adenomectomia: GH nadir in OGTT was 28.1 mU/l (*N*<2.7 mU/l), IGF-1 – 952 ng/ml, PRL – 111 mU/l. Pituitary MRT: endo- and parasella adenoma components 5.7 cm^3 volume. Galactorrhea persists. Long acting somatostatin (Oktreotid-depot) was prescribed in July 2007 in start dose 20 mg/month, and it was increased in Sept 2007 to 30 mg to suppress GH secretion (GH nadir 26.4 mU/l, IGF-1 601 ng/ml). Oktreotid-depot treatment induced a reduction in volume postoperative adenoma components from 5.7 cm^3 to 4.5 cm^3. After 4th Oktreotid-depot injection (30 mg/m) 24 weeks pregnancy has been diagnosed. Oktreotid-depot treatment has been continued up to physiological childbirth at 40^{th} weeks. The child was healthy: weight – 3 250 g, growth – 52 cm, 9 points on Apgar scale. Acromegaly signs did not worsen during pregnancy and after childbirth.

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Ectopic growth hormone-releasing hormone secretion by a neuroendocrine tumor causing acromegaly: long-term follow-up results

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Less than 1% cases of acromegaly is caused by ectopic secretion of growth hormone releasing hormone (GHRH).
A 25-year-old woman was admitted to the hospital, with iron deficiency anemia, acromegaly and a 6×6 cm mass in infrahepatic location near to the pancreatic head. Sellar magnetic resonance (MR) imaging indicated pituitary enlargement without obvious evidence of a pituitary adenoma. The patients underwent abdominal exploration. Histopathological diagnosis was a well-differentiated neuroendocrine carcinoma of duodenum with the invasion of tunica muscularis and 9 metastatic lymph nodes (the greatest size 5×5×2.5 cm in diameter). Neoplastic cells showed cytoplasmic immunoreactivity to GHRH and GHRH-receptor. Because increased IGF-1 concentrations persisted after the operation, octreotide LAR 20 mg/month was begun. Growth hormone and IGF-1 levels normalized. At the end of six years of follow-up, a left paraaortic mass, showing uptake of $indium^{111}$ octreotide was detected. Operation showed a metastatic lymph node of 2.5×1.5×1.4 cm in diameter. After the operation IGF-1 concentration was mildly elevated. Octreotide–LAR 10 mg/day was begun and continued until the present time.
We suggest that octreotide treatment may lead to a delay in tumor growth and the clinical and radiological diagnosis of recurrence and have a beneficial effect on disease course during a total follow-up of 7 years for our case. Expression of both GHRH and GHRH-R may indicate the autocrine/paracrine role of GHRH for proliferation of tumor tissue itself.

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Misleading, difficult and lucky path towards a diagnosis in a medullary thyroid carcinoma case

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MTC represents still a diagnostic challenge in thyroidology. A case is here reported with some misleading signs that was finally solved with a calcitonin assay.
A 50 years women was referred for a thyroid nodule incidentally discovered at echography in the right lobe; it appeared round, hypoechoic, without alo sign, well-definite edges, with a diameter of 7 mm. At the first evaluation with echocolordoppler there was no appearance of intralesion vascularization. TSH was 0.19, thyroid antibodies were absent. A Tc99 scintiscan demonstrated an iso-uptake area in correspondence of nodule. The subject was put in ultrasound follow-up: a size evolution to 12 mm was shown, while ECD demonstrated an

appearance of intra lesion color sign. A citology showed a follicular pattern. At this time a calcitonin assay was done that demonstrated high levels; she was referred to surgery. MTC emerged in the nodule.
Some considerations arise about this case: A) the initial signs were confounding because the presence of low TSH with not clear scintiscan suggesting the possibility of pre autonomous thyroid nodule;and indeterminate result of citology and ECD; C) for this kind of small nodule, even if a cytology is not indicated, an accurate follow-up with ultrasound is recommended, to arise the diagnostic doubt about the nature of the nodule; D) the solution of the diagnostic challenge derived by an intuition of an assay of calcitonin, owing to growing even if minimal, of nodule size.
Once again the importance of calcitonin assay is demonstrated. If screening is no acceptable for cost benefits, this test has to be well present in our mind, both at starting nodule work-up if doubts emerged from ultrasound, ECD,cytology point of view and,like in our case, along the course of echography follow-up, that is advised in guidelines for small nodule less than 1 cm.

P178

Predictive value of interleukin-10 promoter genotypes and haplotypes in determining the susceptibility to nephropathy in type 2 diabetes patients

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Background
The IL-10 promoter polymorphisms −1082G/A, −819C/T, and −592C/A have been consistently associated with type 2 diabetes (T2DM). We examined whether these polymorphisms variants are also associated with progression of diabetic nephropathy (DN).
Methods
These promoter variants were genotyped in 917 T2DM patients comprising 515 DN patients and 402 control patients without nephropathy (DWN), together with 748 non-diabetic control subjects. Haplotype analysis and multivariate regression analysis were employed in assessing the contribution of IL-10 haplotypes to DN risk, using genotype, clinical and biochemical profile, and their interactions as predictors of DN.
Results
Carriers of mutant −592*A* and −819*T* alleles, and −819T/T, −592A/A, and −819C/*T* genotypes were more frequent in T2DM. However, the −819C/T genotype appeared to be protective of DN, since lower frequency −819*T* allele and −819C/T genotype were seen in DN patients. Regression analysis identified −1082G/−819T/−592A (GTA) and −1082G/−819T/−592C (GTC) haplotypes as DN-protective haplotypes. Relative to the −1082G/−819C/−592C haplotype, GTA ($P=0.044$; odds ratio (OR) $=0.54$, 95% confidence interval (CI) : 0.30–0.98) and GTC ($P=0.045$; OR $=0.56$, 95% CI: 0.31–0.99) haplotypes were associated with decreased odds ratio OR for DN, after controlling for a number of covariates (age, sex, body mass index (BMI), hypertension, glucose, HbA1c, DN duration, total cholesterol).
Conclusions
Our results indicate that genetic variations at the IL-10 promoter influence the risk of nephropathy in T2DM patients and thus represent a potential DN genetic-susceptibility locus worthy of replication.

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AIP immunostaining is increased with lanreotide therapy in individuals with acromegaly and predicts changes in IGF-1 levels in female patients

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Background
Recently mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene have been found to occur in familial and sporadic somatotroph adenomas. These tumours tend to respond less well to somatostatin analogues, are diagnosed at an earlier age and behave more aggressively. AIP is expressed in sporadic somatotroph adenomas (Leontiou, JCEM, 2008).
Aim
To evaluate the change in AIP immunostaining in sporadic acromegaly patients treated with lanreotide prior to transsphenoidal surgery.
Methods
About 17 patients with sporadic acromegaly were treated with lanreotide 30 mg weekly or fortnightly for a 16 week period prior to transsphenoidal surgery. 17 patients, who had no pretreatment with lanreotide prior to surgery were matched for age, sex and size of tumour. The change in AIP immunostaining was measured by immunohistochemistry.
Results
After 16 weeks lanreotide treatment there was a $33.9\pm17.1\%$ mean tumour volume reduction, 49.8 ± 33.5 mU/l mean GH change (day curve) and 53.6 ± 93.6 nmol/l mean IGF-1 change. Strong AIP immunostaining was significantly increased in the lanreotide group ($60.3\pm19\%$) versus the control group ($27.9\pm11.7\%$) in both sexes; $P<0.001$. In the lanreotide group as a whole there were no associations between AIP staining and changes in GH or IGF-1 levels, or tumour volume reduction, after lanreotide treatment. However, in female patients there was a positive correlation between AIP staining and changes in IGF-1 levels after lanreotide treatment ($R=0.66$, $P<0.05$).
Conclusion
AIP protein expression was significantly increased in sporadic acromegaly patients who were treated with lanreotide, as compared to controls. In female acromegaly patients AIP immunostaining positively correlated with changes in IGF-1 levels after lanreotide therapy. These results suggest that AIP may play a role in the mechanism of action of somatostatin analogues in sporadic acromegaly patients.

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Papillary thyroid carcinoma associated with thyroid autoimmunity: clinical and molecular characterization

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It is still debated if the coexistence of papillary thyroid cancer (PTC) with a thyroid autoimmune process is associated with a better or worst outcome. Moreover, though a direct relationship between oncogenes and the activation of a pro-inflammatory program has been documented, the genetic background of PTCs with associated autoimmunity is not known.
Aim of the present study was to to investigate the clinical and molecular features of PTCs associated or not with autoimmunity. A large series of PTCs associated or not with thyroiditis and followed up according to recent guidelines has been clinically evaluated. Moreover, the genetic background of these two Groups of tumors was studied by means of RET and BRaf molecular analyses. In some cases, the thyroid tissue of the lobe controlateral to the tumor was also analyzed.
No significant differences were found between the two Groups regarding either the clinical and pathological features, or the outcome. Interestingly, the molecular defects were significantly different among patients with PTC associated or not with thyroiditis ($P=0.001$), being *ret*/PTC1 significantly more represented in patients with PTC and autoimmunity, and BRaf in patients with PTC alone. A *ret*/PTC rearrangement was also found in 41% of non-neoplastic thyroiditis tissues controlateral to tumors harbouring either *ret*/PTC or BRaf or none mutations.
In conclusion, the whole of present findings extend the knowledge about the tight relationships between oncogenes, thyroiditis and thyroid cancer. A significantly different genetic background between PTCs with or without associated autoimmunity was firstly demonstrated, well in agreement with the recent finding that, in normal human primary thyrocytes, *ret*/PTC1 activates a transcriptional program related to inflammation. Moreover, the presence of *ret*/PTC in inflammatory tissues associated with non-*ret*/PTC tumors, indicate that inflammation could predispose to carcinogenesis even if this is driven by different genetic alterations.

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Steroidogenic factor 1 – a valuable diagnostic and prognostic tool in patients with adrenocortical carcinoma

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Objectives
No immunohistochemical marker has yet been established to reliably differentiate adrenocortical tumors from other adrenal masses (e.g. metastases). Thus, a panel of several markers like melan A and inhibin is currently used for this purpose, but suffers from limited diagnostic accuracy. We hypothesized that expression of steroidogenic factor 1 (SF-1), a nuclear transcription factor involved in adrenal development and steroidogenesis, might hold significant diagnostic potential for the differential diagnosis of adrenal masses. Moreover, SF-1 overexpression has been associated with increased cell proliferation and tumorigenesis in an adrenocortical mouse model. Therefore, the prognostic value of SF-1 expression in human ACC was also investigated.
Methods
SF-1 protein expression was assessed by immunohistochemistry with a commercially available specific monoclonal antibody (Perseus Proteomics, Japan) using tissue microarrays and regular tissue slides of formalin-fixed paraffin-embedded tissue samples from 156 ACCs, 15 adrenocortical adenomas, four normal adrenal glands, 50 malignant non-adrenal tissues (including colon, breast, kidney and lung cancer) and normal ovary. We also correlated SF-1 protein expression with clinical outcome in patients with ACC using Kaplan–Maier and Cox-regression analysis.
Results
SF-1 expression was detectable in 150/156 (96%) of ACC samples including 43 (28%) with strong SF1 staining and in 15/15 adrenocortical adenomas. In contrast, SF-1 expression was absent in all of the non-adrenocortical tissues except in granulosa cells of the ovary. In addition, strong SF-1 expression significantly correlated with poor clinical outcome (tumor stage adjusted hazard ratio for death 2.0 (95%CI 1.3–3.3; $P<0.01$).
Conclusion
Our study indicates that SF-1 is a promising immunohistological marker to determine the adrenocortical origin of an adrenal mass with high specificity. In addition, SF-1 expression is of prognostic value in patients with ACC.

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Endocrine gland-derived vascular endothelial growth factor and its receptors in adrenocortical carcinoma

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Objectives
Endocrine gland-derived vascular endothelial growth factor (EG-VEGF; also termed prokineticin-1) has been identified as a mitogen preferable for the endothelium of steroidogenic glands (1). EG-VEGF and its receptors (prokineticin receptors 1 and 2; PKR1 and 2) are highly expressed in the normal adrenal gland and an autocrine mitogenic loop has been proposed (2). Therefore, we investigated the expression of EG-VEGF and its receptors in adrenocortical carcinoma (ACC) samples and correlated theses results with clinical outcome data.
Methods
EG-VEGF, PKR1, and PKR2 mRNA expression was assessed by real-time PCR in 30 ACC and 12 normal adrenal glands. In addition, these factors were analyzed by immunohistochemistry with specific antibodies using tissue microarrays including samples from 151 ACCs, 15 adrenocortical adenomas, and five normal adrenal glands. We also correlated EG-VEGF protein expression with clinical outcome in patients with ACC using Kaplan–Maier and cox-regression analysis.
Results
The mRNA of EG-VEGF and its receptor were highly abundant in most ACC samples and the expression in ACC was comparable to the normal adrenal gland and the adenomas. EG-VEGF protein was detectable in the cytoplasm of 150/151 (99%) and in the nucleus of 131/151 (87%) ACC samples including 90 (60%) and 27 (18%) samples with strong staining. There was no significant correlation with tumor stage. In $>90\%$ of ACC samples at least one of the receptors PKR1 or 2 was detectable. Patients with no nuclear staining for EG-VEGF had a significant better, stage-adjusted overall survival (hazard ratio for death: 0.33 (95% CI 0.12–0.90; $P=0.03$).
Conclusion
EG-VEGF and its receptors are expressed in the vast majority of ACC samples and EG-VEGF expression correlates with clinical outcome. Therefore, EG-VEGF seems to be an interesting therapeutic target for future studies.

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Differential expression of microRNAs in human parathyroid carcinomas compared with normal parathyroid tissue

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Parathyroid carcinoma is a rare cause of primary hyperparathyroidism. Though the loss of the oncosuppressor *HRPT2* gene product, parafibromin, has been involved in the hyperparathyroidism-jaw tumor syndrome and in a consistent set of sporadic parathyroid carcinomas, parathyroid carcinogenesis remains obscure. MicroRNAs (miRNAs) are a new class of small, non-coding RNAs implicated in embryonic development and cancer. A deregulated miRNA can induce the aberrant expression of several target genes. The aim of the present study was to identify differentially expressed miRNAs in parathyroid cancers compared to normal parathyroid tissues. We performed a TaqMan low-density array-based profiling of 4 parathyroid cancers harboring an inactivating mutation in *HRPT2* gene and negative for parafibromin immunostaining. Their miRNA profiling was compared with that of two normal parathyroid biopsies. Out of 362 human miRNAs assayed, 279 (77%) were expressed above background levels in all samples. Unsupervised hierarchical clustering correctly classified the normal specimens from the tumors. Fourteen and 3 miRNAs were significantly down- and over-expressed in parathyroid cancers, respectively. Of these, SAM analysis identified 2 miRNAs (296 and 139) and 2 miRNAs (503 and 222) significantly down- and over-expressed, respectively, with a null false discovery rate. In particular, miRNA-296 was able to discriminate with the highest accuracy between parathyroid cancers and normal glands ($P=0.0012$). To further investigate the expression of miRNA-296, we analyzed its expression profile in 13 parathyroid sporadic adenomas, four atypical adenomas and two metastasis. miRNA-296 expression levels were definitely low in parathyroid cancers and metastasis as well as in atypical adenomas, while in sporadic adenomas they were reduced but not significantly different from normal samples. These results suggest a potential role of miRNA-296 as an oncosuppressor gene and indicate this miRNA as an explorative tumor marker useful for clinical diagnosis.

P184

Recombinant human thyrotropin in follow-up of patients with differentiated thyroid cancer

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Background
Despite good prognosis patients with previously treated well-differentiated thyroid cancer (DTC) require lifelong monitoring for recurrent disease. The

diagnostic value of radioiodine whole body scanning and serum thyroglobulin (Tg) measurement is most accurate during thyroid stimulating hormone (TSH) stimulation. The introduction of recombinant human TSH (rhTSH)-stimulated testing offers the possibility to avoid hormone withdrawal associated with the morbidity of severe hypothyroidism. Recent clinical trials have shown that measurement of the rhTSH-stimulated serum Tg concentration (rhTSH-Tg) alone is the most sensitive way to detect residual or recurrent thyroid cancer.
Objectives
The aim of the study was to investigate rhTSH-Tg in patients considered to be cured with already finished radioiodine treatment 1–3 years ago (routine follow-up) and in patients more years after radioiodine therapy with a new indefinite (mild) suspicion for DTC recurrence.
Patients and methods
RhTSH-Tg was examined in 84 patients (72 women and 12 men) clinically free of disease, 1–3 years after finishing radioiodine therapy. Second group consisted of four patients (2 women and 2 men) 5, 9, 12 and 38 years after ^{131}I treatment with a mild suspicion of DTC recurrence based on routine neck ultrasonography (USG).
Results
RhTSH testing was well tolerated. No adverse events were detected. In the first group clinically free of disease undetectable rhTSH-Tg ((0.2 ng/ml) was found in 77 patients (91.7%), Tg above diagnostic cutoff ((2 ng/ml) in four patients (4.8%) and Tg in the range 0.6–2 ng/ml in three cases (3.6%). In all patients of second group previous indefinite suspicion of DTC recurrence was confirmed by the rhTSH-Tg rise (2.9–7.3 ng/ml).
Conclusion
We detected persistent disease in 4.8% of patients considered to be cured and confirmed recurrent disease in all patients wild mild USG suspicion. In accordance with the literature rhTSH-Tg concentration in combination with neck USG has the highest sensitivity and negative predictive value in detecting residual or recurrent DTC.

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Estradiol influences somatostatin receptor expression and potentiates the effects of SOM230 on prostate cells

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Somatostatin (SS) receptors (SSR) expression may be modulated by estrogens in breast cancer cells. Aim of this study was to evaluate the effects of estradiol (E_2) on SSR levels in prostate epithelial cells (PEC).
Methods
We investigated the effects of E_2 and SS-analogue SOM230 combined treatment on two PEC lines: EPN that expresses both ERalfa and beta and CPEC, showing no ERalfa and very low ERbeta expression. Cells starved in red phenol-free DMEM and 1% charcoal treated FBS for 5d were treated with 20 mM E_2 or 10^{-6} or 10^{-8} SOM230 or 20 mM E_2+ SOM230 (10^{-8} or $+10^{-6)}$ for 48 h. Cells were differently harvested for semiquantitative RT-PCR, Western blot or flow cytometry (FACS) analyses.
Results
In EPN E_2 or SOM 10^{-6} alone induced apoptosis and decreased slightly proliferation; 20 mM E_2+ SOM230 (10^{-8} or $10^{-6)}$ combined treatment induced a stronger rate of apoptosis and a greater decrease of proliferation. The synergistic action correlated to: a reduction in S-phase proliferation with an arrest in G0/G1 phase induced by SOM230 and increased by E_2-SOM 230 co-treatment; a caspase-dependent apoptosis induced by SOM230; a reduction of bcl-2 levels induced after addition of E_2; an up-regulation of SSR 1, 2 and 5 mRNA and proteins induced by E_2 that amplified SOM230 effects at lower doses. In CPEC, expressing SSRs 3, 5, and low levels of SSTR1 and 2, SOM230 induced a modest apoptotic effect; moreover, E_2 administration did not influence SSR expression neither SOM230 effects on cell growth.
Conclusion
Of E_2 increases the inhibitory effects of SOM230 in prostate cellsexpressing ERalfa and beta, acting directly on cell growth and cell death control and up-regulating SSRs. The evaluation of ERs and SSRs should be performed preliminarily in order to verify the possible efficiency of E_2-SOM230 combined treatment in prostate cancer.

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mTOR inhibition influences cell viability of medullary thyroid carcinoma primary cultures

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Effective medical therapy for persistent/recurrent medullary thyroid carcinoma (MTC) is not available, yet. Everolimus (RAD001) is a Rapamycin derivative, a potent mTOR pathway inhibitor. RAD001 has been employed in several clinical studies demonstrating antiproliferative and apoptotic effects in human tumors, both *in vitro* and *in vivo*, also in combination with somatostatin analogs. The aim of our study was to investigate the antiproliferative effects of RAD001 in human MTC primary cultures. Of 10 MTC have been dispersed in primary culture and incubated for 24 h in culture medium without serum. Cells have been then treated without or with increasing concentrations of RAD001 (10 nM – 1 μM) and/or 10 nM SOM230 (a multiligand somatostatin analog) and/or 50 nM IGF-1. Cell viability has been evaluated after 24 h with a colorimetric method. Somatostatin receptor (SSTR) expression has been evaluated by quantitative PCR. We found that RAD001 10 nM slightly (−17%) but significantly reduces cell viability in five MTC, and that this effect is blocked by co-treatment with IGF-1. SOM230 alone did not modify cell viability but enhanced the antiproliferative effects of RAD001 (−23%). In this group, SSTR2 was the mostly expressed SSTR subtype (72×10^3 molecules × μg total RNA), followed by SSTR1 (59×10^3 molecules × μg total RNA), while SSTR3 and SSTR5 were not expressed. Both RAD001 and SOM230 did not affect cell viability in the other five MTC, which expressed SSTR1, SSTR2, SSTR3 and SSTR5 (33, 32, 35, and 16×10^3 molecules × μg total RNA). These results indicate that Everolimus might represent a possible medical therapy aimed at controlling MTC cell growth in some cases, in association with somatostatin analogs.

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Presence and potential pathophysiological relevance of GOAT, the ghrelin *O*-acylation enzyme, in human pituitary tumors

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Ghrelin was isolated from stomach by its ability to stimulate growth hormone (GH) release through the GH-secretagogue receptor (GHS-R1a). However, ghrelin/GHS-R expression in multiple tissues and tumor types suggested additional roles for this tandem. Ghrelin, a 28-aminoacid peptide, requires a unique *O*-acylation at its Ser-3 residue to bind GHS-R1a and release GH. Conversely, unacylated ghrelin (UAG), initially considered inactive, seems to play distinct metabolic roles. Recent identification of GOAT, the enzyme that acylates ghrelin, opens novel strategies to understand and clinically manipulate this axis. Pituitary tumors often express ghrelin and/or GHS-R, yet their pathophysiological relevance is still unclear. To investigate this, expression of GOAT, ghrelin, GHS-R1a, and truncated GHS-R1b was evaluated by qPCR in 35 non-functioning pituitary adenomas (NFPA), 13 somatotropinomas, and seven corticotropinomas. Additionally, functional relevance of ghrelin acylation was assessed by evaluating calcium kinetics in single living somatotropinoma cells in response to acylated ghrelin (AG) or UAG. Results showed that ghrelin was expressed at moderate, comparable levels in the three types of adenomas. Conversely, GOAT expression was higher in somatotropinomas than in NFPA, which expressed similar levels than corticotropinomas. Accordingly, relative GOAT levels only surpassed those of ghrelin in somatotropinomas. GOAT was also expressed in normal human pituitary. GHS-R1a and GHS-R1b were also expressed in all three tumor types, whit somatotropinomas showing the highest GHS-1a levels. GOAT activity can be determinant for ghrelin function, since AG increased [Ca2+]i in single cells derived from three different somatotropinomas, whereas UAG did not alter calcium levels nor affected AG action in these cells. These results demonstrate a differential expression of GOAT in human NFPA, corticotropinomas, and somatotropinomas, and support the notion that, at least in somatotropinomas, GOAT can play a functionally significant role by modulating the effects of ghrelin (AG versus UAG) upon somatotropinoma cells.
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Efficacy of repeat surgery in patients with recurrent adrenocortical cancer

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The optimal treatment of recurrent adrenocortical cancer (ACC) remains to be established since there are discrepant opinions on the value of repeat surgery. We did a retrospective analysis of the outcome of patients who were referred to our units from 1988 to 2006 for a recurrence of ACC, which occurred 2–83 years after radical removal of the tumor. In that period, the treatment policy of ACC recurrence differed among our units, since oncologists were more accustomed to use chemotherapy while endocrinologists recommended surgery more frequently. Patients were stratified in two groups according to the treatment received: group one included 33 patients (18 W, 15 M, aged 21–64 years, median 38) who underwent repeat surgery, while group 2 included 16 patients (8 W, 8 M aged 18–69 years, median 48) who were treated with chemotherapy (EDP + mitotane). Repeat surgery was radical in 25 patients while eight patients were left with residual ACC and were treated with the same chemotherapeutic regimen. The 2 groups did not differ as to demographic characteristics, ACC stage, Weiss score, use of adjuvant mitotane therapy and secreting status, while the disease-free survival (DFS) after the first operation was significantly longer in group 1 (19 mos (5–83)) than in group 2 (10 mos (2–44) ($P=0.05$). Survival after recurrence was significantly longer for group 1 (36 mos (8–168)) than in group 2 (15.5 mos (6–109) ($P=0.001$). In-group 1, 56% of patients are alive at the last follow-up, 27% of whom are free of disease, while only one patients is alive in group 2. DFS after repeat surgery was 22 mos (4–132) in the 25 patients who had radical surgery. Such patients had the greatest survival when compared to patients in whom repeat surgery was incomplete or patients treated with chemotherapy alone ($P<0.0001$). The present data suggests that re-operation for recurrence of ACC is beneficial when a complete removal of tumor can be attained, while debulking does not give any advantage in comparison to medical therapy. Even if this is a retrospective analysis, the patients treated surgically or medically were rather well matched for the most important prognostic factors; however, we cannot exclude selection of less aggressive ACCs in group 1. Notwithstanding these limitations, these data are of interest because they show that surgical treatment of recurrence is worth doing also in some patients with advanced ACCs. An extended DFS following primary surgery may be an important factor for proper selection of patients.

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Tumor/liver standardized uptake values ratio <1.8 on FDG-PET has a high negative predicitive value to rule-out malignancy in patients with incidentally identified non-secreting adrenal tumours

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Purpose
The widespread use of high resolution cross-sectional imaging such as computer tomography (CT) and magnetic resonance imaging (MRI) for the investigation of the abdomen is associated with an increasing detection of incidental adrenal masses. 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) has proved to be an efficient tool in the diagnosis and follow-up of malignancies. We evaluated the ability of FDG-PET to distinguish benign from malignant adrenal masses when CT or MRI results had been inconclusive.
Methods
We included only patients with no evidence of hormonal hypersecretion and no personal history of cancer or in whom previously diagnosed cancer was in prolonged remission.
The visual interpretation, maximal standardized uptake values (SUVmax) and adrenal compared to liver uptake ratio were correlated with the final histological diagnosis or clinico-radiological follow-up when surgery had not been performed.
Results
Of 37 patients with 41 adrenal masses were prospectively evaluated. The final diagnosis was 12 malignant, 17 benign tumours, and 12 tumours classified as benign on the follow up. The visual interpretation and tumour/liver SUVmax ratios were more accurate than SUVmax alone, tumour diameter, or unenhanced density, with a sensitivity of 100% (12/12) and negative predictive value also of 100% (25/25). The use of 1.8 as the threshold for tumour/liver SUVmax ratio demonstrated 100% sensitivity and specificity.
Conclusion
FDG-PET/CT accurately characterises adrenal tumours with an excellent sensitivity and negative predictive values. A negative PET may predict a benign tumour that would potentially prevent the need for surgery of adrenal tumours with inconclusive conventional imaging. A cost-effectiveness study is required.

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Serum chromogranin A assay in the biological diagnosis of pheochromocytomas and/or paragangliomas: results in 146 patients

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The biological diagnosis of pheochromocytoma (P) and/or paraganglioma (Pgg) relies on the identification of excessive secretion of the metanephrines. Chomogranin A (CgA) is a general indicator of neuroendocrine tumours that is highly expressed in P and correlate with tumour mass and secretory activity. The CgA test could be indicated as a useful test in patients with false positive metanephrines results. The aim of our prospective bi-centre study, is to evaluate the performances of the CgA assay in the diagnosis of the P and Pgg according to the hereditary context and the localization of these tumours. One hundred forty six patients (67 females and 79 males) have a P and/or Pgg. All patients have had surgery and the diagnoses were confirmed by histological examinations. Thirsty eight patients (26%) have a hereditary disease.We used a radioimmunometric assay (Cis Bio-international, cut-off level to 120 ng/ml). The overall sensitivity of the CgA was equal to 92.7, 95.3% in a sporadic context and 84% in a hereditary context. The means CgA are significant different between the hereditary or sporadic diseases ($P<0.01$) as for the metanephrines concentrations. In patients with Pgg, (11 sporadic, 19 hereditary) the mean CgA was significant higher in the sporadic than in the hereditary Pgg ($P<0.05$). The means CgA in patients with sporadic P did not differ significantly from those of patients with sporadic Pgg. The means CgA appears also to be different regarding the genetic forms of P and Pgg. In conclusion, CgA assay is a useful tool for the diagnosis of P and Pgg with a high sensitivity, better for sporadic than for hereditary diseases. CgA is a biochemical marker for the diagnosis of the P as for the majority of the Pgg with some variations according to the genetic context.

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Clinical features and outcome of thyroid lymphoma: the Auvergne registry

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Introduction
Thyroid lymphoma is a rare thyroid disease, occurring mostly in the elderly. We report a series of 13 cases from a single centre.
Material and methods
Among our regional registry (1294 cases of thyroid cancer), we report 16 cases of thyroid lymphoma (with sufficient data on follow up for 13).
Results
Mean age was 68.3 years (range 38–85) and included 12 women for 1 man. Nine patients also presented with Hashimoto's thyroiditis. All but three were euthyroid. A cervical rapidly growing mass was the major revealing symptom (10/13). Diffuse giant cell B lymphoma (D G C B L) was more common (10/13) with anti CD 19+, CD20+ and CD30− immunostaining. One patient had DGBCL issued from mucosa associated lymphoid tissue (MALT) and two ad Burkitt lymphoma (BL). Follow up after appropriate therapy, ie conjunction according to staging of chemotherapy, radiotherapy or surgery, lead to remission in BL and MALT lymphoma, and in 3/10 DGBCL. The survival rate was 78.5 and 48.5% at 5 and 10 years respectively.

Conclusion
Thyroid lymphoma is a rare thyroid disorder, traditionally linked with Hashimoto's thyroiditis. Prognosis of lymphoma is far better than anaplastic carcinoma which it may mimic. Yet the most frequent form, DGCBL, keeps a poorer prognosis than BL or MALT lymphoma.

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The comparison of serum endostatin levels between patients with metastatic and non-metastatic well differentiated thyroid cancer

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Tumor growth is limited by its neoangiogenesis, which is dependent on dynamic balance beetween it's activators and inhibitors. One of the most important antiangiogenic factor is endostatin. Therefore we hypothesized, that serum endostatin concentration would differ between patients with metastatic and non-metastatic thyroid cancer, with multinodular goiter and healthy subjects. We also hypothesized that endogenous TSH stimulation would effect serum endostatin level.
The study group consisted of 64 (55 females, 9 men), aged 44. 9 ± 12.3 year, with differentiated thyroid cancer, treated in our department in the years 2003–2006. All patients had undergone total or near total thyroidectomy and radioactive iodine treatment, that had resulted in remission in 52 patients and persistent/recurrent disease in 12 patients. The study included two control groups – 30 patients with non-toxic multinodular goiter and 30 healthy subjects.
Serum endostatin concentration was was significantly higher in patients with distant metastases than in patients with remission (141 95 vs 105 345 ng/ml, $P < 0.05$). This was not observed in patients with locoregional metastases. During endogenous TSH stimulation, endostatin levels significantly decreased (122 94 vs 9360 ng/ml, $P < 0.05$). Serum endostatin levels in patients with metastases correlated with Tg levels. This was not observed in patients with remission.
Serum endostatin levels might be used as an additional marker of thyroid cancer with distant metasases. Endogenous TSH stimulation decreases endostatin levels in patients either with and without thyroid tissue, suggesting its regulatory effects through receptors located outside the thyrocytes.

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Effectiveness and safety of combined therapy with low dose ketoconazole and cabergoline in patients with Cushing's disease partially responsive to monotherapy with cabergoline

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The first-line treatment of Cushing's disease is surgery, although it is effective in inducing a long-term remission in around 50% of patients with Cushing's disease (CD). Nowadays, no pituitary tumor-directed medical treatment is available with the exception of cabergoline, which has been recently demonstrated to control cortisol secretion without major side effects in around 40% of patients with Cushing's disease. Cabergoline has been recently demonstrated to induce cardiac valve insufficiency in patients with Parkinson's disease, usually long-term treated with high dose of the drug. A widely used adrenal-directed palliative medical treatment is represented by ketoconazole, which however can be associated with different side effects mainly including liver damage especially when used at high dose (until 1200 mg/day) for a long period of time. The aim of the current study was to evaluate the effectiveness and safety of the combined treatment with cabergoline and low-dose ketoconazole in patients with Cushing's disease partially responsive to cabergoline mootherapy. Six patients with post-surgical persistent Cushing's disease had been treated with cabergoline at the maximal dose of 3.5 mg/week with a significant reduction but not normalization of urinary cortisol levels (from 530.5 ± 136.2 to 258.0 ± 107.1 μg/day, $P < 0.05$) associated with a partial clinical improvement after 6 months of treatment. Ketoconazole at the initial dose of 50 mg was added to cabergoline in all patients, and increased by 50 mg every month until normalization of urinary cortisol levels had been achieved. After 6 months of combined treatment with cabergoline (3.5 mg/week) and ketoconazole (50–200 mg/day), urinary cortisol levels were 107.8 ± 19.8 μg/day ($P < 0.05$), and were in the normal range in all patients. A significant clinical improvement was observed in parallel with the decrease and normalization of cortisol levels. No cardiac valve disease occurred or worsened during the 1-year treatment with cabergoline, except a worsening of tricuspidal regurgitation in one patient. No liver damage was observed in any patient. In conclusion, the combined treatment with cabergoline and low dose ketoconazole seems to be effective and safe in the management of patients with Cushing's disease, and can be considered in patients who had unsuccessful surgical treatment or are not candidates for alternative definitive treatments.

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Impact of surgery on clinical outcome in patients with recurrence of adrenocortical carcinoma

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Introduction
The role of surgery for recurrent ACC is not well defined. Therefore, we used the German ACC Registry to evaluate treatment modalities after first recurrence in patients amenable to surgery.
Methods
Patients with recurrence after radical resection and follow-up data were included. Patients with extensive metastasized disease (>2 tumoral organs, peritoneal carcinomatosis) were excluded. Progression-free and overall survival (PFS/OS) were analysed using the Kaplan–Maier and cox regression methods.
Results
In 351/506 patients registered with the German ACC Registry radical resection was performed. Of 223 of these patients experienced recurrence during follow-up and 76 fulfilled all inclusion and exclusion criteria. Patients presented with local recurrence ($n = 33$), liver, lung, or lymph node metastases ($n = 15$, 11, 2 respectively). In 15 patients two organs were affected. Median follow-up was 30 (6–250) months. Of 68 patients underwent second surgery (R_0 $n = 29$; R_1/R_x $n = 32$; R_2 $n = 7$). Patients voting against surgery were treated with mitotane ($n = 6$) or mitotane plus cytotoxic drugs ($n = 2$). Of 68 patients experienced progressive disease after a median of 7 (2–144) months. PFS after recurrence was prolonged in patients with time to first recurrence (TTFR) >12 months (14 vs 6 months; $P < 0.001$), but PFS was not significantly associated with surgery, resection status, or number of lesions. In contrast, TTFR and surgery were associated with reduced risk for death after recurrence (HR 0.23 (95% CI 0.11–0.50) and HR 0.38 (0.16–0.94), respectively). However, in multivariate analysis only TTFR was of prognostic value (HR 0.25 (0.11–0.56) $P = 0.001$). There was a clear trend favouring patients with R_0 resection (HR 0.44 (0.14–1.37)), but not with R_2 resection (HR 1.43 (0.42–4.88)).
Conclusion
In ACC, after first recurrence 90% of patients experience progression of disease independent of therapy. The best predictor for survival after recurrence is time to first recurrence. Our study suggests that surgery is of benefit only if complete resection is feasible.

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Microalbuminuria and insulin resistance in nondiabetic acromegalic patients

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Growth hormon (GH) counteracts the effects of insulin on glucose metabolism and GH excess may lead to insulin resistance (IR). Impaired glucose tolerance (IGT) and diabetes mellitus (DM) are frequently associated with acromegaly. Microalbuminuria (MAU) is a well established cardiovascular (CV) risk factor and a predictor of CV mortality in both diabetic and nondiabetic subjects.
The aim of this preliminary study was to investigate the MAU levels as a marker of CV disease in patients with acromegaly but without DM.

Forty-one acromegalics without DM with a mean age of 43.86 ± 11.7 years, mean BMI 29.74 ± 4.7 kg/m^2 and median disease duration 48 (IQR 21–108) months and age, sex and BMI matched 18 healthy controls were included. Patients and controls underwent OGTT and hormonal/biochemical evaluation and 24-hour urinary microalbumin excretion was measured. IR was evaluated with the homeostasis model insulin resistance index (HOMA-R). HOMA-R was not different between patients and controls ($P > 0.05$). Mean nadir GH level was 4.36 ± 14.71 ng/ml. In nondiabetic patients with normal IGF-1 levels for age, HOMA R was found to be lower than nondiabetic patients with elevated IGF-1 levels for age (1.06 ± 0.99 and 1.82 ± 1.27, $P < 0.05$). However, in nondiabetic patients HOMA-R values were not statistically different between patients who achieved the nadir GH level of less than 1 ng/ml after OGTT and who did not achieve the nadir GH level ($P = 0.063$). We demonstrated a positive but weak correlation between HOMA-R values and MAU in nondiabetic acromegalic patients ($R = 0.105$, $P < 0.05$), but not in the control group. In conclusion, daily urinary albumin excretion in acromegalic patients seems to be correlated with IR (HOMA-R) even before devoloping overt DM. Since, both IR and presence of MAU are CV risk factors, MAU may be important in the assessment of nondiabetic acromegalic patients.

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Safety of long-term combined therapy with somatostatin analogues and cabergoline (CAB) on cardiac valve in acromegaly: an echocardiography study

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The aim of the present study was to evaluate cardiac valve insufficiency prevalence after 12-month combined treatment with somatostatin analogues (SA) and CAB in acromegalic patients partially responsive to high-dose and long-term SA monotherapy. Twenty-four patients entered the study. A standard echocardiography was performed in all patients at diagnosis, after high-dose and long-term SA therapy and 12 months after CAB addition to SA to evaluate ejection fraction (EF) and mitralic (M), tricuspidal (T), aortic (A) and pulmunar (P) valve regurgitation (R). CAB was added at the initial dose of 1 mg weekly, then increased up to 0.5 mg daily after 3 months on the basis of GH and IGF-I levels. Compared to baseline, SA treatment induced a significant decrease, but not normalization, in GH ($P < 0.001$) and IGF-I ($P < 0.001$). After CAB addition, GH ($P < 0.001$) and IGF-I ($P = 0.002$) were furtherly decreased until normalization in all patients. Compared to baseline, EF was increased ($P < 0.001$) after SA monotherapy, whereas CAB addition induced only slight, but not significant, further increase in EF. At the study entry, patients showed: mild MR in 85%, moderate MR in 4.2%, mild TR in 41.2%, moderate TR in 8.3%, mild AR 8.3%, moderate AR in 8.3% and mild PR in 16.6% of patients, respectively. After treatment with SA, mild MR ($P = 0.002$), moderate TR ($P < 0.05$) and mild PR ($P < 0.05$) were decreased compared to baseline. After the addition of CAB to SA, mild MR and AR were furtherly reduced ($P < 0.001$ and $P < 0.01$ respectively); mild PR was increased ($P < 0.05$) compared to SA therapy. However, no case of severe or moderate to severe valvular abnormality was observed. In conclusion, valve dysfunctions, particularly mild MR and AR, seem to be improved after CAB addition, although a slight impairment in mild PR was found. Therefore, long-term combined treatment with SA and CAB is effective and safe in acromegaly.

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Incidence of benign and malignant neoplasms in acromegalic patients at a single institution

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Introduction
In acromegalic patients the incidence of benign and malignant neoplasms, appears to be higher than that in the standard population. Our aim was to evaluate the incidence of tumours in acromegalic patients treated at our Department.
Materials and methods
Over the years 1983–2008, 101 acromegalic patients (mean age 51.8 ± 15.4 years), were diagnosed and treated in our Department. Pituitary macroadenoma and microadenoma were stated in 63.4 and 25.7% of these patients, respectively (no data available for 10.9%). Mean observation period was 9.4 ± 6.5 years. We only scored neoplasms in patients primarily diagnosed with acromegaly. Our study was based on retrospective analysis of patient history and on recent screening for colon, breast, thyroid and prostate cancer.
Results
The median concentrations of hGH and IGF-1 prior to treatment were 20.2 (IQR = 34.9) ng/ml and 764.5 (IQR = 569.6) ng/ml, respectively. The current median hGH and IGF-1 concentrations were 2.1 (IQR = 4.0) ng/ml and 304.3 (IQR = 397.3) ng/ml, being statistically different from the former values ($P <$ Per 101 patients we observed incidences of: nodular goitre-63.0%, polyps of the colon-13%; uterine myoma and polyps-12% and 4.0%; prostate adenoma-2.0%; meningioma-4.0%; adrenal adenoma-2.0%; parathyroid adenoma-1.0%. Thyroid cancer, endometrium and cervix cancer were the most frequent malignant tumours (3% each); colon cancer incidence was 2.0%. Single cases of breast, stomach, skin and small-cell lung cancer were also observed. The dependence of the number of malignant neoplasms on the duration of uncontrolled disease: less than 5 years and over 5 years, was found to be statistically significant ($P < 0.05$).
Conclusions
1. We suggest an overall increase of tumour incidence in acromegalic patients. Prospective studies are required to resolve the significance of this observation.
2. The number of malignant neoplasms was significantly higher in patients with over 5 years of uncontrolled disease. No difference was found in the number of malignant neoplasms over the total duration of acromegaly (< 10 years and > 10 years).

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Gastric neuroendocrine tumors – new diagnostic and therapeutic approach

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The incidence of gastric neuroendocrine tumors (GNT) is increasing, what can be explained by the increased detection caused by the common use of the endoscopy and the pervasive use of acid suppressive therapy leading to enterochromatofine like cells proliferation. There are numerous new diagnostic/therapeutic GNT methods in use like: EUS, SRS, somatostatin therapy and 90Y/177Lu-DOTA-TATE radiotherapy.
Materials and methods
In 1998–2008 37 patients were diagnosed with the hist.path. confirmed GNT (mean age − 61 ± 12; 27F, 10 M). Gastroscopy, CT/MRI, EUS 99Tc-EDDA/HYNIC-Octerotate scintigraphy, chromogranin A serum level, clinical manifestation of the disease and type and efficacy of the therapy were assessed.
Results
Among 37GC patients in 26 patients type I in 2 type II and in 3 type III was diagnosed. During 4 years of the observation seven patients died (two patients-type I, death not related to GC, 2- type II and 3 -type III). The best detective value was found for the 99Tc-EDDA/HYNIC-Octerotate scintigraphy both for the primary and the metastatic lesions. The mean increased level of chromogranin A was found (366.1 ± 587.2 U/l; n:2–18 U/l), with maximum value in patients with dissemination (over 1000 U/l). In 43% of patients partial/total gastric resection was performed. However in four patients with type I GNT treated with the somatostatin analoque complete endoscopic remission was observed.
Conclusion
As the number of GNT is increasing the extensive diagnostic and therapeutic methods development are needed. However the endoscopic or surgical gastric resection are still a basic treatment, the use of somatostatin in type I, somatostatin and 90Y/177Lu-DOTA-TATE radiotherapy in nonoperative, disseminated cases seems to be very promising. Due to the different clinical course of the disease it seems that the treatment should be individually tailored to reach the best and optimal effect.

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The German NET-registry: an audit on the diagnosis and therapy of neuroendocrine tumours

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Introduction
Clinical experience with neuroendocrine tumours (NET) is difficult to acquire because they are rare and heterogeneous. The impact of recently published guidelines on diagnosis and therapy of NET is not known. The German NET-Registry offers a unique possibility to analyse data on diagnostic/therapeutic performance in a wide range of institutions. This study posed three questions: who provides the care for patients with NET; do the diagnostic/therapeutic procedures comply with guidelines; and are the results comparable to the literature?
Patients and methods
Centres were defined as any institution that cares for at least five NET patients. Data were accrued from patients' files by two study-nurses and transferred to a dedicated database (160 questions). Between 2004 and 2007, 1263 patients from 21 centres were included.
Results
Data on tumour location, age and sex, compared well with published data. Most patients were cared for in very large (>100 patients, 47.9%) or large (20–99 patients, 46.1%) centres. Imaging results (MRI, CT, US) were available for 79% of the patients, laboratory tests (chromogranin A, 5-hydroxyindolacetic acid, specific hormones) for 67%, somatostatin receptor scintigraphy for 56% and pathological findings for 79%. High-quality pathology reports were rare (2%). Surgery was the first therapy in 70.9%, nonsurgical treatment the second therapy in 45.7% of the patients. Peptide radio-receptor therapy was used more often than ablative therapy as second-line treatment. Median follow-up was 2.8 year (0.4–6.4), median overall survival was 2.5 year (0.34–6.3). Mortality was unrelated to tumour location.
Conclusions
Very large centres treated the majority of patients. These centres adhered best to the guidelines. However, there were still significant deficiencies in the documentation of diagnostic results, mainly concerning pathology reports. These deficits may negatively interfere with therapeutic decision-making. The therapeutic strategies used were comparable between the centres. These data provide a basis for quality management in NET.

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Adrenocortical carcinoma: results of surgical treatment and clinico-morphological prognostic factors

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The aim of the study was analysis of long-term results of surgical treatment in patients with adrenocortical carcinoma as well as definition of prognostic factors. From 1998 to 2008, examination and treatment of 53 patients with adrenocortical carcinoma (31 women and 22 men, mean age 52.8 years) was carried out in our institute: 13 patients had Cushing syndrome, 1 – virilization, 2 – total adrenohypercortisolism, and 37 – nonfunctioning tumors. The mean tumor diameter was 8.7 cm (range, 3–21), weight – 301.2 g (range, 21–2000). Four patients were at stage I, 8 – at stage II, 25 – at stage III, and 16 – at stage IV; 48 patients were operated on, and in five cases, surgical intervention was abandoned because of the multiple distant metastases (the diagnosis was confirmed by cytologic analysis of the fine-needle aspiration biopsy tumor samples). Radical surgery with complete resection of the primary tumor was performed in 42 patients. Among them, four patients underwent combined resection en bloc with adjacent organs (kidney, spleen and pancreas), three patients – liver segmentectomy, and one – lung resection. Incomplete tumor resection was performed in two patients. In four other patients, only tumor biopsy was performed. Patients not treated surgically died 3–7 months later diagnosis. Patients, who underwent incomplete tumor resection or only tumor biopsy, died 5–24 months later surgery. The mean follow-up after radical surgical treatment was 48.2 months (range, 2–86): 28 patients are still alive and 14 died. The 5-year overall and disease-free survival, calculated by the Kaplan–Meier method, was 58.3 and 55.1%, respectively. There were no reliable differences among overall and disease-free survival rates and tumor functional activity depending on patient age and sex. The 5-year overall and disease-free survival rate for patients at stage I and II was 100%, for patients at stage III – 51.9 and 47.4%, for patients at stage IV – 0%. The 5-year overall and disease-free survival for patients with tumors less than 10 cm (60.6 and 59.2%) was significantly higher than those for patients with tumors more than 10 cm (0%).
Complete resection of primary tumor (with adjacent organs and, when feasible, solitary metastases) and pathological tumor stage are the most significant prognostic factors in patients with adrenocortical carcinoma.

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Parathyroid cancer

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From 1987 to 2008, 226 patients were operated on for primary hyperparathyroidism (pHPT). In 17 (men and women, aged 21–71 years) of them, parathyroid carcinoma (PC) was verified by histologic analysis. Mixed pHPT form was noted in 12 patients, visceropathic one – in 4, and asymptomatic – in 1. Hypercalcemia was revealed in 14 of 17 patients, and elevation of parathyroid hormone (PTH) level (426–1160 pg/ml) – in 12. In 5 patients operated on before 1990, serum PTH level was indefinable. Neck palpation revealed tumor-like neoplasm in 14 patients. US neck scan showed tumor-like neoplasms in 10 patients. CT demonstrated one tumor in anterosuperior mediastinum, the second one – behind trachea (at the level of C_{VI}). Three patients underwent only tumor resection because thyroid invasion wasn't suspected by that time. In addition to PC resection, one patient underwent thyroid lobe resection, 4 – hemithyroidectomy, 3 – subtotal thyroidectomy, and 5 – total thyroidectomy. In one patient, sternotomy allowed to reveal a tumor with cystic degeneration in the right superior thymic limb. It was woody-dense and intimately connected with a sternoclavicular joint, subclavian artery, and brachiocephalic vein. Thymectomy and subtotal thyroid resection were performed. In postoperative period, in 16 of 17 patients, clinical manifestations of differently pronounced hypoparathyroidism developed and was confirmed by laboratory data. One patient died after operation due to pancreanecrosis. Remote outcome (later than 6 months – 15 years after operation) was studied in 11 patients. By the time of examination, all these patients were alive and no signs of tumor recurrence or regional and remote metastases were revealed. When suspecting PC, careful following ablation technique during surgery is needed which can help avoiding capsule damage especially as parathyroid tissue is markedly capable of implantation. After urgent histologic investigation and diagnosis verification, it's necessary to be sure that operation was radical enough. As a rule, resection of PC is associated with simultaneous removal of adjusting thyroid lobe. Revision of ways of the regional metastatic spreading is obligatory as well as lymphadenectomy, if necessary.

P202

A National survey of neuroendocrine lung tumors

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Neuroendocrine lung tumors represent approximately 20% of all lung tumors. They range from low-grade well-differentiated NETs and well-differentiated neuroendocrine carcinomas (typical and atypical carcinoids) to aggressive poorly differentiated small-cell and large-cell neuroendocrine carcinomas. They can develop different clinical syndromes due to ectopic hormone secretion.
We analyzed 178 patients with neuroendocrine tumors (age range: 17–79 years, 51.4 mean) treated at our department in last 5 years. The diagnosis was based on histological and immunohistochemical examinations and they were classified according to the WHO classification. Neuroendocrine lung tumors occurred in 35 (19.7%) patients (age range 24–79, 51.4 mean). Among those, 14 (40%) patients had well-differentiated neuroendocrine tumor, 16 (45.7%) had well-differentiated neuroendocrine carcinoma, SCLC was found in 4 (11.4%) cases, and one patient (2.8%) had mixed endocrine/adenocarcinoma of the lung. Two patients (8.6%) with well-differentiated NETs had ectopic secretion of ACTH. Four patients had clinical presentation of MEN1 syndrome while no mutations in MEN-1 gene were found. Eight patients (22.8%) had carcinoid syndrome. At the time of diagnosis in 12 (34.3%) patients distant metastases were evident: 9 with well-differentiated neuroendocrine carcinoma, 2 with SCLC, and 1 with mixed carcinoma. Primary tumor was operated in 21 (60%) patients. In 4 of them (11.4%) locally recidivant tumor occurred (occurrence range: 8–120 months, mean 37.5), 2 with atypical and 2 with typical carcinoid tumors. Four patients with atypical tumors (11.4%) developed distant metastases after surgery (occurrence range: 48–120 months, mean 96.0). They mostly metastasized in the liver (55.5%), bones (33.3%) and adrenal glands (33.3%). Twelve patients (34.3%) with metastatic disease died durring this period, 7 with atypical carcinoids and 3 with poorly differentiated carcinomas; 2 patients died due to non-tumor related causes.
Our data are in concordance with literature, local recurrences and distant metastases are more frequent in aggressive tumors. Tumor biology as defined by WHO classification is most relevant prognostic factor.

P203

Efficacy and safety of ^{90}Y-DOTATATE therapy in neuroendocrine tumours (NETs)

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Therapy with labeled somatostatin analogues is the modern approach to patients with disseminated or unresectable NETs expressing somatostatin receptors (SSTR). Octreotate is the somatostatin analogue with high affinity to SSTR type 2, most commonly present in NETs. The aim of the study was to assess the efficacy and toxicity of peptide receptor radionuclide therapy (PRRT) with the use of ^{90}Y labelled Tyr^3-octreotate, (^{90}Y-DOTATATE) in NETs.
Material
Thirty-six patients with positive ^{99}mTc-Hynic-Tate receptor scintigraphy (23 females, 13 males; Karnofsky's index $>$70–83%, $<$70–17% of the patients) were referred to the therapy. The study group comprised 22 patients with foregut, 11 with midgut, 2 with hindgut tumours, 1 with NET of unknown origin and 3 patients with unresectable tumour, but no metastases.
Methods
Each patient received 7.4 GBq/m^2 (200 mCi/m^2) of ^{90}Y-DOTA-TATE divided in 3 to 7 doses (most often in 4–5 cycles) repeated every 4 to 9 weeks. For nephroprotection amino-acids formula, before and after each cycle of PRRT was administred.
Results
After the PRRT partial remission was observed in 45%, stabilization in 24% and disease progression in 31% of patients. Seven patients died before completing PRRT. No worsening in renal function was observed after PRRT. In 5 cases after 18 months the creatinin level increased. A drop in WBC was observed mostly after 3–4 cycle of PRRT, with transient grade 3 toxicity in 4 patients. Mean PLT count was within normal limit during the therapy. In 3 patients the value of Hb was assessed as toxicity grade 3. One patient, previously with chemotherapy developed myelodysplastic syndrome. In 76% patients chromogranin A level decreased after therapy.
Conclusions
(1) Therapy with ^{90}Y-DOTA-TATE results in partial remission or stabilization of the disease in most patients. (2) Treatment with labelled somatostatin analogue usually does not induce clinically important haematological or renal toxicity.

P204

The variability of clinical presentation of multiple endocrine neoplasia syndrome type 1 as the reason of the underestimated diagnosis

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Introduction
Multiple endocrine neoplasia (MEN) is a rare disease. Apart from the well recognised MEN2 syndrome the MEN1 is less common diagnosed. The MEN1 gene is localised on the 11q13 chromosome and encodes menin. There is no simple definition of MEN1 syndrome because of heterogenous combination over 20 different endocrine and non-endocrine tumours. According to the Gubbio consensus, MEN1 is diagnosed by the occurrence of two of the three main MEN1-related endocrine tumours: parathyroid adenomas, enteropancreatic tumours (GEP) and pituitary tumours ('3P') independently from different endocrine and non-endocrine tumours in the same patient. It seems to be over-simplified definition leads to MEN1 incidence being underestimated.
Materials and methods
Over the years 1994-2008, 42 cases (11 males and 31 females) of MEN (non MEN2) syndrome were diagnosed at our Department of Endocrinology. Hormone tests, and imaging (USG, CT, MRI, SRS) were carried out in all patients.
Results
The most common pathology was parathyroid adenoma or hyperplasia (26 patients) and pituitary adenoma (26 patients: 4 somatotropic, 4 lactotropic and 18 non-secretory). GEP tumours were diagnosed in 16 patients: in 2 patients glucagonoma, in 3 gastrinoma, in 2 somatostatinoma in 7 serotoninoma and in 2 patients non-secretory tumours. In 14 patients (4 males and10 females) of mean age 49.8 $\pm$ 14 years 'classic' MEN1 was diagnosed. Three patients mean age 56 $\pm$ 12 years had three tumours of the main glands. The others 16 patients (7 male and 22 females) mean age 52 $\pm$ 9 years, had only one main MEN1-related tumour and other less essential, but connected with MEN1, abnormalities. The last group of 6 patients mean age 47.3 $\pm$ 8 years had no main MEN1- related tumours but had adrenal tumours (pheochromocytoma, adenoma or adrenal carcinoma) and adenoma or thyroid carcinoma.
Conclusions
(1) Variability of the clinical presentation of MEN1 syndrome and variability of occurrence at different times, lead to underestimation of MEN1 incidence; (2) better specification of criteria for diagnosing the MEN1 syndrome need to be urgently established; (3) long-term observation of MEN1 patients may increase the number of cases established and decrease mortality due to tumour progression to malignancies.

P205

Acromegaly in the Swedish pituitary register: background data and up to 10 years follow-up

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Background
The aim of the Swedish Pituitary Register – the Swedish Pituitary Study Group's quality register – is to guarantee that all patients with pituitary tumours get equivalent diagnostic evaluation and treatment, as well as to evaluate given therapy. In this study, patients diagnosed with acromegaly from 1991 are described.
Methods
Data from 557 patients (275 men/282 women), median age 51 years (range 4–87), with acromegaly were registered.
Results
The incidence 1991–2007 was 3, 7 cases/million per year. Thirty-eight percent were classified as micro- and 58% as macroadenomas. According to SIPAP classification system for tumour extension 44% had suprasellar, 27% infrasellar, 28% parasellar, 6% anterior and 6% posterior extension. Twenty-one percent had visual field defects and 13% impaired visual acuity. Pre-operatively LH/FSH, ACTH, TSH and ADH deficiencies were reported in 21, 6, 7 and 1%. Four hundred and three patients were operated once, and 47 patients 2–3 times. Fractionated radiotherapy was given to 28 patients and treatment with Gamma Knife to 44 patients. One hundred and seventy-one patients received medical treatment. Follow-ups after 1, 5 and 10 years were registered for 91, 75 and 51%. The overall cure rate (normal IGF-1 for age and mean GH $<$ 5 mU/l or 2.5 μg/l) after 1, 5 and 10 years was 42, 51 and 62%. Improvement was reported in 44, 36 and 24%. Of these, further 31, 54 and 58% normalized their GH- and IGF-1 values on medical treatment. After 1, 5 and 10 years, 51, 58 and 68% of cases with primary surgery were cured, and well controlled in further 9, 17 and 11%.
Conclusion
Baseline registration appeared to be complete and treatment in accordance to international guidelines. Number of cured patients seemed to increase over time. Further efforts will be made to increase the frequency of follow-up registrations also after long time.

P206

Percutaneous laser ablation for palliative treatment of neuroendocrine liver metastases

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Background
Liver metastases occur in about 40–85% of patients with neuroendocrine tumours (NET). NET usually run a rather indolent course but the 5-year survival is about 40% in patients with liver metastases versus 75–99% in subjects free of hepatic lesions. The most effective management and timing of treatment for patients with surgically unresectable metastases remains still unsettled.

Purpose
To evaluate the feasibility, safety, and clinical benefits of percutaneous laser ablation (PLA) in patients with unresectable and progressive NET hepatic metastases.
Patients and methods
Eleven patients (6 male, mean age 54, range 24–79) with NET hepatic metastases and progressive disease under medical treatment underwent PLA. Primary tumors were localized in the pancreas and intestine in 4 and 2 patients respectively, five patients had lung NET. The lesions treated with PLA were 15; the number of treatments was 24. The mean diameter of metastases was 3.5 cm (range 1.5–12 cm) at baseline. Nine of eleven patients had symptoms related to either hormone secretion or mass effect. PLA was performed under ultrasound guidance. The treatment efficacy was assessed by CT and contrast-enhanced Ultrasound examination 24 h after PLA. Clinical and CT controls were performed every 3 months.
Results
Mean follow-up was 84 months (range 18–200). Nine hepatic tumors $\leq$4.0 cm appeared completely ablated by PLA treatment, while larger metastases (diameter from 5.5 to 12 cm) showed over 60% ablation. Most patients (88%) reported symptom relief. Progression-free mean survival after PLA was 16 months (range 2–48). No major complications were observed during and after the procedures.
Conclusions
PLA is a feasible and well tolerated procedure for the palliative treatment of unresectable NET hepatic metastases. In the present series, most patients showed an improvement in their performance status and a long progression-free survival. Further controlled studies are required to evaluate the impact of PLA on the overall survival.

P207

The colonic polyps in the patients with acromegaly

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Aim
To evaluate the frequency and morpho-histological characteristics of the colonic polyps (CP) in acromegaly patients (pts).
Materials and methods
We studied 53 pts (50 female, 3 men) aged 23–76 (median – 47) with active acromegaly. The mean disease duration was 10.7$\pm$4.3 years. Somatotropinoma was diagnosed in 51 pts and somatoprolactinoma in two. The activity of acromegal? was confirmed on the base of the clinical and hormonal data. Basal GH (median – 17.4 (6.7; 23.5) mU/l) and IGF-1 levels (according to the aged reference significances) were elevated. GH secretion in standard OGTT wasn't suppressed (GH nadir $>$2.7 mU/l). Videocolonoscopy with biopsy of identified CP had been performed. Hematoxylin-eosin staining was used for histological samples.
Results
The hyperplastic CP were revealed in 16 from 53 (30.2%) cases. The solitary CP was diagnosed in 13 (81.35%), multiple – in 3 (18.7%) patients. CP size varies from 0.5 to 2.0 cm in diameter. The majority of CP was localized in c. sigmoideum (7 pts) and rectum (4 pts). Besides, CP were situated in c. transversum (1 pts), in cecum (1 pts), in c. ascendance, (1 pts), in c.descendence (1 pts), in rectosigmoid (1 pts), in ileocecal valve (1 pts). Pseudopolyps were revealed in two pts. Tubulo-villous adenoma was identified in one case. The colon cancer wasn't diagnosed in our patients.
Conclusion
The prevalence of the colonic polyps achieves 30.2% among our patients with predominant localization in c. sigmoideum and rectum. The malignancy index of CP was zero.

P208

Laboratory diagnosis of gastrinoma remains difficult

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Before the use of potent acid suppressing drugs and in particular proton pump inhibiters (PPI), most patients with gastrinoma presented with Zollinger–Ellison syndrome and diagnosis was problematic in only a few. In recent years, the syndrome is rarely seen and gastrinoma patients present with less overt symptoms and hypergastrinaemia which may be mild. Increasingly patients present later.

In the population, hypergastrinaemia most commonly occurs because of stimulation of antral G cells generally due to the lack of negative feed back when gastric acid is absent. In the diagnostic laboratory hypergastrinaemia is frequently recorded due to; post-prandial specimen collection at clinics, H-pylori infection, acid suppressing drug administration, atrophic gastritis and less frequently with impaired renal function, or gastrinoma.
Chromogranin A (CgA) is the best general marker for endocrine tumours and is raised in the circulation of patients with gastrinoma. However, CgA is also raised when gastric acid is absent.
In a tertiary referral laboratory for the diagnosis of endocrine tumours of gastoenteropancreatic origin we have studied requests for gastrin and chromogranin A assay in patients under investigation for gastrinoma (N=500). Chromogranin A is raised in 46% of these patients. Five percent were gastrinomas. The remaining patients had unsuspected endocrine tumours other than gastrinoma (frequently serotonin producing tumours of the ileum or colon), atrophic gastritis, were on PPI therapy or had no known relevant diagnosis.
Gastrin was raised in 28% of patients 5% being gastrinoma.
We have also studied gastrin in several groups of subjects (N=25–50), normal healthy controls with or without H-pylori infection and patients with duodenal ulcer during and after PPI therapy, autoimmune atrophic gastritis, idiopathic achlorhydria, renal failure and gastrinoma and compared circulating gastrin both fasting and post-prandially in these groups using three regional specific antisera. This has illustrated that gastrinoma remains a difficult laboratory diagnosis in many patients.

P209

R171Q MEN1 polymorphism in patients presenting with hyperparathyroidism

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The change of aminoacid Arginine (CGG) to Glutamine (CAG) at position 171 (R171Q) in the *MEN1* gene has been occasionally reported in MEN1 carriers, but also in 1.4 to 5% subjects among the general population, therefore it is still unclear whether it might represent a polymorphism and/or it has a role in tumourigenesis. The aim of our study was to evaluate the presence of the R171Q polymorphism in patients with MEN1-related states presenting for MEN1 genetic screening. Fifty-seven patients (16 men, 41 women, mean age 55.1$\pm$2.3) were evaluated for the R171Q polymorphism, that was detected in 1 patient presenting with a parathyroid adenoma and an ACTH-secreting pituitary adenoma, in her two sons, and in her father. The R171Q polymorphism was found in an unrelated patient with parathyroid adenoma and mild hyperprolactinemia, in her unaffected father, and in a third patient with parathyroid adenoma and paraganglioma. Moreover, the same polymorphism was found in an unrelated family, with primary hyperparathyroidism, non-functioning pancreatic neuroendocrine tumour and a non-functioning adrenocortical adenoma. As controls, a panel of 50 healthy subjects from the same geographical area was screened, and the R171Q aminoacid change was not detected. The R171Q was present in 8 out of 57 (14%) patients undergoing MEN1 genetic screening, in 34.8% of the cases presenting with hyperparathyroidism. Our results indicate that MEN1 patients carrying this genetic alteration, as well as clinically unaffected carriers, should undergo a careful endocrine investigation and a close clinical and biochemical follow-up.

P210

Recurrence in patients with pituitary nonfunctioning adenoma

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Nonfunctioning adenomas (NFA) are 30% of all pituitary adenomas. Transphenoidal surgery is the first line therapy, but recurrences are frequent (12% al 69%). NFA treatment and follow-up are controversial. Aim of our study was to evaluate the recurrence prevalence and the factors associated with tumor aggressiveness in patients with NFA. We studied 30 patients that underwent surgery: 14 patients (group A, 7F, 3.92$\pm$12.48 years) with and 16 patients (group

B, 6F, 56.5 ± 12.31 years) without recurrence, with a follow-up of 7 ± 4.49 years. At baseline, signs frequently observed were visual field defects (90%), headache (33%) e reduced energy (27%). In our study recurrence prevalence was 47%, mostly within 48 months after surgery. Only one patient recurred 156 months after surgery. In group A and B, 29% and 37% patients had normal pituitary function, respectively. In group A PRL level was significantly increased ($P < 0.05$) compared to group B. No significant difference in neuroradiologic imaging was observed between the two groups, but suprasellar extension and chiasmal compression were more frequent in group A, where we also observed cavernous sinus invasion. Tumor size was greater in group A compared to group B ($P < 0.05$). In group A, LH immunostaining was more frequently observed ($P < 0.05$), while group B showed a higher number of null-cell adenomas ($P < 0.05$). In our study 6 patients were treated with radiotherapy after first surgery and 3 patients after second or third surgery, but recurrences were observed also in early radiotreated patients. In conclusion, in this study, we observed that tumor size, cavernous sinus invasion, suprasellar extension, chiasmal compression, and LH immunostaining are associated to a higher recurrence rate after surgery. Our data confirm that long term follow-up is necessary, mostly within the first 5 years after surgery.

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Clinical feature and genetic testing in patients with multiple endocrine neoplasia syndrome type 2

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Background
Multiple endocrine neoplasia syndrome type 2 (MEN2) is a rare disease characterised by inherence in each patient medullary thyroid carcinoma (MTC), pheochromocytoma and in type MEN 2a primary hyperparathyroidism, in type MEN2b marfanoid habitus and neurofibromas. Mutation in RET proto-oncogene at chromosome 10 is a molecular cause of MEN2 syndrome.
Methods
Eighteeen patients with MEN2 syndrome were enrolled: (10 women and 8 men) mean age 22 ± 7 years. An average follow-up period was 10 ± 9 years. Every patient was examined by the thyroid gland ultrasonography and computed tomography of the abdomen. TSH, fT4 and fT3, PTH, calcium and calcitonin serum level were measured. Also methoxycatecholamines in urine and genetic testing were undertaken. Furthermore, scintigraphy scans were performed (SRS, DMSA, MIBG).
Results
Multiple endocrine neoplasia syndrome type 2 was primary diagnosed in 7 patients and the rest 11 patients had MEN2 diagnosed because of positive genetic findings. Currently 5 patients are in remission of disease after the thyroid gland surgery and do not reveal symptoms of pheochromocytoma so far. Rest of patients, even though early diagnosis was established and the operation was performed in preclinical stadium, have MTC relapse and symptoms of pheochromocytoma. Two patients with MEN2b syndrome died because of progression and complication.
Conclusions
The first symptom, both of MEN2a and 2b syndrome is MTC, but the cause of the first consultation are symptoms related to pheochromocytoma. Early treatment based on genetic tests improve asymptomatic survival and extend remission period. The prognosis in MEN2b is worse than in MEN2a syndrome.

P212

Pancreatic neuroendocrine tumors: a national survey

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Pancreatic neuroendocrine tumors (PETs) occurs in approximately 1 per 100 000 people per year and account for only 1–2% of all pancreatic tumors. Most of them are hormonally active, while about 30% secrete no detectable hormons and are discovered due to a tumor mass effect.
Material and methods
We analysed 178 patients with neuroendocrine tumors (NETs) treated at our department in the last five years. The diagnosis was made by pathohistological examination and patients were classified according to the WHO criteria.
Results
In our group of patients with NETs there were 45 (25.3%) patients with PETs (age range 25–71, 51.8 mean). MEN1 was diagnosed in 5 (11.1%) patients and one patient had VHL-syndrome. Almost half of them (44%) were functional (11 insulinomas, 5 gastrinomas, 4 somatostatinomas). According to WHO classification, 17 were well-differentiated tumors (37.7%), 20 well-differentiated carcinomas (44.4%), 6 poorly-differentiated carcinomas (13.3%) and two mixed endocrine–exocrine carcinomas (4.4%). At the time of diagnosis in 19 (42.4%) patients metastatic disease was diagnosed. Primary tumor was operated in 26 (57.7%) patients. Tumor recidive was diagnosed in 7 (15.5%) patients (occurrence range 1–30 months, 10 months mean). All of these patients had partial pancreatic resections, with one having pathologically proven infiltration of operative margins. Metastatic disease developed in 6 (13.3%) patients (occurrence range 11–60 months, 2.7 years mean). All the patients with locally recidivant tumor or who developed metastases postoperatively belong to the groups of well- or poorly-diffentiated carcinomas. Unoperated patients were treated with chemotherapy, biotherapy, PRRT or combined. During this period 15 patients died, one patient with well-differentiated tumor died due to non-tumor related cause.
Conclusion
Our data are concordant with the data previously presented in literature. We conclude that the tumor biology as defined by WHO classification is most relevant for the clinical outcome of these patients.

P213

Image-guided radioiodine therapy of HCC following AFP-promoter targeted *in vivo* sodium iodide symporter (NIS) gene transfer

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Due to limited treatment options the prognosis of patients with advanced hepatocellular cancer (HCC) has remained poor. We therefore examined the feasibility of radioiodine therapy of HCC after human sodium iodide symporter (hNIS) gene transfer, using the tumor-specific alpha-fetoprotein (AFP) promoter for transcriptional targeting. For this purpose NIS gene transfer was performed *in vivo* in human HCC cell (HepG2) xenografts, using replication-deficient adenoviral vectors carrying the NIS gene linked to the AFP-promoter fragment (Ad5-AFP-NIS). Functional NIS expression was confirmed by immunostaining as well as *in vivo* ^{123}I gamma-camera imaging followed by application of a therapeutic ^{131}I dose. HepG2 cell xenografts in nude mice injected intratumorally with Ad5-AFP-NIS accumulated 10–15% ID/g (percentage injected dose per gram tumor tissue; 3×10^9 PFU) with an average biological half-life of 8.3 ± 1.8 h resulting in a tumor-absorbed dose of 215 ± 77 mGy/MBq. After Ad5-AFP-NIS-mediated NIS gene transfer in HepG2 cell xenografts administration of a therapeutic dose of 55.5 MBq of ^{131}I resulted in a significant reduction of tumor growth associated with significantly improved survival. We conclude that a therapeutic effect of ^{131}I was demonstrated *in vivo* in HCC cell xenografts after adenovirus-mediated induction of tumor-specific iodide accumulation by AFP promoter-directed hNIS expression.

P214

The retrospective analysis of the pheochromocytoma diagnostic procedures in patients after laparoscopic adrenalectomy

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The pheochromocytoma (PH) diagnosis is often very difficult, particularly in asymptomatic or oligosymptomatic patients. Every subject with adrenal tumor should be screened for PH, although many factors may interfere with biochemical

evaluation. The false positive testing for PH may result in unnecessary surgical treatment.
Methods
Retrospective analysis of 45 patients after laparoscopic adrenalectomy due to suspicion of PH (based on biochemical testing) was performed. The subjects were divided into 2 groups according to the results of histopathological examination: group 1–27 PH positive patients (mean age 46.8±14.4 years), group 2–18 PH negative patients (mean age 55.7±13.7 years). Following parameters were analyzed: presence of PH related symptoms, urinary excretion of metanephrine (MN), normetanephrine (NMN), MN+NMN and pre-operative imaging (multi-phase CT) results.
Mean value of NMN, NM and MNM+NM were statistically higher in group 1 (*P* value: 0.002, 0.001, <0.001 respectively). The highest sensitivity (81.5%) for PH diagnosis, as well as true negative predictive value, had MN+NMN. The highest true positive predictive value was stated for NM. The extimated cut-off levels for our laboratory: were: 713.6, 1598.6, 1396.1 for NM, NMN, NM+NMN, respectively.
Adrenal CT had the highest sensitivity for PH diagnosis (100% for cut off point of 59 HU in venous phase) and highest specificity (100% for cut-off point 48 HU for wash-out phase).
Results
The biochemical testing alone may be misleading particularly for only slightly elevated metanephrines excretion. Imaging results are the most sensitive tool for proper diagnosis of PH, and should be always considered while deciding on surgical treatment of patents suspected for pheochromocytoma.

P215

Neuroendocrine disorders of patients with pituitary tumours

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Aim
Aim of research is to study special features of pituitary adenoma (PA).
Materials and methods
Seventy-four operations on CST were performed. Outcomes of surgery in 66 patients with PA were analyzed. Age of patients at surgery was from 18 to 71 years. Mean age of patients – 44 years. Four patients (6%) had a corticotropinoma, 11 patients (17%) had somatotropinoma, 11 patients (17%) had prolactinoma and remaining 40 patients (60%) had non-functioning pituitary adenomas (NFPA). Analyzed patients with PA undergone transnazall selective hypophysisectomy surgery (TSH).
Results
In patients with NFPA manifestation of disease signs of cerebral hypertension (100%), visual disturbances (83.2%), secondary hypopituitarism (59%) and hyperprolactinemia (56.5%). Among operated patients with ACTH-secreting PA, all patients were females including 3 cases (75%) with obesity, 4 cases with hyrsutism (100%), 3 women with amenorrhea and 1 female (25%) with myopathy. Fifty-six cases (85%) neurological symptoms regressed right after TNS, in 8 cases (12%) focal signs regressed partially and worsening of presented neurological deficiency have seen in 2 cases (3%). Eleven patients with acromegaly after TSH have shown signs such as changes in facial appearance (100%), headaches (88.9%), increased sweating (68.7%), in men, decrease of libido and erectile dysfunction (91.2 and 58.7% respectively).
Conclusions
(1) During the analysis of clinical manifestation and hormonal activity of PA there characteristic features revealed in clinical and neurological course. (2) Among the histological types of pituitary tumors in prolactinomas and somatotropinomas there are acidophilic adenomas prevail in comparison with less rare chromophobe adenomas. (3) As somatotropinomas have invasive growth and recurs postoperatively there is need in radiotherapy. (4) NFPA characterized with consecutive manifestation; initially, brain and focal symptoms occur as the result of tumor focal effects and then endocrine symptoms appear which are result in secondary endocrine deficiency with obliterated flow.

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Insulinomas: experience of Coimbra's University Hospital, Endocrinology Department

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Background
Insulinomas are rare neuroendocrine tumours (4 cases/million patients per year), representing an important cause of hyperinsulinemia. Usually are benign and sporadic, but can be part of multiple endocrine neoplasias. To establish the diagnosis it is essential to document inappropriately high levels of insulin during episodes of hypoglycaemia.
Aim
Retrospective analysis of the clinical files of the patients followed in our department since January 1997.
Patients and methods
The analysed parameters were: age, gender, clinical presentation, biochemical and imaging diagnosis, treatment and follow-up.
Results
We studied nine patients (5M: 4F), mean age 57.2±18.1 years old and body mass index 31.7±7.5 kg/m^2. Six patients were symptomatic, all with neuroglicopenic symptoms, four of them also with adrenergic symptoms and one reporting increase of weight. Three patients were asymptomatic, but fasting hypoglicemias were detected in routine analysis. One patient was already diagnosed as MEN-1. Eight patients performed the 72-hours fasting test (minimum glicemia 31.9±6.0 mg/dl, insulinemia 15.7±7.2 μUI/ml and C peptide 4.2±2.3 ng/ml). Mean HbA1c was 4.8±0.6%. Tumour localization: pancreatic head (2), body (3), tail (2), body–tail transition (2). Mean diameter was 2.6±2.2 cm. Three patients were submitted to preoperative medical treatment with octreotide, and one of them also with diazoxide. Surgical procedures were the following: pancreaticoduodenectomy (1), tail pancreatectomy (1), body–tail pancreatectomy (4) and enucleation (3, one of them reoperated – partial pancreatectomy). There were several surgical complications: one ileum perforation and death, three pancreatic fistulas, one transverse colon perforation, two pseudocysts and one case of pancreatic abscess. Surgery was curative in all patients alive. Anyone developed diabetes.
Conclusions
The authors emphasize the serious difficulties in the diagnosis, namely the preoperative localization of the insulinoma, which sometimes is only recognized during surgery. Treatment is also a delicate matter, since these surgical procedures are extremely invasive, with a high level of complications.

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The ectopic adrenocorticotropic hormone syndrome in carcinoid tumors (case report)

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Ectopic production of adrenocorticotropic hormone by carcinoid tumors is relatively uncommon. This report describes a woman who had Cushing syndrome from the ectopic secretion of adrenocorticotropic hormone by a carcinoid tumor. Before her hospitalization the patient's conditions was misdiagnosed as disease of connective tissue and thus the patient was treated inadequately. The untreated hypercortisolism caused bilateral pneumonia and sepsis. There are three instructive elements of this case: (1) the recognition of Cushing syndrome, (2) the association of Cushing syndrome with thymic carcinoma (3) the need to treat the hypercortisolism and its complications as well as the tumor.
In April 2007, a 23-year-old woman presented to hospital with following symptoms: general weakness, progressive hyperpigmentation and facial rounding. Physical examination revealed moon face, violaceous striae, easy bruising, hirsutism, pododema, hypertension, *tachycardia*; the auscultation revealed diminished breath sounds. Laboratory results showed hypokalemia, hypoproteinemia, hyperglycemia, and anemia. A random serum ACTH and cortisol levels revealed significant elevation (ACTH=387 pg/ml (N<46), Cortisol=1592 nmol/l (N 119–618)). The computed tomography (CT) of the chest showed thymic tumor and bilateral multisegmental pneumonia. She was treated with aminoglutethimide, insulin, antihypertensive, potassium, albumin, antimicrobial therapy, ketokonazole. In spite of the treatment the patient developed an abscess of the right upper lobe of the lung. In July, the patient underwent surgery (thymectomy with right upper lobectomy). Histological examination revealed the presence of small-cell carcinoma with invasion to surrounding fatty tissue. Immunohistochemical staining defined the tumor as an ACTH-secreting (Ki67-14%). After surgery and further treatment with Octreotide and *chemotherapy* the patient's symptoms completely resolved and the ACTH and Cortisol levels were normalized. For the last 6 months the patient has not been given any therapy, her general condition remains satisfactory.
This case demonstrates the successful diagnosis and treatment of the ACTH-ectopic tumor as well as hypercortisolism and its complications.

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Growth hormone deficiency problems in adult patients with pituitary adenomas

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Aim of the research
To study neuroendocrine disorders at growth hormone deficiency (GHD) in adult patients with various pituitary tumours.
Materials and methods
There 27 adult patients with GHD due to different sellar region neoplasms were evaluated in 2008. Among them, there were 20 women and 7 men. Average age of patients constituted 36.3 years.
All patients evaluated with clinical, biochemical, hormonal, instrumental, roentgenologic (CT in 15 patients and MRI of pituitary in 23 patients) methods as well as evaluation of quality of life according to questionnaire (QoL).
Results
Study shown that non-functioning pituitary adenomas seen in 21 (77.7%) patients whereas prolactinoma, astrocitoma, craniopharyngioma and germinoma have seen in 2, 1, 1 and 1 patients respectively. Sixteen patients undergone transnasal surgery and 1 bifrontal surgery for pituitary tumor. Our patients revealed various neuroendocrine disorders: partial hypopituitarism in 81.5%, panhypopituitarism in 7.4%, postoperative GHD in 72.7%, postoperative panhypopituitarism in 29.6%, secondary hypocorticism in 14.8%, secondary hypogonadism in 11.1%, functional hyperprolactinemia in 11.1%, secondary amenorrhea in 29.6%, diabetes insipidus in 15%, bitemporal hemianopsia in 59.2%. Quadrant hemianopsia in 14.8% scotoma in 3.7% ptosis in 3.7% and so on.
Conclusions
1. GHD with various sellar region neoplasms revealed in 81/5% cases (22 patients of 27 evaluated) whereas postoperative GHD have seen in all operated patients in early postoperative period – 16 patients (72.7%).
2. The feature of GHD manifestation is significant decrease of psycho-emotional condition of patients along with neuroendocrine disorders.
3. Patients with GHD require biochemical evaluation, hormonal measurements, stimulating tests for GHD evaluation, quality of life evaluation, anthropometry (BMI, WV/HV), CT, MRI of pituitary, densitometry.
4. Patients with non-functioning pituitary adenomas with postoperative GHD require GH replacement therapy.

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Parathyroid surgery a paradigm shifts from inpatient to a day case surgery

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Introduction
There has been an increasing trend towards outpatient and short stay surgery in UK in last 20 years. This leads to reduced costs, reduced inpatient waiting lists, increased availability of inpatient beds and has the psychological benefit of avoiding prolonged hospitalisation.We liked to evaluate the feasibility of day case and short stay surgery for parathyroid disease in our district general hospital.
Material and method
It was retrospective audit of 48 patients in our district general hospital undergoing parathyroid surgery over the period of 7 years (2000–2007) by a single endocrine surgeon.
Results
In our study the average hospital stay was ~1.5 days with minimal morbidity of 0.5% and no mortality rate. Most of the patients had preoperative scan with either sestamibi or ultrasound or both for pre operative localization of the gland.
Conclusion
It can seen that in our district general hospital the Parathyroid surgery can be performed with minimal morbidity with an early post operative discharge. The hospital stay can further be reduced by using focussed parathyriodectomy in our hospital after adequate preoperative localization of the gland.
In most of the regional centres there is a shifting trend to perform focussed-parathyriodectomy as a day case procedure after adequate preoperative localization of the gland for uni glandular adenoma. However, we feel that this procedure needs to be discussed and made easily available to patients in all endocrine units performing parathyroid surgery. There need to be a national consensus with clear guideline made available to surgeons performing these complex procedures, so that, more and more patients can benefit by day case surgery especially for adenoma in all units performing these operations.

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Oktreotid-depot therapy of acromegaly with long action somatostatin analogue

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Aim
To estimate efficiency of long acting somatostatin analog (Oktreotid-Depot, Ltd 'FarmSyntez', Russia) in acromegaly treatment.
Material and methods
Twenty-five patients with the confirmed acromegaly diagnosis, active phase, receiving Oktreotid-Depot therapy within 6–12 months in a single dose of 20–40 mg/month. Every three months dynamics of clinical signs, basal IGF-1 and GH levels and pituitary adenoma size were analyzed.
Results
The most pronounced and significant ($P<0.0001$) decrease in clinical and laboratory acromegaly signs was observed in first three months of treatment: GH from 18.1 (7.7; 3.3) to 4.2 (1.9; 13.5) mU/l, IGF-1 from 566 (445; 813) to 235 (188; 391) ng/ml. By third month of Oktreotid-Depot therapy, IGF-1 normalisation was observed in 52% patients, by sixth month in 32%, to the ninth – in 40%, to the twelfth – in 53% patients. In all patients with normalised IGF-1 level basal GH level was normal (<2.5 ng/ml) too. In group of patients with normalised IGF-1 level by third month, its normal values were supported till the end of the period of supervision in the majority of patients. In other patients the incidental deviation from norm was insignificant usually no more than 10%. In patients with elevated IGF-1 level in 3 months its decrease was more than 50% and remains at the reached level within 12 months.
Before Octreotid-Depo treatment the median macroadenomas size was 2.3 (1.1; 4.85) cm^3 and in 6 months therapy it decreased to 1.4 (0.75; 4.6) cm^3. In three of eight patients a reduction of the adenoma size was significant: 21, 39.2, and 74%. In four patients in 6 months of treatment a stabilisation tumour sizes was marked.
Conclusion
Oktreotid-Depot – an effective medication for acromegaly treatment in active phase which in some cases causes reduction of tumour size.

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Severe elevation of testosterone serum levels as unique finding in occult Sertoli-Leydig ovarian cell tumors

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Hirsutism affects 5–10% of women of reproductive age and may be the initial sign of an androgen disorder. We describe two cases of occult Sertoli-Leydig ovarian cell tumor suspected only on the basis of clinical and laboratory features.
The first patient, a 42 year-old woman came to our attention for hirsutism. Several blood samples showed a very high testosterone concentration (~4 ng/ml), while non-ovarian causes of hyperandrogenism were excluded. Abdominal CT scan and transvaginal US showed a bilateral ovarian hyperthecosis, confirmed by diagnostic laparoscopy with bilateral ovarian biopsy. However, signs of hyperandrogenism and testosterone levels were suggestive of a possible occult ovarian cancer. Therefore, the patient underwent bilateral ovariectomy, with histological diagnosis of a right 1.8 cm Sertoli-Leydig cell tumor. After surgery, laboratory data showed a drop in serum testosterone (<0.1 ng/ml). The patient is now on HRT with careful biochemical and clinical observation.
The second patient, a 54 year-old woman, was recently referred to us because of very high testosterone values (~10 ng/ml). An adrenal hyperandrogenism was excluded while abdominal CT scan showed only an uterine fibromatosis, without ovarian malignant features. In spite of this radiological finding, the patient underwent hysterectomy and bilateral ovariectomy, with histological diagnosis of a left 1.7 cm Sertoly-Leydig cell tumor. After surgery, there was a drop in serum testosterone levels. The patient is now in follow-up.
Sertoli-Leydig cell tumor represents less than 0.5% of ovarian tumors: the presence of testicular structures producing androgens can cause virilization. Surgical treatment is recommended for all patients, except in cases of metastatic disease.
We described two cases of severe hyperandrogenism caused by an androgen secreting tumor diagnosed only after bilateral ovariectomy. In fact, despite negative morphological data, in presence of severe clinical features and very high serum testosterone concentration, an occult malignancy should be suspected.

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Treatment of active acromegaly with the somatostatin analogue lanreotide SR

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Background and aims
The long-acting somatostatin analogs represent are nowadays the first-line medical treatment of acromegaly. To assess the efficacy and tolerability of lanreotide-SR (LSR) in the treatment of active acromegaly.
Subjects and methods
Eleven patients (2 men and 9 women; aged 27–75 years, median 47.4 years) were treated in whom active acromegaly with during disease from 1 to 34 years. All patients had the macroadenoma of hypophysis including six patients with relapse of adenoma. Patients were treated for a median period of 12 weeks with i.m. injections of lanreotide SR 30 mg given every 14 days. Blood samples for GH, IGF-1 measurements were taken in fasting state. Mean GH and IGF-I levels were measured at baseline and every 4-weeks together with symptom score assessment.
Results
Eleven patients were treated for at least 12-weeks and, in these, GH levels fell from 40.55 ± 25.69 ng/l (Me 33.17) at baseline to 17.52 ± 11.58 ng/l (Me 4.6) ($t = 2.71$; $P = 0.013$) and IGF-I levels from 563.87 ± 157.35 ng/l (Me 582.62) to 380.4 ± 202.74 ng/l (Me 418.0) ($t = 2.37$; $P = 0.02$). GH response to treatment was better in elderly patients (age 58.00 ± 1.58) compared to younger patients but neither sex, pre-treatment GH levels, previous surgery nor previous radiotherapy influenced the response. Treatment resulted in a significant improvement in the symptoms of active acromegaly in the majority of patients. Treatment was well-tolerated by the majority of patients, side effects were mainly transient gastrointestinal symptoms. There were minor effects on glucose tolerance which were not of clinical importance. There were minor effects on glucose tolerance which were not of clinical importance. During treatment LSR one patient give up insulinotherapy, indemnification of carbohydrate exchange was attained on a diet.
Conclusion
The treatment of acromegaly with lanreotide SR is effective in controlling GH and IGF-I levels and symptoms and is well tolerated in the majority of patients.

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Should we offer combination scan in all patients with parathyroid disease: a district general hospital experience

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Introduction
Tc 99m sestamibi scan and ultrasound are been used most frequently than other imaging technique in the pre operative localization of parathyroid gland in primary hyperparathyroidism. We liked to evaluate our hospital experience with the scan in preoperative localization of the glandular disease.
Method and material
It was a retrospective audit of 7 years from 2000–2007 of 48 patient who underwent open explorative procedure by single surgeon.The scans were compared with that of histology report and data analysed.
Result
Parathyriod adenoma was the most common pathology in our patient cohort (44/49–88%). Approximately half of the patients had combination scan (sestamibi and ultrasound) with high preoperative localization in 92% (24/26). Ultrasound and sestamibi scan done alone had low preoperative sensitivity in gland localization.
Conclusion
We can conclude that if we use the combination scan then there is high probability (93% from our study) of gland localization preoperatively. We should offer the combination scan routinely to all patients in preoperative work up our patients. This is more likely to localize gland early and help surgeon in discussing focussed parathyroidectomy as a day case surgery in a unigalndular parathyroid adenoma. It would widen the treatment option for the patients with uniglandular adenoma for short stay and day case surgery.

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Lanreotide effects on glucose metabolism in evolutive acromegaly in remission during chemotherapy

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Lanreotide has long been used in the therapy of GH secreting pituitary adenomas and other somatostatin receptor positive neuroendocrine tumors.
Aims
To determine the impact on glucose metabolism of the 6 months of lanreotide therapy, beside of the antisecretory and antiproliferative effects.
Patients and methods
Seven patients with active acromegaly treated with lanreotide, admitted in the Department of Neuroendocrinology, Institute of Endocrinology, Bucharest. They were evaluated by oral glucose tolerance test (OGTT) with serum glucose, GH (IRMA-sensitivity 0.02 ng/ml) and insulin (RIA-sensitivity 1 mU/ml), serum IGF1/upper limit for age and sex ratio, HOMA-IS and insulin sensitivity index during OGTT (ISI_{OGTT}) were calculated for insulin sensitivity measuring and computed tomography.
Results
Seven patients (3 males), aged 44 + 19 years were treated with lanreotide for 6 + 3 months after unsuccessful surgery and radiotherapy. Basal GH decreased to normal values (< 2.5 ng/ml) in 5/7 patients, most being after minimum 6 months after radiotherapy. In 3/7 patients, nadir of GH during OGTT decreased to < 1 ng/ml and IGF1/ upper limit for age and sex ratio became < 1. Tumor diameters did not change with > 25% in neither of patients. Before lanreotide treatment, 1 patient had secondary diabetes mellitus and 1 had impaired glucose tolerance. During chemotherapy, basal glycemia insignificantly decreased from 115.8 + 49.3 to 103.5 + 39.9 mg/dl and 120 min glycemia after glucose upload from 138.2 + 90.7 to 111.1 + 83.9 mg/dl. Indexes of insulin sensitivity increased statistically insignificant, but the patient with impaired glucose tolerance showed normal glucose tolerance after 6 months of lanreotide therapy. HOMA IS was 0.19 + 0.05 vs 0.47 + 0.031 mmol/l*mU/ml and ISI_{OGTT} was 2.2 + 0.7 vs 5.3 + 0.3 during treatment.
Conclusion
Six months of lanreotide, a long acting somatostatin analogs therapy showed antisecretory effect in 5/7 acromegalic patients, without altering glucose metabolism.

Bone/Calcium

P225

The study of bone turnover biochemical markers in delayed puberty

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Background
The delayed puberty is defined by absence of secondary sexual characters until the age of 16 or lack of puberty development until the limit of + 2DS in regard to the age when the puberty begin normal at considered population. After the major factor implied in delayed puberty ethiology, can be distinguished three mechanisms: hypothalamic, hypophyseal and gonadal. It is recognized the fact that the osteoporosis process is, in the first place, dependent and interdependent by the deficiency of one or of all sexual hormones, arised during the ontogenesis process.
Methods
Have been included in the study 26 cases with delayed puberty, with ages between 12 and 35 years old, where 14 cases (53.85%) with hypergonadotroph hypogonadism (female Turner syndrome – 10 cases; Klinefelter – 4 cases) and 12 cases (46.15%) with hypogonadotroph hypogonadism (hypophyseal dwarfism with sexual infantilism – 3 cases; functional adipose-genital syndrome – 7 cases; tumor-like hypophyseal insufficiency – 2 cases). Was evaluated the plasmatic level of the 2 markers of bone turnover (osteocalcine and CrossLap) trough ELISA method. The measuring of the bone mineral density was made by dual absorption with X-rays.
Results
Were identified trough DXA, 10 cases (38.46%) with osteoporosis, where the osteocalcine values (29.4–112.96 ng/ml) and CrossLap (0.197–1.768 ng/ml) were comparable with those of women in postmenopausal period, 6 cases (23.08%)

with osteopenia, and at 10 cases (38.46%). *T* score value and of biochemical markers were in normal limits.
Conclusions
The paperwork is suggesting two major objectives in therapeutically strategy of existent osteoporosis/osteopenia at delayed puberty cases: precocious diagnosis of gonadal insufficiency, in the purpose of some prophylaxis measures for bone modifications beginning from pre-pubertal, for insuring the stabilization or amounting of bone mass corresponding to sex and age; therapeutically solution associates estro-progestative/androgenic substitution with antiresorbtion or proformation medication.

P226

The new biology of pituitary natriuretic peptides: novel signalling from guanylyl cyclase-B (GC-B) receptors

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Backgound
ESRD is associated with reduce bone mineral density compared with aged-matched healthy population. DXA is the standard noninvasive method to assess BMD. It is expensive, needs special equipment, X ray exposure, movement of the patients. QUS is inexpensive, mobile, easy to perform, radiation free, recognize for screening abilities and risk fracture prediction in normal population. This study assessed the ability of QUS versus DXA in determine BMD in haemodialised population.
Materials and methods
Patients randomly selected from all patients active in the evidence of the Haemodialysis and Renal Transplantation Center form the County Hospital no.1, performed DXA (anteroposterior technique, Delphi W device, Hologic Inc.), and also QUS (Sahara device, Hologic Inc.). Correlation between DXA and QUS parameters were performed. Receiver operator characteristic curves (ROC) were plotted for BUA, SOS and QUI and used to define cut-off values for best sensitivities and specificities for all parameter. WHO *T* score diagnosis of osteoporosis and osteopenia were used. We also used the UK NOS strategy to define the interval of the best QUS diagnostic parameter, to identify with 90% sensitivity and 90% specificity different degrees of bone demineralization.
Results
One hundred and thirty-one patients (63 females and 68 males), mean age 47 776$\pm$12, 32 years, being in haemodialysis for a mean period of mean 51 488$\pm$4686 months. BU A (r=0.613/0.447) and QUI (r=0.613/0.502) seem to be the parameters of choice when considering BMD at cortical level. Areas under ROC for BUA and SOS in diagnosis of osteoporosis and osteopenia, have a sensibility of 76, 1%-76, 1%, respectively a specificity of 72, 5%-77, 8%. The values for osteoporosis are even better, for 77% and 84%. The identified cutoff levels for QUI are 76.1 (osteopenia) and 69.6 (osteoporosis). The diagnostic value of QUS)when reporting QUI=are even higher when we did define the proper interval.
Conclusion
DXA and QUS parameters correlate significantly. The best QUS diagnostic parameter is QUI. The high negative predictive value of different cut-off point suggests a very good screening power of QUI in identifying cases without bone demineralization. Cut-off values for QUI associated a high sensitivity (between 60 and 80%) respectively specificity of over 75% in diagnosis osteopenia and or osteoporosis. Using the 90–90 approach, we identify the precise interval for QUI values that allows the best diagnostic of bone demineralization.

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Experience with cinacalcet in primary hyperparathyroidism: results from the Swiss primary hyperparathyroidism cohort study

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Objective
Cinacalcet, a calcimimetic that reduces parathyroid hormone (PTH) secretion and serum calcium (S-Ca) levels by increasing the sensitivity of calcium-sensing receptors has been introduced for the treatment of patients with persistent or recurrent primary hyperparathyroidism (PHPT). Within a prospective, non-interventional cohort study we identified patients with newly diagnosed PHPT who have been started on cinacalcet. Patient characteristics, treatment indications and biochemical follow-up are presented.
Methods
The Swiss Primary Hyperparathyroidism Cohort Study is an ongoing prospective project initiated in June 2007. Clinical, biochemical and densitometric data are recorded systematically at least every 6 months according to NIH guidelines. Currently 110 patients with PHPT (74% female) have been included. Thirteen patients with PHPT (12%) have been started on cinacalcet treatment.
Results
As compared to the entire cohort of patients with PHPT, patients starting cinacalcet were younger (57.7$\pm$17.1 vs 70.0$\pm$14.4 years, P=0.02) and had higher S-Ca levels (3.19$\pm$0.61 vs 2.74$\pm$0.30 mmol/l, P<0.001). Serum iPTH levels were comparable (19.0$\pm$10.6 vs 16.9$\pm$13.4 pmol/l, P=0.28). Reasons for starting cinacalcet were progressive (n=7) or symptomatic (n=3) hypercalcemia, patient refusal to parathyroidectomy (PTX, n=2), or recurrent PHPT after unsuccessful PTX (n=1). Median daily cinacalcet dose was 60 mg (range, 30–420 mg). During cinacalcet therapy S-Ca levels decreased from 3.19 to 2.55 mmol/l (P=0.008); normocalcemia (S-Ca <2.60 mmol/l) was achieved in 55% of patients. The treatment was well tolerated. All patients had at least partial relief of hypercalcemia-related symptoms (depression, fatigue, musculo-skeletal symptoms).
Conclusion
Our preliminary results show that cinacalcet use results in biochemical and clinical improvement in patients with PHPT and therefore may have the potential as a non-surgical alternative in patients with recurrent disease or in case of surgical contraindications. Furthermore, cinacalcet may be warranted in the preoperative management to test reversibility in patients with symptomatic hypercalcemia.

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Use of Cinacalcet in patients with intractable primary hyperparathyroidism (PHPT): a UK budget impact analysis

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Introduction
Persistent or intractable PHPT can lead to neuromuscular and psychiatric symptoms and may increase the long-term risk for cardiovascular disease and fractures. Cinacalcet (Mimpara/Sensipar) represents an innovative treatment option for this small group of patients, as it directly acts on the calcium sensing receptors on cell surfaces, reducing the severity of hypercalcemia.
Purpose
To estimate the UK budget impact of cinacalcet in patients with intractable PHPT.
Methods
A 5-year budget impact model was developed in Microsoft Excel. Total treatment costs were estimated for two types of patient populations with intractable PHPT: failed PTX surgery and contraindicated for PTX surgery. Total treatment costs incurred in the first year were used for incident cases, costs in subsequent years (average of 9 years) were used for prevalent cases. Cost-offsets were modelled for short- and long-term consequences: Short-term (ST) costs included re-operation, pharmaceuticals, physician visits and lab tests/procedures. Long-term (LT) costs included the cost of treating hypertension, fractures, anxiety, and CVD events. The ability of cinacalcet to induce cost offsets was based on the efficacy of cinacalcet as estimated in clinical trials.
Results
Using epidemiological information, there are ~755 intractable PHPT patients in the UK. The budget impact models shows, assuming a 3 to 15% market share in years 1 to 5 in both populations, increased total costs associated with the launch of cinacalcet from 7% in year 1 to 34% in year 5 compared to not using cinacalcet or £119 000 additional costs in year 1 to £602 000 in year 5.
Conclusion
The overall budget impact of cinacalcet is limited due to the restricted population affected with this serious form of PHPT, the conservative market share estimates and the cost-offsets from LT events.

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Swiss primary hyperparathyroidism cohort study

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Objective
The Swiss Primary HyperParaThyroidism (PHPT) Cohort Study is an ongoing, prospective, non-interventional project collecting clinical, densitometric, biochemical and outcome data in patients with PHPT. The aims are to describe the profile of these patients particularly neurobehavioral and cognitive symptoms, changes in calcium and PTH over time and treatment modalities.
Methods
Patients newly diagnosed with PHPT and with high serum calcium levels were enrolled. Physicians recorded data on a web-based system (https://www.phpt-registry.ch). Follow-up data were recorded at least every 6 months according to NIH guidelines. If a parathyroidectomy (PTX) was decided, a final visit took place within 6 months after surgery. Changes in neuropsychological functioning are evaluated yearly and within 3 to 6 months after PTX.
Results
From June 2007 to September 2008, 99 patients (mean age 69.3±14.6 years; 73% female) have been included. Median preoperative calcium and PTH levels were 2.72 mmol/l (Inter-Quartile Range: 2.61–2.86 mmol/l, normal range: 2.20–2.60 mmol/l) and 13.8 pmol/l (IQR: 9.2–17.6 pmol/l, *N*: 1.1–6.8 pmol/l), respectively. Densitometric osteoporosis was documented in 27 cases (out of 66 measurements). Forty eight patients presented with a classic symptomatic form of PHPT (history of renal lithiasis, low-trauma fractures, or muscle weakness). Twelve asymptomatic patients were considered as candidates for surgery, having at least one of the 2002 NIH criteria. Of the 39 patients who were not candidates for surgery (with no NIH criteria), 10 had nonspecific symptoms and 29 were truly asymptomatic. Twelve patients were started on cinacalcet, including one post-operatively.
Conclusion
Our preliminary results show that although many patients undergoing parathyroidectomy have a paucisymptomatic profile of PHPT, the classic form remains frequent. Sixty patients were symptomatic or fulfilled at least one criterion of the NIH guidelines, and were referred for surgery. Among the medical options, cinacalcet was prescribed in 12% of patients.

P230

Bone mineral density among patients with primary hyperparathyroidism with or without diabetes

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Introduction
The relationship between diabetes mellitus (DM) and osteoporosis is controversial. Both Type 1 DM and Type 2 DM have been associated with higher risk of fractures, whereas BMD is increased in Type 2 and decreased in Type 1 DM. In primary hyperparathyroidism (PHPT) a higher prevalence of Type 2 DM has been found but the influence of DM on BMD is unknown.
Subjects and methods
In a consecutive series of 262 patients with PHPT (age, mean±SD, 59.1±13.6 years; BMI: 25.5±5.2 kg/m^2; PTH: 200.9±164.8 pg/ml; Ca:11.1±1.1 mg/dl) we measured BMD by DXA at forearm, lumbar spine and femur as well as biochemical parameters of the illness and HbA1C levels. We compared data of patients with DM (DM+, $n=29$, F/M 23/6; age: 64.7±11.5 years; BMI: 28.7±6.3 kg/m^2) with those without DM (DM−, $n=233$; F/M 172/61; age: 58.4±13 years; BMI: 25.1±4.9 kg/m^2).
Results
DM+ were older ($P<0.01$) and had higher BMI ($P<0.0008$) than DM−, while gender ratio, PTH, Ca, creatinine, 25OHD3 and calciuria were not different. In DM+, BMD at femur was higher than in DM− ($P<0.02$) even after adjustment for age and BMI, while BMD at forearm and lumbar spine were similar. In DM+, HbA1C was negatively associated with BMD at lumbar spine ($r=-0.615$; $P<0.03$) and this association was confirmed in a multivariate analysis including age and PTH (beta −0.632; $P<0.03$).
Conclusions
Our data indicate that in PHPT, as reported in the general population of Type 2 DM, the presence of diabetes is associated with increased BMD at femur and thus with a reduced fracture risk; on the other hand, a poor glycemic control seems to influence negatively BMD at lumbar spine, indicating a peculiar sensitivity of trabecular bone to this metabolic impairment.

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Bone and mineral metabolism before and after kidney–pancreas transplantation in patients with type 1 diabetes

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Aims
End stage renal disease is associated with disorders of calcium and phosphate metabolism that favor the loss of bone mass.
Kidney transplant may alter this unbalance restoring bone mass. Nevertheless, recent studies showed that 48 months after transplant, the loss of bone mass still is superior to general population. Post-transplant corticosteroid therapy is considered the main responsible for the loss of bone mass.
The authors present a 5 year retrospective analysis of markers of bone metabolism after kidney–pancreas transplant.
Methods
The study included 40 transplant recipients (25 women; 15 men), age between 20 and 47 years (33.6±6.3 years), that have completed 5 years of follow-up.
We analyzed the following markers of bone metabolism until the 5th year post-transplant:
–Lumbar spine and hip Bone mineral density (BMD) determined by DXA;
–Plasma levels of calcium, phosphorus, parathyroid hormone (PTH), vitamin D, bone-specific alkaline phosphatase, osteocalcin and Beta Cross Laps
Results
Five years post-transplant, lumbar spine and femoral BMD (T score) increased 42.2% and 15.9% respectively.
Plasma levels of calcium, phosphorus and PTH decreased 5.4%, 25% e 66.8%, respectively.
Plasma levels of bone-specific alkaline phosphase, osteocalcin and beta cross laps decreased 63%, 65.8% e 93% respectively.
Vitamin D levels increased 86.7%.
Conclusions
In our population of transplant recipients, there was an increase in BMD at the 5th year post-transplant, bone turnover decreased favoring bone formation.

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Clinical and molecular characterization of Spanish patients with pseudohypoparathyroidism

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Pseudohypoparathyroidism (PHP) is a term applied to a heterogeneous group of disorders whose common feature is resistance to parathyroid hormone. Most of the PHP forms are caused by defects in *GNAS*: PHP-Ia (characterized by PTH and TSH resistance with Albright Hereditary Osteodystrophy) is caused by heterozygous inactivating mutations in those exons of *GNAS* encoding the α subunit of the stimulatory G-protein, and the autosomal dominant form of PHP-Ib (PTH and TSH resistance without phenotypic manifestations) is caused by alteration in the methylation pattern of the locus, usually associated with microdeletions at STX16 gene that are maternally transmitted.
Aim
To analyze the complete GNAS locus, including deletions at *STX16*, in order to investigate the underlying molecular mechanisms involved in the etiology of pseudohypoparathyroidism.
Methods
$G_{s\alpha}$ activity, *GNAS* mutation and haplotype, and *GNAS* methylation analyses were performed for the probands and family members.
Results
The genetic and epigenetic study of 60 PHP patients revealed 25 point mutations (all associated with PHP-Ia), two paternal 20qUPD (one PHP-Ia and one PHP-Ib)

and 23 loss of imprinting at GNAS locus (nearly half of them associated with PHP-Ia), only 5 associated to previously described STX16 deletions. A 2q37 deletion was also identified.
Very preliminary studies on genotype–phenotype correlations showed that patients with epigenetic alterations are diagnosed latter than those with genetic mutations.
Conclusion
There seems to be an overlap between the molecular and clinical features of PHP-Ia and PHP-Ib as molecular alterations previously associated with PHP-Ib are also present in patients diagnosed as PHP-Ia.

P233

Is parathyroid function abnormal in active acromegaly?

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Introduction
Acromegaly is associated with skeletal changes that are characterized by appositional bone growth, increased bone dimensions and increased bone turnover. PTH is an important regulator of bone remodeling and its anabolic action requires the presence of GH. The strong correlation found between PTH concentrations and bone turnover markers in active and treated acromegaly has led to the suggestion, that the effect of GH on bone turnover may be mediated by PTH. Successful treatment of acromegaly results in a reduction of bone turnover markers but previous reports on the effect of PTH concentrations have been inconsistent. The aim of the present study was to evaluate parathyroid function in patients with acromegaly after surgical treatment.
Patients and methods
We studied 47 acromegalic patients (27 females and 20 males) aged $62+8$ who were treated with transphenoidal surgery. Serum concentrations of IGF-1, iPTH, calcium, phosphorus, alkaline phosphatase, creatinine and albumin as well as 24 h urinary calcium and creatinine were measured. Patients were divided in 3 groups according to GH levels after an oral glucose tolerance test with 100 g glucose: group A 18 patients (11F-7M) with GH <1 ng/ml, group B 17 patients (10F-7M) with GH between 2 and 10 ng/ml and group C 12 patients (6F-6M)with GH >10 ng/ml.
One way analysis of variance (ANOVA) was used for comparisons between the three groups and correlations were sought using Pearsons correlation coefficient.
Results
As expected, GH and IGF-1 levels were significantly different between the three groups of patients ($P<0.001$). iPTH levels did not differ between groups and no correlation was found with any of the measured variables. Serum calcium, phosphorus and alkaline phosphatase levels were not different between groups while 24 h urinary calcium was significantly higher in group B compared to patients in group A (250.2 ± 22.4 vs 184.4 ± 21.4, $P<0.04$). In all patients, a positive correlation was found between serum calcium with GH and IGF-1 levels ($r=0.33$, $P<0.05$ and $r=0.35$, $P<0.05$ respectively) and alkaline phosphatase with IGF-1 levels ($r=0.4$, $P<0.02$).
Conclusions
In active acromegaly, parathyroid function as expressed by iPTH levels does not seem to be influenced while the augmented levels of GH and IGF-1, have a positive effect on bone metabolism via the increased calcium intestine absorption on one hand and the increased osteoblastic activity on the other.

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The circulating concentration of adiponectin in post-menopausal women with and without osteoporosis and its association with body mass index and biochemical markers of bone metabolism

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Introduction
There is increasing evidence suggesting that adiponectin plays a role in the regulation of bone metabolism. We have studied the changes in the circulating concentration of adiponectin in lean and obese post-menopausal women with and without osteoporosis and its association with a marker of bone formation – type 1 procollagen amino-terminal pro-peptide (P1NP) and a marker of bone resorption – type 1 collagen C-telopeptide (βCTX).
Methods
Venous blood samples were obtained from 37 non-osteoporotic and 34 osteoporotic post-menopausal women who had been fasted. All subjects had bone mineral density (BMD) measured as part of an osteoporosis screening program. Total- and high molecular weight (HMW) adiponectin and osteoprotegerin (OPG) were measured by ELISAs; βCTX and P1NP by ECLIA. The relationship between total and HMW-adiponectin, body mass index (BMI), BMD, OPG, βCTX and P1NP was investigated.
Results
We observed a positive correlation between BMI and BMD ($r=0.44$, $P<0.001$). There was no difference in the circulating adiponectin concentration in those with or without osteoporosis. However, when stratified for BMI the concentration was lower in obese compared to lean subjects but a statistically significant difference was only observed in the HMW/Total adiponectin ratio with lean patients without osteoporosis having a higher ratio when compared to their obese counterparts ($P<0.05$). There were significant negative correlations between HMW-adiponectin and HMW/Total adiponectin ratio with BMI ($r=-0.25$, $P=0.040$ and $r=-0.27$, $P=0.030$ respectively) and between HWM/Total adiponectin ratio with OPG ($r=-0.44$, $P<0.001$).
Conclusions
Our data suggest that there is no significant difference in the circulating concentration of fasting early morning adiponectin in post-menopausal women with or without osteoporosis. The correlation between HMW/Total adiponectin ratio and OPG may indicate that adiponectin could influence bone metabolism by altering osteoblast production of OPG thereby affecting osteoclast mediated bone resorption.

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Glucose tolerance, insulin secretion and insulin sensitivity before and after radical treatment of primary hyperparathyroidism

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It was previously shown that patients with primary hyperparathyroidism (PHPT) are insulin resistant.
Aim
The aim of our study was to evaluate the effect of surgical treatment on glucose tolerance, insulin secretion and sensitivity (SI) in patients with PHPT.
Material and methods
In 26 patients with PHPT (age: 57.15 ± 9.54 years, BMI 26.00 ± 4.55 kg/m^2, PTH 276.61 ± 64.83 ng/l, Calcium 2.95 ± 0.19 mmol/l)) AIR and SI were determinated before and 4 months after surgical treatment. Insulin sensitivity was evaluated using euglycemic hyperinsulinemic clamp (M index), while acute insulin response (AIR) was calculated as the mean increment above basal of insulin values measured at 2, 3, 4, 5, 6, 8 and 10 min after intravenous glucose bolus (IVGTT). Glucose and insulin response during OGTT were evaluated as area under the curve (AUC). AUCs was calculated using Trapezoidal rule. Paired *t*-test and Wilcoxon test were used for statistical analysis, as well Pearson correlation test. Statistical analysis.
Results
After operation PTH (51.47 ± 8.57 ng/l) and serum calcium (2.33 ± 0.12 mmol/l) were normalized. There was significant improvement in insulin sensitivity (M index: 3.91 ± 2.01 vs 6.08 ± 4.88, $P<0.05$), while there was no significant difference in AIR (44.77 ± 6.71 vs 35.14 ± 9.77, $P>0.05$), AUC GLUCOSE 855.88 ± 188.37 vs 823.84 ± 139.39, $P>0.05$) and AUC INSULIN (6270.27 ± 3870.05 vs 6351.55 ± 3820.44, $P>0.05$) after surgical treatment. There was no change in BMI after operation (26.00 ± 4.55 vs 26.36 ± 4.31, $P>0.05$). There was no correlation between PTH and M index ($r=-0.169$, $P>0.05$), AIR ($r=0.160$, $P<0.05$), AUC GLUCOSE ($r=0.231$, $P>0.05$) and AUC INSULIN ($r=-0.110$, $P>0.05$) as well as between serum calcium levels and M index ($r=0.214$, $P>0.05$), AIR ($r=-0.167$, $P>0.05$), AUC GLUCOSE ($r=0.298$, $P>0.05$) and AUC INSULIN ($r=-0.009$, $P>0.05$).
Conclusion
Radical treatment improves SI in patients with PHPT. Non-significance of changes in AIR, AUC GLUCOSE and AUC INSULIN might be due to fact that testing was performed relatively short period after surgical treatment.

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Bone mineral density and bone turnover markers in patients with schizophrenia treated with atypical antipsychotics

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According to the novel concept bone remodeling is centrally regulated (CNS). Leptin the adipocyte derived hormone acts on hypothalamus and by increasing sympathetic activity acts on osteoblasts to regulate bone formation. In patients with schizophrenia several factors have influence on bone metabolism. Schizophrenia *per se*, as a disease of central nervous system is associated with increased occurence of osteoporosis. Hyperprolactinemia as consequence of antipsychotic treatment in these patients has impact on both bone mineral density (BMD) and bone metabolism by increasing bone resorption. On the other side, weight gain which is the commonly observed on antipsychotic therapy may be protective factor against osteoporosis.

Aim

The aim of our study was to investigate the effects of BMI and insulin on bone mineral density and markers of bone metabolism in 23 patients (12 males, mean age 32.2 ± 1.3 years, BMI 29.2 ± 1.0 kg/m^2) with schizophrenia treated with atypical anypsychotic – depo risperidone 100 mg monthly, during 1.4 ± 0.3 years. The control group included healthy 35 individuals sex, age and BMI matched (11 males, mean age 32.2 ± 1.4 years, 28.0 ± 1.2 kg/m^2). After fasting in the morning serum leptin, prolactin-PRL, osteocalcin-OCL, ßcross laps-BCL, IGF 1, PTH and 25-OH-vitamin D levels were measured. Both groups were tested by oral glucose load (OGTT) with measuring insulin levels. In all patients DEXA (Hologic) was performed to measure bone mineral density-BMD. We used for statistical measurements General Linear Model for repeated measures. *P* values below 0.05 were regarded as significant.

Results

We did not find a statistical difference in serum levels of OCL, BCL, leptin, PTH and IGF1 between two groups ($P > 0.05$). Vitamin D levels were lower ($P < 0.05$) and prolactin levels were significantly increased in patients with schizophrenia ($P < 0.05$). We found significant positive correlation between BMI and BMD in healthy control ($P = 0.051$). BMI had positive correlation with *Z* score of the spine in controls ($P = 0.027$). Correlation between insulin levels during OGTT (AUC) and parameters of BMD and *Z* score of spine in control ($P < 0.0001$; $P = 0.001$, respectively) was confirmed. On the other side in patients with schizophrenia no significant correlation between BMI and insulin levels (AUC) with BMD and *Z* score were found.

Conclusion

BMI and insulin levels did not affect bone mineral density and bone metabolism in patients with schizophrenia which is different from healthy control. Central control of bone remodeling might be disturbed in schizophrenia.

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Thin healthy women have a similar low bone mass as women with anorexia nervosa

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Association between anorexia nerviosa (AN) and low bone mass has been demonstrated. Bone loss associated with AN involve hormonal and nutritional impairments, though their exact contribution is not clearly established. We compare bone mass in AN patients with women of similar weight with no criteria for anorexia nervosa, and a third group of healthy, normal-weight, age-matched women. The study included 48 patients with AN (DSM-IV criteria), 22 healthy eumenorrheic women with low weight (LW Group; BMI < 18.5 kg/m^2) and 20 healthy women with BMI > 18.5 kg/m^2 (Control Group), all of similar age. We measured by DEXA lean body mass, percentage of fat mass, total bone mineral content (t BMC) and bone mineral density in lumbar spine (BMD LS) and total (BMD T). We measured anthropometric parameters, leptin and GH. The control group had greater BMD T and BMD LS than the other groups, with no differences between the AN and LW groups. No differences were found in BMD T, BMD LS and t BMC between the restrictive ($n = 25$) and binge-purge type ($n = 23$) in AN patients. In AN, minimum weight ($P = 0.002$) and percent of fat mass ($P = 0.02$) explained BMD LS variation (r^2: 0.48) and minimum weight (r^2: 0.42; $P = 0.002$) for BMD T in stepwise regression analyses. In LW group, BMI explained BMD LS (r^2: 0.72; $P = 0.01$) and BMD T (r^2: 0.57; $P = 0.04$). We concluded that patients with AN had similar BMD as healthy thin women. Anthropometric parameters could contribute more significantly than estrogen deficiency in achievement of peak bone mass in AN patients.

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Evaluation of the association between bone turnover markers and OPG/sRANK-L levels in relation with the changes of thyroid function in women with thyroid cancer

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Aim

Thyroid hormones play an important role in bone remodeling. The aim of the study was to evaluate the changes of bone markers and osteoprotegerin (OPG)/sRANK-L levels during follow-up in a group of patients with thyroid cancer.

Material and methods

Twenty euthyroid women with the diagnosis of differentiated thyroid cancer were enrolled to the study (39.25 ± 13.46 years, 6 postmenopausal) before thyroidectomy. Samples were collected before the operation (euthyroid status) (EU), before radioactive iodine administration (hypothyroidism) (HYPO) and under thyroxine supressive therapy (subclinical hyperthyroidism) (SHYPER). In addition to OPG and sRANK-L, the markers of bone formation (BALP, osteocalcin and P1NP) and resorption (urinary DPD) were also evaluated. To determine the independent effect of bone formation markers and TSH on OPG, a multiple logistic regression model was used after pooling all the 3 visits' data. Statistical analysis were performed on SPSS 15.0.

Results

The level of all bone formation markers except urinary DPD decreased in HYPO when compared to EU period ($P = 0.047$ for BALP, $P = 0.003$ for osteocalcin, $P = 0.024$ for P1NP). However no significant change was observed in urinary DPD. In SHYPER period all of the bone formation markers increased and reached to the levels of EU period. OPG levels increased in HYPO period ($P < 0.001$) and returned to comparable levels in SHYPER period. RANK-L levels did not change during the 3 different periods of the study. In multiple regression analysis, the only significant variable was TSH ($r^2 = 0.446$, $P = 0.001$).

Conclusion

In this study, we tried to investigate the relationship of thyroid dysfunction and skeletal system. The results indicate that there is a decrease in bone formation and an increase in OPG levels in HYPO but no change in SHYPER period. The increase in OPG level was related to increase in TSH but not to any of the bone formation markers.

P239

The results of cinacalcet therapy in patients with severe or refractory hypercalcemia due to primary hyperparathyroidism

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Refractory or severe hypercalcemia is important clinical problem as it can lead to serious complications such as arrhythmias, acute or chronic pancreatitis, gastric ulcer, water and electrolyte balance disturbances, osteoporosis, psychoses and even to hypercalcemic crisis. Most often it is diagnosed in parathyroid cancer (PC). It is also observed in benign primary hyperparathyroidism (HPTH) in case of difficulties with adenoma's localization. Routinely treatment includes forced diuresis and/or bisphosphonates. Calcimimetics are a new group of drugs which increase the sensitivity of the calcium sensing receptor (CaR) to extracellular calcium and due to that reduce serum calcium and PTH level.

Aim

The aim of the study was to assess the efficacy of cinacalcet in the treatment of refractory hypercalcemia due to primary hyperparathyroidism.

Material and methods

Seven patients (6 women and 1 man, mean age 49 years) have been treated with cinacalcet for 3–117 weeks (median 31 weeks). In 1 patient PC, whereas in 6 of them benign HPTH were stated. Two of them were operated before cinacalcet therapy, In 3 patients the treatment was carried out to prepare them for surgery.

Applied doses of cinacalcet ranges from 30 to 180 mg. In all patients forced diuresis and/or bisphosphonates were given before and during treatment.
Results
All patients responded to cinacalcet therapy. Serum total and Ca++ levels decreased significantly ($P<0.05$) whereas serum PTH level did not differ before and during the treatment. Only in one calcemia was normalized. Mean serum PTH, total and Ca++ before therapy were 744 pg/ml, 2.75 mmol/l, 1.47 mmol/l and during the treatment respectively 780 pg/ml, 2.55 mmol/l, 1.37 mmol/l. Usually the medication was well-tolerated. Most common adverse events were nausea and vomiting, especially at the beginning of therapy.
Conclusion
Cinacalcet is an effective, safe and well-tolerated treatment of patients with severe or refractory hypercalcemia due to primary hyperparathyroidism.

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The effect of short term human chorionic gonadotropin treatment on the improvement of bone mass in patients with hypogonadotropic hypogonadism

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Background
Idiopatic hypogonadotropic hypogonadism is a congenital abnormality due to GnRH deficiency which is associated with severe osteoporosis. Testosterone replacement has been shown to improve the loss in the bone mass. The aim of the present retrospective analysis was to measure the effect of human chorionic gonadotropin treatment on the improvement of bone mineral density.
Methods
A total number of 96 young male (mean age 21 ± 3.7 years) patients with hypogonadotropic hypogonadism who were not currently under any drug treatment were enrolled. The baseline bone mineral densities were measured from the lumbal region and femur neck by using dual X-ray absorbtiometry (DEXA). All patients were treated with human chorionic gonadotropin 1500 Units 3 times/week for 6 to 9 months.
Results
The patients had severe osteoporosis according to the measurements from different regions (L1–4 BMD: 0.742 g/cm^2, L1–4 *Z*-score: -3.04; Femur Neck BMD: 0.809 g/cm^2, *Z*-score: -0.81; Distal Radius BMD:0.622 g/cm^2, *Z*-score: -3.38). After the treatment period (7.4 ± 2.1 months), significant improvements were observed the lumbal regions and femur neck ($P<0.001$ for both), but no significant short term effect was seen in the distal radius.
Discussion
The results of the present study show that short term human chorionic gonadotropin improves bone mineral density in the lumbal vertebrae and the femur neck but has no significant effect on distal radius. Further studies in different regions with longer periods are warranted to assess the differential effect of gonadotrophins on the bone mineral density.

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Role of vitamin D replacement on serum FGF-23 levels in patients with osteomalacia due to vitamin D deficiency

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Introduction
Fibroblast growth factor (FGF)-23 is a peptid, released from bone tissue and osteogenic cells. Phosphatonins regulate phosphate homeostasis in some phosphate wasting disorders such as X-linked hypophosphatemia, otozomal dominant hypophosphatemic rickets, and tumor-induced osteomalacia.
FGF-23 is a possible phosphatonin: it regulates phosphate homeostasis by PTH independent actions.
Objective
In the present study, we aimed to determine the effect of serum FGF-23 levels before and after D Vitamin replacement therapy in Vitamin D deficient patients.
Materials and methods
Eighteen premenouposal female patients who had diagnosed as osteomalacia due to Vitamin D depletion were included to the study. Blood samples were collected before (Group 1: mean age 29.1 ± 9.9 years) and 6 weeks after a standard treatment protocol (Group 2) (oral Vitamin D 150 000 IU once for all and subsequently 880 IU D3+1000 mg calcium carbonate for 6 weeks). Nineteen healthy premenouposal women who had normal levels of serum 25 OH D_3 Vitamin (>30 ng/ml) composed control group (Group 3; mean age 28.5 ± 5.2 years). Serum levels of kreatinin, calcium, albumin, phosphate, alkaline phosphatase (ALP), intact parathyroid hormone (iPTH), bone alkaline phosphatase (BALP), 25(OH) D Vitamin, $1,25(OH)_2$ D_3 Vitamin, FGF-23 and 24 h urine calcium, phosphorus, kreatinin were measured. The study was approved by local Ethical Committee and informed consent was obtained from each subject.
Results
After Vitamin D+calcium replacement, serum corrected calcium levels (9.0 ± 0.3 vs 9.3 ± 0.3 mg/dl; $P<0.001$), $1,25(OH)_2$ D_3 Vitamin (41.6 ± 15.2 vs 94.0 ± 31.5 pmol/l; $P<0.01$), urine calcium excretion (145 ± 110.8 vs 297 ± 138.4 mg/day; $P<0.01$) were significantly increased. Serum BALP (27.8 ± 29.6 vs 18.6 ± 20.1 mg/l; $P<0.05$), iPTH (78.5 ± 51.6 vs 44.8 ± 15.1 pg/ml; $P<0.01$) and FGF-23 (33.6 ± 43.0 vs 15.6 ± 12.5 RU/ml; $P<0.05$). levels decreased significantly. In group 1, FGF-23 had positive correlation with serum calcium levels ($P<0.05$, $r=0.30$).
Conclusion
FGF 23 levels decrease while $1,25(OH)_2$ D_3 Vitamin increase durıng Vıtamın D replacement. The present study was supported by the Research Fund of Istanbul University (Project No: 573).

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Peculiar features of bone disease in thalassemia: comparison with anorexia nervosa

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In thalassemic patients individual values of BMD measured by traditional DEXA are lower than those determined by QCT. The reason for this discrepancy is still controversial.
Aim
To investigate bone features in a large group of thalassemic patients, compared with patients with anorexia nervosa, also characterized by precocious osteoporosis.
Study design
Forty-six adult thalassemic subjects and 25 anorectic women were studied. In all patients lumbar BMD was determined by DEXA and standard QCT. In a subset of 22 thalassemic and 13 anorectic patients, a modified QCT was also performed: this technique allows to include the measurement of the cortical component of the vertebra, at variance with standard QCT which measures volumetric BMD in a limited trabecular portion.
Results
In the whole group of thalassemic patients the mean lumbar *Z*-score measured by QCT was significantly higher than that measured by DEXA. On the contrary, in anorectic women the mean *Z*-score values measured by the two techniques were not significantly different. While in thalassemic patients the correlation between QCT and DEXA values was weakly positive, in anorectic women the same correlation was highly significant. Interestingly, when considering the BMD values determined by the modified QCT, these correlations were highly significant in both groups.
Conclusions
(a) Our data point to the peculiarity of bone disease in thalassemia in comparison with other forms of juvenile osteoporosis. (b) In thalassemic patients the degree of lumbar osteopenia appears to be more severe when estimated by DEXA compared to standard QCT; however, the correlation between the two techniques improves when including the whole vertebra using QCT measurement. (c) The discrepancy between the two methods might be accounted for by a greater involvement of cortical bone in thalassemia. (d) Standard QCT seems to underestimate the degree of bone damage in this haematological condition.

P243

The influence of other hormonal disturbances on the bone density and turnover in women with hyperprolactinemia of various origin

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Hyperprolactinemia may lead to bone loss, both due to hypogonadism and other hormonal disturbances.
Aim
Aim of the study was the analysis of influences of hormonal profiles associated with hyperprolactinemia on bone mineral density (BMD) and bone turnover in women with hyperprolactinemia of various origin. The subjects were 32 patients with prolactinoma, 43 ones with functional hyperprolactinemia and 29 healthy controls. All of them were studied BMD (lumbar spine, proximal femur, forearm, total body) using DXA; bone turnover markers (BAC, OC, ICTP) and hormones levels (prolactin, estradiol, LH, FSH, SHBG, testosterone, DHEA-S and i-PTH) using Spearman's correlation analysis and multiple regression analysis model. Correlations revealed the anabolic influence of PTH on lumbar spine in women with prolactinoma, and on ultradistal radius in functional hyperprolactinemia. DHEA-S correlated positively, and SHBG negatively with ICTP in prolactinoma patients. In multiple regression analysis, estradiol had greatest influence on lumbar spine and total body BMD. Moreover, positive influence of testosterone, SHBG on spine BMD, and of estradiol, testosterone, SHBG and DHEA-S on total body BMD were observed in patients with prolactinoma. LH had positive, FSH and estradiol negative influences on BAP, LH had positive and estradiol, testosterone and FSH negative influences on OC in patients with prolactinoma.
Conclusion
Hormonal disturbances associated with hyperprolactinemia influence both bone mineral density and bone turnover more in patients with prolactinoma than these with functional hyperprolactinemia.

P244

Effect of one year treatment with strontium ranelate on bone mineral density in women with established osteoporosis previously treated with teriparatide

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Teriparatide (TPTD – recombinant human parathyroid hormone 1–34) markedly increases bone mineral density (BMD) and reduces fracture risk. Sequential treatment with an antiresorptive agent is believed to preserve or further increase BMD. Strontium ranelate (SR) is thought to uncouple bone remodeling resulting in increased BMD and reduced fracture risk. In this prospective study, we aimed to evaluate the effect of SR on BMD in women with established osteoporosis previously treated with TPTD. Nineteen postmenopausal Caucasian women (aged 65.9 ± 1.8 years) with established osteoporosis previously treated with TPTD, 20 μg daily for 18 months, sequentially received SR 2 g daily for 12 months. Lumbar spine BMD was measured by dual-energy X-ray absorptiometry (DXA) pre- and post-TPTD administration, as well as twelve months post-SR administration. Blood samples for bone-specific alkaline phosphatase (BSAP) and C-terminal telopeptide of type 1 collagen (CTx) were obtained at the same time points. Lumbar spine BMD increased significantly after 18 months of TPTD ($P<0.001$) and further improved with sequential SR treatment ($P=0.033$). Serum BSAP and CTx increased significantly with TPTD ($P=0.008$ and 0.017, respectively) and reduced to baseline levels after SR treatment ($P=0.031$ and 0.019, respectively). The change in BSAP was positively correlated with the change in CTx during both TPTD ($r=0.641$, $P=0.007$) and SR treatment ($r=0.539$, $P=0.026$). In conclusion, our data suggest that SR following TPTD administration further increases BMD and could be used as an alternative to bisphosphonates' sequential treatment.

P245

Body composition and bone mineral density in hemodialysis patients

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Aim/hypothesis
Chronic renal failure maintenance hemodialysis (HD) patients are at risk for low bone mineral density (BMD) and fractures. Parathyroid hormone (iPTH) plays a pivotal role in the pathophysiology of uremic bone disease, but in healthy population body weight and particularly body composition are more important determinants of bone mass.
Patients and methods
We studied BMD (results were recorded as g/cm^2) and T score using DEXA osteometer (DTX-200 Osteometer) on forearm in 42 patients (19 females, 15 post-menopausal and 23 males), who had been on HD for a mean of 42.3 ± 23.2 months. Body composition was evaluated by bioelectrical impedance analysis and quantifies body fat, lean body mass and total body water ($FAT_{\%}$; $LBM_{\%}$; $TBW_{\%}$) using a TBF-110 Body Fat Analyser (Tanita).
Results
The prevalence of osteoporosis was high (84%) in women, and we found significant negative correlation between BMD and age and positive correlation with $LBM_{\%}$. In the multiple regression analysis $LBM_{\%}$ was most significant, independent predictor of BMD in women (β coefficient $=1.047$, $P=0.001$). The prevalence of osteoporosis was also high (76%) in men, and besides negative correlation between BMD and age we found positive correlation between bone mass and iPTH, as well as with LBM%. In the multiple regression analysis, only iPTH independently predicted BMD in men (β coefficient $=0.812$, $P=0.02$).
We conclude that the lean body mass compartment is the most important component of body composition that determines BMD, particularly in post-menopausal HD women. This study suggests also that secondary hyperparathyroidism, confirmed in our group of patients, seems to be independent predictor of bone mass in HD men.

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The relationship between serum TSH, free T4 and bone mineral density in pre- and postmenopausal women

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Background
Thyroid-stimulating hormone (TSH) might influence bone mineral density (BMD) through its regulation of the thyroid hormones thyroxine (T4) and triiodthyronine (T3). Direct effect of TSH on bone turnover, mediated through its receptors on both osteoblast and osteoclast precursors, has been suggested. We wanted to explore the relationship between serum TSH, fT4 and BMD in women with different TSH levels after a long-term observation of thyroid function and bone status.
Methods
This study included 151 premenopausal women (age 36.4 ± 6.8 years) and 153 postmenopaual women (age 60.9 ± 7.7 years) with valid measurements of BMD at the hip and lumbar spine by dual energy X-ray absorptiometry (DEXA). Based on the TSH levels (ref. 0.32–5.0 mU/l), Premenopausal women were divided into two different groups: group 1 ($n=101$) with normal serum TSH (2.38 ± 1.24 mU/l) and group 2 ($n=50$) with low TSH (<0.5 mU/l). Postmenopausal women were divided also into two groups: group 1 ($n=79$) with the upper normal TSH (2.5–4 mU/l) and group 2 ($n=74$) with lower normal TSH (0.5–2.5 mU/l) and we compared these groups each other.
Results
After multivariate adjustment, in premenopausal women, the group 1 with normal serum TSH, had significantly higher TSH ($P<0.001$), lower fT4 ($P<0.001$) and higher BMD at the lumbar spine and hip ($P<0.01$), as compared to group 2. In postmenopausal women, group 1 had significantly higher serum TSH ($P<0.001$), higher BMD at the lumbar spine and hip ($P<0.05$). No difference between these two groups has been found in the level of fT4.
Conclusions
Generally, we have demonstrated that lower serum TSH (In premenopausal women TSH <0.5 mU/l, and in postmenopausal women TSH <2.5 mU/l) was associated with significant decrease of BMD. Serum TSH below 2.5 mU/l was associated with higher BMD mainly in postmenopausal women. The results support clinical attention toward skeletal health in the patient with low TSH.

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Changes in adipokines serum levels after anticatabolic drugs in postmenopausal osteoporosis

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Adiponectin and leptin have been described as potential contributors to bone metabolism. The effect of anticatabolic drugs on these adipokines and their relationship with bone metabolism have not been clearly clarified.
Aims
(1) Evaluate adiponectin and leptin levels in osteoporosic postmenopausal women and their relationship with BMD, bone turnover and osteoclastogenesis markers. (2) Analyze changes on adiponectin and leptin levels after treatment with raloxifene or alendronate. We selected 53 untreated women (63 ± 7 years) with postmenopausal osteoporosis divided into two groups: women treated with raloxifene (60 mg/day; $n=20$) or alendronate (70 mg/week; $n=33$) during one year. All of them received calcium and vitamin D supplements. We determined at baseline and after 12 months of treatment: anthropometric data, OPG, E2, IGF-I, adiponectin, leptin, 25-hydroxyvitamin D, iPTH, osteocalcin, BALP, ALP, TRAP and BMD in lumbar spine (LS), femoral neck (FN) and total hip (TH).
Results
At baseline, leptin and adiponectin serum levels were 1371.4 ± 822.4 pM/ml and 42.24 ± 26.1 μg/ml, respectively. Adiponectin was significantly correlated with BAP (r: -0.413; P:0.003), OPG (r: 0.51; $P<0.001$), years since menopause (r: 0.295; P: 0.039), but was not with BMD in any site. Leptin was significantly related to weight (r: 0.41; $P<0.01$), BMI (r: 0.47; $P<0.01$) and waist (r: 0.38, P: 0.01), osteocalcin (r: 0.285; P: 0.038) and iPTH (r: 0.33; P: 0.016). Leptin was correlated with LS T score (r: -0.301; P: 0.04) and BMD LS (r: -0.266; P: 0.05) after adjustment for age and weight. After 12 months, no changes were observed in leptin (P: 0.46) and adiponectin (P: 0.55) in alendronate group; however, a significant increase in leptin levels (973.47 ± 637.37 vs 1305.7 ± 793.4 pM/ml; P: 0.031) was detected in the raloxifene group, whereas adiponectin levels showed no significant changes (P: 0.46). Moreover, the percentage changes of adiponectin levels did not differ between the two groups (P: 0.79); while the percentage changes in leptin levels were near significance, between the two groups (P: 0.07).
Conclusions
Adiponectin and leptin levels contribute at least in part to BMD in patients with postmenopausal osteoporosis. Changes in leptin levels after raloxifene treatment could be indirectly implicated in raloxifene bone effects.

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Influence of age, menopause and body composition on bone mineral density in non-obese healthy Romanian subjects

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The strong link between bone mass and body composition is widely recognized but only few studies were selectively performed on healthy subjects with body mass index within normal limits. We aimed to evaluate the influence of body composition on bone mass in apparently healthy young non-obese men and women ($n=40$). To reveal the effect of menopausal transition on the fat mass content, fat mass distribution and fat mass-bone mineral density relationship we compared body composition parameters of young healthy non-obese women to body mass index-matched postmenopausal women ($n=20$). Despite normal values of body mass index, large variability of the whole-body fat mass content was noted in our study, with limits ranging between 18.6 and 49.7% in women and 22–40.3% in men. Age appeared not to influence significantly the amount of whole-body fat tissue. Fat mass was not related to bone mineral density of the lumbar spine, hip or whole-body; in contrast, bone mineral density at all sites was positively associated to fat-free mass in young non-obese women (L1–L4: $r=0.53$, $P=0.003$; femoral neck: $r=0.34$, $P=0.04$; hip: $r=0.53$, $P=0.003$; total body: $r=0.52$, $P=0.003$). Despite a tendency towards higher whole-body and trunk fat mass values in postmenopausal women in comparison to BMI-matched eugonadal young women, the difference between groups reached no statistical significance. Leg fat mass was better represented in young women in comparison to postmenopausal women ($P=0.04$). To conclude, in subject with normal body mass index, both fat mass content and fat distribution are highly variable. In young, healthy, non-obese women, fat-free mass appears to be the main body composition contributor to bone mass. Menopause was not associated with major changes of whole-body fat and trunk adipose tissue, although we noticed a decrease in peripheral fat mass content and a tendency towards a central distribution of adiposity.

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Bone mineral density, bone turnover, serum osteoprotegerin and soluble receptor activator of nuclear factor kβ ligand levels in patients with differentiated thyroid cancer

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Thyroid hormones play an important role in bone metabolism. The potential action of prolonged levothyroxine therapy on bone mass reduction is stil a matter of debate.
The aim of our one year prospective study was to elucidate whether longterm suppressive thyroid hormone therapy in patient with differentiated thyroid cancer (DTC) affects bone metabolism, osteoprotegerin (OPG) and soluble receptor activator of nuclear factor kβ ligand (sRANK-L) and is a risk factor for osteoporosis. Forty-nine patients with DTC (17 premenopausal, 22 postmenopausal women, 10 men) were investigated. All of them had undergone a total thyroidectomy and subsequent I-131 radio-iodine ablation therapy. The levels of free triiodothyronine (fT3), free tetraiodothyronine (fT4), thyrotropin (TSH), parathormone (PTH), serum calcium (Ca), phosphorus, alkaline phosphatase (ALP), osteocalcin (OC), OPG, sRANK-L, urinary deoxypyridinoline (DPD) and 24-hour urine calcium were assessed before and after one year suppressive thyroid hormone therapy. Bone mineral density (BMD) (g/cm^2) in lumbar spine (L1–L4), femoral neck, trochanter and total hip was measured by dual-energy X-ray absorptiometry (DXA) before tratment and after one year of treatment.
In the first year of suppressive thyroid hormone therapy, a statisticaly significant increase was found in serum Ca, ALP, urinary DPD and calcium in each of the three subgroups; and a statisticaly significant decrease was found in serum OPG levels in pre- and postmenopausal groups. No difference was noted in serum sRANK-L before end after one year of treatment in each group. We detected significant decreases at post treatment DXA values in comparison to basal DXA values in lumbar BMD in premenopausal women (1.12 ± 0.10 vs 1.08 ± 0.10, $P=0.01$) and similarly at post treatment femoral neck BMD (1.12 ± 0.27 vs 1.02 ± 0.16, $P=0.02$) in men.
In conclusion, the results of our study revealed that longterm suppressive thyroid hormone therapy in patients with DTC may affect bone metabolism and OPG/RANK-L system.

P250

Colecalciferol loading dose guideline for vitamin D deficient adults

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Introduction
Severe vitamin D deficiency is very common in northern Europe. It is not limited to the elderly, but occurs in a large variety of subjects. Colecalciferol dosing guidelines for rapid correction of vitamin D deficiency are not available.
Objective
To assess the optimal Colecalciferol dose regimen, based on body weight, for rapid correction of vitamin D deficiency, in a variety of subjects.
Materials and methods
One hundred and twenty-three subjects (age ranging from 20 to 90 years, female/male ratio 1.9:1, body weight ranging from 41 to 175 kg) with vitamin D deficiency (defined as serum vitamin D level <50 nmol/l) were treated with solubilised Colecalciferol 50.000 E/ml, in a dose of 25.000 IU every 2 weeks during 8 weeks (total dose 100.000 IU), 25.000 IU every week during 6 weeks (total dose 150.000 IU), or 25.000 IU every week during 8 weeks (total dose 200.000 IU). The Colecalciferol dose per kilogram body weight ranged from 625 to 4000 IU/kg. Serum creatinine, calcium, phosphate, albumin, PTH, 25-OH-D_3 were measured at baseline and 10 days after the final dose of Colecalciferol.
Results
Mean 25-OHD_3 increased from 20.2 ± 0.7 to 69.5 ± 2.9 nmol/l (mean $\pm$ S.E.M., $P<0.0001$). Serum calcium, phosphate, albumin and PTH levels did not change significantly. The Colecalciferol dose required to achieve the optimal serum level of 75 nmol/l was related to the vitamin D deficit (Δ25-$OHD_3 = 75$ – actual

25-OHD_3 level) and body weight. The dose per kg body weight required to achieve normalisation of serum 25-OHD_3 was:
Dose (IU/kg) = 40 (Δ25-OHD_3) + 400 ($R^2 = 0.42$, $P < 0.0001$).
Conclusion
Correction of vitamin D deficiency by Colecalciferol should be based on the degree of vitamin D deficit and body weight.

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The incidence of osteoporotic hip fracture in north west of Iran

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Introduction
Osteoporotic hip fracture is one of the serious complications of bone loss. These fractures constitute almost 20% of orthopedics wards admissions. Due to lack of precise statistical data of osteoporotic hip fracture rates in IRAN, and absence of previous investigations regarding the problem in Tabriz (a large city in north west of IRAN), we planed a study to survey frequency of this epidemic in over 50 years old residents of Tabriz.
Material and methods
In a retrospective – descriptive study we reviewed medical records of all over 50 years old patients who was inhabitant of Tabriz, and admitted with hip fracture in citywide hospitals (private or governmental), during 24 months from March 2005 to February 2007. Data regarding age, sex, type of trauma, type of fracture, and in-hospital morbidity and mortality were extracted. Data analysis was preformed by SPSS[14] software.
Results
During the study period there were 878 admissions for hip fracture in over 50 years old subjects. There were 779 patients with nontraumatic hip fracture including 398 males and 381 females with a mean age of 75.1 ± 9.1. It is estimated that, the rate of nontraumatic hip fracture in over 50 years old citizens of Tabriz to be 175 for each 100 000 population. The rate was 174 for females and 176 for males with a female to male ratio of 0.96.
Conclusion
The frequency of nontraumatic osteoporotic hip fracture in over 50 years old population of Tabriz (a large city in northwest Iran) is high. These rates are lower than those reported from Sweden and the Netherlands, and similar to France and Portugal. Age related surge of osteoporotic hip fracture occurs 10 years earlier in our country. Female to male ratio is lower than those of other countries.

P252

Metabolic and cardiovascular risk in primary hyperparathyroidism

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Background
Primary hyperparathyroidism (PHP) is associated with increased rates of cardiovascular (CV) risk. Moreover, it is not fully clear whether surgery can attenuate CV risk. The intima-media thickness (IMT) of carotid vessels is considered a marker of atherosclerosis and CV events.
Aim
Of the study was to evaluate IMT and some metabolic parameters in PHP patients.
Subjects
Of 56 subjects were studied. Patients were divided into two groups: those with normal calcium levels (Gr 1, $n = 31$) and those with still elevated levels (Gr 2, $n = 25$) after surgical or medical therapy. Surgery was performed in 60% and 71% of patients from Gr 1 and Gr. 2, respectively.
Protocol
In all subjects, we measured BMI, blood pressure (BP), IMT by color-duplex sonography, serum Ca, PTH, HOMA-IR, HbA1c, serum lipids, and osteoprotegerin.
Results
BP was similar in both groups. Gr 2 patients were significantly older (65 ± 3 years) than Gr 1 patients (56 ± 3 years; $P = 0.02$). BMI > 30 kg/m^2 was found in almost five times as many patients in Gr 2 (47%) as in Gr 1 (10%). Serum Ca levels were 9.8 ± 0.1 mg/dl in Gr 1 and 12.4 ± 0.4 mg/dl in Gr 2 ($P < 0.0001$). PTH levels were elevated in 68% and 52% of Gr 1 and 2 patients, respectively. Osteoprotegerin, total cholesterol, triglycerides, and HOMA-IR levels were higher and HDL-cholesterol was lower in Gr 2 than in Gr 1 patients, while HbA1c levels were similar in both groups. A significant increase in IMT was observed in 22% and 32% of Gr 1 and Gr 2 patients, respectively.
Conclusion
Age and BMI seem to be the best predictors of the increase in IMT, osteoprotegerin and some other factors involved in CV risk in PHP patients. These clinical and biochemical abnormalities persist after surgery and may explain the lower survival in PHP patients than in the general population.

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Vitamin D inadequacy in patientes who are screened for osteoporosis

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Low concentrations of vitamin D leads to secondary hiperparathyroidism, bone loss, and an increase of osteoporotic fractures in populations at risk. Adequate vitamin D and calcium intake is considered an essential component of postmenopausal osteoporosis management. Several epidemiological studies have assessed the prevalence of low serum vitamin D concentrations, indicating that vitamin D inadequacy (< 30 ng/ml) is a problem world-wide.
Aims
To evaluate vitamin D inadequacy in patients who are screened for osteoporosis, and in postmenopausal osteoporotic women after one year of treatment.
Patients and methods
In 126 postmenopausal women (mean age 63 ± 7 years) who were evaluated for osteoporosis at the Bone Metabolic Unit we determined: BMD by DXA (Hologic QDR 4500 w) at lumbar spine, femoral neck and total hip, bone turnover markers, PTH and 25(OH) vitamin D. 76% of the women were diagnosed of osteoporosis (*T*-score –2.5 S.D.) and started treatment with antirresoptives, calcium and vitamin D (1200 mg and 800 UI daily).
Results
At baseline 90% of the women had serum levels of 25 (OH) vitamin D less than 30 ng/ml, and 42% less than 15 ng/ml. There was no correlation between vitamin D levels and age. After one year of treatment, serum 25(OH) vitamin D was less than 30 ng/ml in 68% of patients, and less than 15 ng/ml in 12%. There was no correlation between vitamin D levels and BMD changes after treatment.
Conclusions
There is a high prevalence of vitamin D inadequacy among women screened for osteoporosis. A significative percentage of osteoporotic patients treated during one year including calcium and vitamin D supplementation remains with inadequate levels of serum vitamin D.

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Renal function before and after radical treatment of primary hyperparathyroidism

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Objective
It was previously shown that primary hyperparathyroidism (PHPT) might induce impaired renal function and chronic renal failure. The aim of our study was to evaluate the effect of surgical treatment on parameters of calcium and phosphate renal metabolism and renal function in patients with PHPT.
Material and methods
In 26 patients with PHPT (age: 57.15 ± 9.54 years, BMI 26.00 ± 4.55 kg/m^2, PTH 276.61 ± 64.83 ng/l, Calcium 2.95 ± 0.19 mmol/l) serum creatinine, urine calcium levels, phosphate (CPH) and creatinine clearance (CCR), tubular phosphate reabsorption (TRP), proteinuria and microalbuminuria were determinated before and 4 months after surgical treatment. Paired *t*-test and Wilcoxon test were used for statistical analysis, as well Pearson correlation test. Statistical analysis.
Results
After operation PTH (51.47 ± 8.57 ng/l) and serum calcium (2.33 ± 0.12 mmol/l) were normalized There was significant improvement in urine calcium levels (398.37 ± 181.2 vs 107.07 ± 53.41 mg/day, $P < 0.05$), CPH (22.27 ± 11.67 vs 12.41 ± 5.24 ml/min, $P < 0.05$) and TRP (70.55 ± 10.56 vs 82.31 ± 11.37,

$P>0.05$) after surgical treatment. There was no change in serum creatinine (87.90±30.71 vs 91.76±27.90, $P>0.05$) and CCR (78.02±38.83 vs 80.19±30.71, $P>0.05$). There was nonsignificant reduction in proteinuria (131.78±32.84 vs 111.68±27.79 mg/day, $P>0.05$) and microalbuminuria (54.07±21.14 vs 39.60±15.05 mg/day) after operation. There was significant negative correlation between serum calcium levels and CCR ($r=-0.492$, $P<0.05$).
Conclusion
Radical treatment improves parameters of calcium and phosphate renal metabolism and renal function in patients with PHPT.

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Preoperative parathyroid hormone levels are correlated with parathyroid adenoma volume, bone mineral density but not serum calcium levels

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The aim of this study was to determine the relationship between biochemical parameters, parathyroid adenoma volume, and bone mineral density with respect to intact parathyroid hormone (iPTH) levels in patients with primary hyperparathyroidism. Data were collected retrospectively from patients with primary hyperparathyroidism who were diagnosed and followed-up at our clinic between 2004 and 2008. Forty-eight (female/male=42/6) patients with a mean age of 52.8±13.1 (range 23–75) years were enrolled into the study. Bone pain was the most common presenting feature in 41.7% of patients, while 45.8% of patients were asymptomatic. The mean serum calcium and iPTH concentrations were 11.5±2.2 mg/dl and 657.1±682.0 pg/ml, respectively. The mean total *Z*/*T* scores of DEXA scan at femur and lumbal spine were −0.4±1.6/−1.0±1.7 and −1.4±1.6/−2.2±1.5, respectively. Parathyroidectomy was performed in 39 patients while nine patients were observed with medical treatment. The sensitivity of ultrasound and Tc-99 m sestamibi scintigraphy for parathyroid adenoma localization was 31 and 79%, respectively. Preoperative iPTH levels were correlated with serum phosphate ($r=-0.412$, $P=0.005$), alkaline phosphates ($r=0.698$, $P=0.0001$), femur ($r=-0.402$, $P=0.020$) and lumbal spine total *Z* scores ($r=-0.441$, $P=0.013$) whereas parathyroid adenoma volume was correlated with iPTH ($r=0.367$, $P=0.036$) and alkaline phosphates ($r=0.570$, $P=0.001$). There was no correlation between iPTH, serum calcium levels and total *T* scores at femur and lumbal spine. Serum 25-hydroxyvitamin D (25-OHD) levels were below <10 ng/ml in 16 patients. After excluding patients with 25-OHD insufficiency there was still no correlation between serum iPTH and calcium levels. Parathyroid adenoma volume, serum iPTH and calcium levels were also not different between patients with and without 25-OHD insufficiency.
In conclusion, these results suggest that iPTH levels may be useful to predict parathyroid adenoma volume and it is also well correlated with femur and lumbal spine total *Z* scores.

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The change of bone mineral density in postmenopausal women treated by combined agent alendronate and alfacalcidol assessed by digital X-ray radiogrammetry

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Hypovitaminosis D is frequent in population over 65 years age, producing a secondary hyperparathyroidism, increase the bone remodeling, bone loss and osteoporotic fractures, loss of the muscle force.
Daily dose of 0.5 μg alfacalcidol was effectively in improved muscle strength in elderly and reduced falls. The bisphosphonates have been shown to increase Bone Mineral Density (BMD) and reduce bone turnover in postmenopausal osteoporosis. The combined agent Bisphosphonates and Alfacalcidol can be an alternative treatment. We are proposed to evaluate the effect of combinat agent Alendronate (Fosamax®) and Alfacalcidol (Alpha $D_3^®$) on BMD changes in postmenopausal osteoporosis compared with Fosamax® only.
We studied 10 105 healthy women with ages between 20 and 89 years referred to our department of densitometry using Digital X-Ray Radiogrammetry – BMD (DXR-BMD), 7103 from them being in postmenopause; 830 from them presented osteoporosis (WHO criteria). Among 414 patients selected from Fosamax® - treatment 70 mg/week, 138 were under the treatment after 5 years or more. 104 women were treated with Fosamax® 70 mg/week and Alpha $D_3^®$ 1 μg/daily. The mean age of osteoporotic treated women was 61.3±7.8 years.
BMD by DXR was measured at each 12 months. The BMD mean changes under Fosamax® was: +3.5% after 1 year; +4.6% after 2 years; +5.4% after 3 years; +6.3% after 4 years and +7.2% after 5 years. The BMD mean changes at the patients treated with Fosamax® and Alpha $D_3^®$ was: +3.8% in the 1 year; +5.8% after 2 years; +6.7% after 3 years; +7.4% after 4 years and +8.3% after 5 years. Three patients (2.1%) suffered a fracture under Fosamax®. We conclude that BMD changed under the both treatments. The combined – treatment Fosamax® + Alpha $D_3^®$ increased BMD significantly more that Fosamax® only. DXR-BMD is a good method for diagnosis and monitoring osteoporosis therapy.

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Primary hyperparathyroidism surgery: team report 2002–2008

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Introduction
Single adenoma is the most frequent cause of Primary hyperparathyroidism (HPT1). Surgical excision has a high success rate, particularly when associated with a fast intraoperative intact parathyroid hormone assay (PTHi).
Aim
Evaluate the success rate of primary hyperparathyroidism surgery in our unit.
Methods
Descriptive study of the last 33 consecutive HPT1 patients who undertook surgery, 27 of which with PTHi. The PTHi assays were performed at 10, 15 and 30 min after adenoma excision. Mean age was 57.56±14 years.
Results
The sensitivity of the localization exams was >75%. Minicervicotomy was the preferred surgical approach, and was associated with a >50% decrease in the PTHi intra-operatória at 10 min in all patients. No surgical complications were subsequently observed (mean follow-up 13 months, range 3–48). On average, calcemia levels decreased from 10.9±0.8 (range 9.2–13) pre-surgery to 9.2±0.5 (range 8.5–10) mg/dl at the last follow-up appointment. Similarly, PTH decreased from 203±131.2 (range 89.4–754) to 53.3±30 (12.3–151) pg/ml. Symptoms improved in all patients.
Conclusions
Pre-surgery localization exams increased the number of unilateral and mini-invasive explorations, making this the preferred surgical approach. Intraoperative PTHi played a crucial role in the success rate of the approach by decreasing the operative time and the post-operative hypocalcaemia.

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Growth hormone secretory status and bone mineral content in postmenopausal women

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Background
Although the decline in sex steroid levels, particularly estradiol, may be largely responsible for age-related bone loss and osteoporotic fractures in older women, the insulin-like growth factor (IGF) system may also play a key role. This study aimed at evaluating the relation between the secretory status of growth hormone (GH) with the bone mineral content (BMC) in postmenopausal women.
Methods and materials
In a descriptive cross-sectional study, 150 postmenopausal healthy women out of 1328 patients referred to Tabriz Sina Hospital for bone densitometry were selected. They were a matched population of normal, osteopenic and osteoporotic subjects. The GH response to provocation by clonidine was assessed in all patients. The radioimmunoassay (RIA) employed to measure the serum level of the GH. Bone Mineral Content was measured DEXA using LUNAR version DPC-MD apparatus. The correlation between basal and stimulated GH and BMCs of femoral and lumbar bones were studied.

Results
One hundred and fifty patients with a mean age of 65.6 ± 6.6 years were enrolled in this study. The correlation coefficient of BMC of total lumbar area with basal GH, GH 60 and 90 min were −0.04, −0.06 and 0.04 respectively. The correlation coefficient of BMC of total femoral area with basal GH, GH 60 and 90 min were −0.07, −0.08 and −0.09 respectively. None of the correlations were statistically significant (all Ps were > 0.05).
Conclusion
Based on this study results we cannot show any correlation between BMC of evaluated skeletal areas and secretory pattern of GH in a population of postmenopausal women composed of osteoporotic, osteopenic and normal subjects.

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Vitamin D status in healthy postmenopausal Iranian women
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There have been few epidemiologic studies on vitamin D status in postmenopausal women of Middle East countries.
The purpose of this study was to investigate the 25-hydroxyvitamin D levels in postmenopausal women of Iran. By using the records of the local household registry, a sample of 300 subjects was drawn by simple random sampling. Serum 25-hydroxyvitamin D levels were determined with full automated chemiluminescent immunoassay. In addition, the study included survey questions regarding age, body weight and height, occupation, use of skin protection, clinical and reproductive histories.
Means of age and duration of menopause were 63.4 ± 4.64 and 16.7 ± 6.15 years, respectively.
The mean concentrations of 25-hydroxyvitamin D were 23.75 ± 24.06 ng/ml. Hypovitaminosis D (25(OH) D < 10 ng/ml) affected 38.3% of our population.
These findings indicate that 25(OH) D levels in postmenopausal women of Iran are low.
Studies to elucidate and assess the dietary intake of vitamin D in Iranian women can be of further benefit.

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Could neonatal hypocalcamia have a cultural origin?
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Introduction
A newborn baby boy of a family of Asian origin with an uncommon, but logical diagnosis.
Case
Of 8 days old, term baby boy. Normal pregnancy, spontaneous delivery, uncomplicated postnatal period. On day 5, jerking movements of the whole body. Fully breastfed, not feeding well lately. Medical and neurological clinical examination normal. Tonic-clonic epileptic fit during the 1 h of admission.
Blood results: Calcium 1.48 mmol/l, phosphate 3.52 mmol/l, magnesium 0.57 mmol/l, alkaline phosphatase 301 U/l.
EEG: During the EEG left sided focal fit with cyanosis; rhythmic 3/s spike-waves predominantly central with movements to the right side. Inter-ictal EEG normal.
EEG-monitoring: Series of rhythmic spike waves, lasting 1–3 min up to 10 times/h with bilateral focal fits.
Therapy: IV Calcium gluconate and magnesium. Convulsions stopped after the calcium level normalised.
Usual differentials were excluded. Observation of the mother revealed the likely diagnosis as she is of Asian origin and wears a full facial veil for religious reasons.
Further blood results: Parathyroid hormone 28 ng/l (normal 12–45 ng/l), 25-hydroxy-vitamin D 1.0 μg/l (normal 20–70 μg/l).
Mother's blood results: Calcium 2.19 mmol/l, Phosphate 4.2 mg/dl und alkaline phosphatase 173 U/l, (all normal), 25-hydroxy-vitamin D < 1.0 μg/l and Parathyroid hormone 61 ng/l (normal 12–45 ng/l).
Diagnosis
Congenital vitamin D deficiency with hypocalcaemic convulsions in a child born to a mother with vitamin D-deficiency and secondary hyperparathyroidism.
Discussion
Vitamin D deficiency in pregnant and lactating veiled immigrants is usually precipitated by the lack of sunlight due to religious dress codes. Newborns of veiled mothers do have significant lower vitamin D levels e.g. 10.4 μg/l (25 nmol/l) than newborns of unveiled mothers: 63 vs 15.8%.
Pregnant women with poor exposure to sunlight should have a good vitamin D-substitution. Newborns of veiled mothers should be examined and treated for vitamin D deficiency as soon as possible.

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Bone mineral density and bone metabolism in hemodialysis patients. correlation with pth, 25ohd3 and leptin
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Background
Bone metabolism is affected in hemodialysed patients (HD) and PTH plays central role. Additionally leptin which is increased in renal failure may be linked with bone metabolism. We investigated the BMD and bone metabolism in comparison with serum PTH, 25OHD3 and leptin in HD patients.
Methods
We measured in 37 HD patients bone alkaline phosphatase (bSAP), NTx, PTH, 25OHD3 and leptin. We evaluated BMI and BMD in lumbar spine (LS) and in femoral neck (FN) by DXA. Correlation coefficients were calculated by simple regression analysis.
Results
1. Osteopenia had 32.1% in LS and 50% in FN and osteoporosis had 14.3 and 21.4% respectively. LS or FN *Z* score had no correlation with HD duration.
2. Bone markers, PTH, phosphorus and leptin were increased.
3. 25OHD3 was low and had no correlation with NTx, bSAP or PTH.
4. PTH correlated with bone markers and *Z* score in LS and FN.
5. Leptin as expected, was strongly correlated with BMI. In contrast leptin had no correlation with bone markers or *Z* score.
Conclusions
In hemodialysed patients bone metabolism is increased in relation with the increased PTH resulting to low bone density which is independent of high serum leptin or 25OHD3 deficiency. Additionally the duration of hemodialysis does not seem to affect bone density.

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Thyrotoxicosis presenting as severe life-threatening hypocalcaemia – a case report
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Introduction
Hypocalcaemia is a rare presentation of thyrotoxicosis. We describe a patient with severe life-threatening hypocalcaemia and suggest pathogenic mechanisms.
Case report
A 66-year old lady with long-standing insulin-treated type 2 diabetes presented acutely with generalised weakness and a collapse at home. Prior to admission, she had been treated for an infected neuro-ischaemic plantar ulcer with broad-spectrum antibiotics. She had developed nausea and diarrhoea during the week before admission. She had chronic kidney disease (stage 4) with an eGFR of 25 ml/min. In the past she had undergone partial thyroidectomy for nodular goitre. There was also a history of hypertension and IHD. On examination, T 34.1 °C, Trousseau sign + ve, Chvostek + ve, BP 90/60. Investigations: Hb 10.2 g/dl, WCC 11.6×10^9/l, urea 39.6 mmol/l, creatinine 260 μmol/l, eGFR 15 ml/min, CK 720 rising to 3406 U/l after 2 days, calcium 0.85 mmol/l, corrected calcium 0.99 mmol/l, albumin 33 g/l, phosphate 2.78 mmol/l, alkaline phosphatase 141 U/l, parathyroid hormone 61.3 pmol/l (NR 1.5–7.5), Vitamin D 24 nmol/l, FT_4 43.8 pmol/l, TSH < 0.05 mU/l. She received intravenous saline, intravenous calcium 2 g/d for 72 h, oral Vitamin D and calcium supplements, and carbimazole 20 mg daily. By the time of discharge her renal function had returned to pre-morbid levels and she was eucalcaemic on oral Vitamin D with planned radio–iodine therapy for her toxic remnant goitre.
Discussion
The cause of this lady's profound hypocalcaemia is likely to be multi-factorial. We postulate that impaired phosphate excretion as a result of her worsening renal function combined with a high phosphate load from rhabdomyolysis secondary to her fall and to thyrotoxic myopathy, led to hyperphosphataemia. This in turn, resulted in hypocalcaemia by precipitating calcium and inhibiting parathyroid hormone-mediated bone resorption. Clinicians need to be aware of hyperthyroidism as a rare but treatable cause of severe hypocalcaemia.

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Bone resorption and antiresorptive effect of bisphosphonates related to homocysteinaemia

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Introduction
Some recent data indicates that high levels of homocysteine (Hcy) can be independent risk factor for osteoporosis and fractures in elderly. There are few explanations how homocysteine influence bone resorption and probably modify effect of therapy for osteoporosis. 1. Hcy interferes with collagen cross-linking. 2. High levels of Hcy specially stimulate resorptive activity of osteoclasts *in vitro*. 3. Hcy interfere bisphosphonates binding to hydroxyapatite.
The aim
To investigate is there a connection between levels of homocysteine and changes in levels of osteokalcin, β-crosslaps and ionized calcium during the therapy.
Materials and methods
We examined 40 women's with diagnosed osteoporosis (DXA of lumbar spine or hip: *T* score or X-ray of the spine). All patients use alendronate 10 mg per day with 500 mg of calcium and 0.25 μg of activated vitamin D. Levels of homocysteine, osteocalcin (OC), β-crosslaps (CL) and ionized calcium were measured on baseline and after 6-8 weeks during the therapy. Results were linearly correlated.
Results
There was no significant correlation between baseline levels of homocysteine and osteocalcin ($r=-0.1618$, $t=1.011$, $P>0.05$) and β-crosslaps ($r=-0.0199$, $t=0.123$, $P>0.05$) before therapy. There was no correlation between levels of homocisteine with change in levels of β-crosslaps and osteocalcin during the therapy (CL: $r=0.0737$, $t=0.3696$, $P>0.05$, OC: $r=-0.11262$, $t=0.55526$, $P>0.05$). Homocysteine levels before therapy are slightly negatively correlated with levels of ionized calcium before therapy. ($r=-0.306$, $t=1.982$, $P>0.05$, $P<0.1$).
Conclusions
In this preliminary study, there was no significant correlation between levels of homocysteine and biochemical bone markers before and during the antiresorptive treatment with alendronate. Although there is a suggestion for further investigations with more patients included.

P264

A simple method for osteoporotic fracture risk assessment in women of all ages, using computed clinical and densitometrical data

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Introduction
The combined influence of risk factors on osteoporosis being known, the problem whether it is correctly identified and quantified arises, aiming to the improvement of populational screening. The correct judgment related to osteoporotic pathology refers to fracture risk assessment.
Materials and methods
We investigated 2149 women aged 20–91, without treatment for osteoporosis, for anthropometric, anamnestic and bone densitometry features. Relations between fracture history, clinical factors, anamnesis, bone mineral density (BMD) at different sites, were computed using bivariate analysis (chi-square or ROC curve method) and stepwise logistic regression, in order to assess the probability of clinical osteoporotic fracture.
Results
Of 271 women had a history of frailty fractures, mostly nonvertebral. They are statistically negatively associated to lumbar spine BMD, total femur, femoral neck, whole body and distal radius BMD; also, there are associations to numerous clinical factors. We retained, as independent predictors, function of the site taken into the logistic equation: fractures in first-degree relatives, renal lithiasis, age, body height and weight. We computed the probability of existing clinical osteoporotic fractures and drawn risk maps for each densitometric region of interest. These maps can be used as quick screening methods.
Discussion
Describing fracture risk groups, even in nonosteoporotic patients, by the means of a tool like the fracture risk map, adapted to regional populational features, is a step towards improving therapeutic protocols in osteoporosis.
Conclusions
BMD stays as the most powerful predictor of osteoporotic fractures, but it should be combined with clinical risk factors in order to improve the strategy of diagnosis. The presence of certain described risk factors is sufficient for recommending bone densitometry in individual cases.

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Lipid levels before and after radical treatment of primary hyperparathyroidism

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Objective
It was previously shown that patients with primary hyperparathyroidism (PHPT) have atherogenic dyslipidaemia. The aim of our study was to evaluate the effect of surgical treatment on lipid levels in patients with PHPT.
Material and methods
In 26 patients with PHPT (age: 57.15±9.54 years, BMI 26.00±4.55 kg/m^2, PTH 276.61±64.83 ng/l, Calcium 2.95±0.19 mmol/l) Total cholesterol (TC), HDL-C, LDL-C, triglyceride (TG), ApoA1, ApoA2, ApoB, ApoE and Lp(a) levels were determinated before and after surgical treatment. Paired *t*-test and Wilcoxon test were used for statistical analysis, as well Pearson correlation test. Statistical analysis.
Results
After operation PTH (51.47±8.57 ng/l) and serum calcium (2.33±0.12 mmol/l) were normalized, there was no change in BMI index before and after operation (26.00±4.55 vs 26.36±4.31 kg/m^2, $P>0.05$). There was increase in ApoE levels (46.20±11.46 vs 57.49±13.86, $P<0.05$) after operation. There was no change in TC (6.02±1.33 vs 6.00±1.11 mmol/l, $P>0.05$), HDL-C (1.26±0.32 vs 1.28±0.30, $P>0.05$), LDL-C (3.84±1.07 vs 3.94±1.01, $P>0.05$), TG (1.78±0.76 vs 2.04±0.97, $P>0.05$), Apo A1 (1.637±0.305 vs 1.627±0.302, $P>0.05$), Apo B (1.165±0.276 vs 1.145±0.325), Apo A2 (288.57±56.42 vs 305.08±52.56, $P>0.05$) and Lp(a) (0.19±0.05 vs 0.16±0.04, $P>0.05$) after operation. There was significant correlation between PTH and Lp(a) levels ($r=0.534$, $P<0.05$). CONCLUSION, radical treatment did not improve dyslipidemia in our group of patients with PHPT.

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Vitamin D levels in young healtly premenopausal females in Slovakia

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Background
There is increasing body of evidence suggesting for low vitamin D levels in humans. The levels of vitamin D in nonselect populations of Europe, Middle East, Asia and Latin America had been repeatedly shown to be as low as 20-30 μg/l.
Aim
The aim of our study was to examine serum 25-hydroxyvitamin D [25-(OH)D] levels in young healthy premenopausal women in Slovak population.
Subjects and methods
The participants ($n=162$, mean age 34.0 years), regularly cycling, fertile females with normal BMD and no risk factors of osteoporosis were recruited in six centres. The blood procedures were performed during one month (October 2007), after highest exposure to sunlight in our region. Serum 25-(OH)D levels were measured by HPLC method (Shimadzu, Chromsystem).
Results
The mean 25-(OH)D levels were 32.6 μg/l (min. 6.7 μg/l, max. 69.5 μg/l). In females at the age of 25–40 years, tendency to decrease of 25-(OH)D levels with increasing age was observed. Up to 80 (49.4%) subjects had their 25-(OH)D levels lower than 30 μg/l, the level generally accepted as the lower limit of normal for 25-(OH)D.
Conclusions
In conclusion, the prevalence of low 25-(OH)D concentration in healthy young female in Slovakia is very high and general vitamin D supplementation in our population should be considered.

Clinical case reports and clinical reports

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Graves' disease accompanied by pheochromocytoma presenting with normal levels of catecholamines: report of a case

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We present a rare case of Graves' disease accompanied by pheochromocytoma, which showed normal urine and serum levels of catecholamines and their metabolites. A 45-year-old woman was referred to our hospital for the evaluation of a right adrenal incidentaloma detected by computed tomography. She was diagnosed with Graves disease at 6 months ago. Initially she got an antithyroid medication include methimazole, but her thyroid function could not control easily. So, radioactive iodine therapy was performed then her thyroid function returned normal values. She had no symptoms of pheochromocytoma such as hypertension or a history of hypertension attack. Two consecutive 24-hour urine samples were sent for measurement of catecholamines, both of which showed normal levels of free cortisol, metanephrine, VMA, epinephrine and norepinephrine. After right adrenalectomy was performed, the final pathologic diagnosis was adrenal pheochromocytoma. This case suggests that the Graves' disease may be associated with excess catecholamine secreted by pheochromocytoma. In addition, although the conventional method for detecting pheochromocytoma is to identify an increase in urine catecholamines, physicians should be aware of the possibility of false negativity.

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Toxic liver damage after antithyroid drugs – 2 cases. The role of 131-I in treatment

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Antithyroid drugs frequently used in management of hyperthyroidism may lead to liver damage. Hepatotoxicity is a rare but potentially fatal complication. The aim of this study was to present two cases of severe liver damage and the role of 131-I treatment in such cases.
Case 1
A women 49 years of age with Graves hyperthyroidism was treated with metimazol (30 mg next 10 mg). After 1 month the utricarial skin rush was observed and the drug was changed to PTU 100 mg/day. After 6 weeks, the patient developed cholestatic jaundice. Lab tests: total bilirubin 17.8 (N 0.3–1.1 mg/dl) GOT 31U/l (N 1–37U/l) GPT 421(N 1–40 U/l) AP 394 (N 30–123 U/l) GGTP 97(N 9–37 U/l). Toxicological and immunological labs were negative. The tests showed that she had suffered from viral hepatitis A and B. Despite of drug discontinuation the increase of total bilirubin was observed during 2 weeks. The treatment with glucocorticoids was ineffective. The recovery started after introduction of urodesoksycholic acid. 7.7 mCi 131-I was administred at 10 day of hospitalization. Normalization of liver and thyroid parameters was observed after 12 weeks.
Case 2
A women 49-years of age with Graves disease was treated with metimazol (15 mg next 10 mg). After 3 months of treatment jaundice was observed and the drug was changed to PTU. The worsening of jaundice was observed. At admission to our hospital lab tests: total bilirubin 35.3 mg/dl, GOT 47 U/l, GPT 82 U/l The viral and immunologic labs were negative. The treatment with glucocorticoids and urodesoksycholic acid was ineffective. 131-I was administered at 16 day of hospitalization. Normalization of liver and thyroid parameters was observed after 12 weeks.
Conclusions
The liver dysfunction can progress even after discontinuation of the drug. We should not change the antithyroid drug when the liver damage is observed. In our observation there was no improvement after glucocorticoids. In 1 case, we observed improvement after urosdesoxycholic acid. The best treatment of thyrotoxicosis in such cases is the 131-I therapy.

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Lymphocytic hypophysitis case who developed empty sella to follow up

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Lymphocytic hypophysitis (LH), an uncommon pituitary disorder that is considered an autoimmune disease. The disease shows a striking female predilection of ~9:1 and commonly affects young women during late pregnancy or in the postpartum period. Less frequently, it has also been observed in men and postmenaposal woman. Partial or total hypopituitarism can be in LH. In the early stage, the pituitary gland is enlarged like a pituitary tumor, from which it cannot be distinguished on magnetic resonance imaging (MRI) scanning. Spontaneous resolution of both the mass and the hypopituitarism may be possible. In the later stages, the gland may atrophy, leaving an empty sella, as occurs in Sheehan's syndrome. A 53-year-old postmenaposal woman had image mimic adenoma on pituitary MRI and total pituitary insufficiency. Biopsy was offered the patient but she declined this procedure. Total pituitary deficiency was observed in dynamic tests of patient and L-thyroxin 100 mg/d and prednisolon 5 mg/d was started. She stopped treatment herself after 2 months. She did not come to control for 5 years. Five years later, in dynamic tests of patient was observed that there is a recovery for hypocortisolism without treatment and MRI imaging adenoma was disappeared and empty cella has developed.

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Liver manifestation of poorly controlled Type 1 diabetes mellitus: hepatic glycogenosis

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We report an 18 years old female with hepatomegaly and elevated liver function tests. She had been diagnosed with Type 1 diabetes mellitus when she was 13 years old. She was referred to our division because of diabetic ketoacidosis. Evaluation of her previous records revealed the presence of liver function abnormalities for 4 months. At physical examination she had an enlarged liver. A1c level was 13%. She was treated initially with intravenous insulin. Following the achievement of acceptable plasma glucose levels, negative urinary ketone bodies and normal bicarbonate levels, insulin detemir and insulin aspart were suggested. Ultrasonography revealed the presence of hepatomegaly with 200 mm longitudinal axis. Viral hepatitis markers including hepatitis B, hepatitis C and CMV, ANA and AMA were negative. Serum alpha-1 AT, ceruloplasmin, copper, iron and ferritin levels were in normal ranges. Liver biopsy revealed glycogen deposition that was consistent with hepatic glycogenosis. Subsequent to the achievement of glycemic control, liver enzymes started to decline and 50% reduction was achieved in ALT in 1 week.
Hepatic glycogenosis is associated with poor metabolic control and high amount of insulin that is required to maintain euglycemia. Hepatic glycogenosis may resolve following glycemic control.

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Concurrent thyroid medullary, papillary carcinoma and Hashimoto thyroiditis: case report

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Introduction
The incidence, cell origin, histopathologic features and prognosis of papillary and medullary carcinoma are considered to be completely different. Simultaneous occurrence of medullary and papillary thyroid carcinoma in the same patient is rare. Here, we present a patient with synchronous medullary thyroid carcinoma and papillary microcarcinoma occurring in a thyroid with chronic lymphocytic thyroiditis.
Case
A 47 years old woman with no history of chronic illness and no pathologic sign except palpable nodules in thyroid applied with swelling and intermittant pain in the neck. She was euthyroid both clinically and laboratuary. In thyroid ultrasonography, multiple hypoechoic nodules with microcalcifications in left lobe of thyroid were detected. Because, fine needle aspiration biopsy of the 13×10 mm nodule in superior posterior left lobe was reported as suspicious for medullary carcinoma, she underwent bilateral total thyroidectomy and left radical neck dissection. In pathologic examination, in superior part of left lobe a

medullary thyroid carcinoma of 15 mm with thyroid capsule infiltration and lymphovascular invasion was found. Tumor cells were strongly positive for calcitonin, chromogranin and carcinoembryonic antigen, immunohistochemically. Additionaly, there was a papillary microcarcinoma foci of 1 mm in lateral part of the same lobe. Three lymph nodes were positive for medullary carcinoma metastases also, and chronic lymphocytic thyroiditis was detected in remaining thyroid tissue. MEN was excluded with laboratory and imaging studies. Postoperative serum calcitonin was <2 pg/ml. She was treated with radioactive iodine.

Conclusion

It is still not obvious whether coexistence of medullary carcinoma and papillary carcinoma in thyroid is just incidental or due to a common stem cell or genetic alteration. Also, role of lymphocytic infiltration in this coexistence remains unidentified. Further investigations and genetic analyses are needed to explain the pathogenesis of simultaneous lymphocytic thyroiditis and papillary and medullary carcinoma in the same thyroid.

P272

The study of an immunohistochemical aggresivity marker in mammary carcinomas

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Introduction

The mammary cancer is the most frequent malign tumor encountered in females, characterized by a high distant metastasis tendency. Among the potential prognosis factors, we mention the biomarkers that measure or are associated with biologic processes involved in the tumorous progression. The study analyzes the p53 protein's positivity in correlation with the mammary cancer's classical prognosis factors: the hystologic type, the histopathologic degree, the clinical stage and the status of the axial lymphonodules.

Purpose

The immunohistochemical evaluation (IHC) of an aggresivity marker in mammary cancer.

Methods

Using the immunohistochemical method of ABC Elite avidin-biotin complex staining and the p53 human anti-protein mouse monoclonal antibody, the DO 7 clone (1:500 dilution) on tissue sections fixed in 10% formaldehyde and included in paraffin, we have obtained a red staining of the tumorous cells' nuclei.

Results

Out of 40 mammary carcinomas where we have immunohistochemically determined the p53 protein, we have assessed that 29 of them proved to be negative, six had a moderate staining and five had an intense staining. Several studies estimate that the over-expression of the p53 protein is comprised between 25 and 50% of the cases.

The p53 immunoreactivity was more frequently encountered in pre-menopausal women and in invaded axial lymphonodules tumors. We remarked a strong connection between the p53 over-expression and the studied tumors' grading.

Conclusions

The results of the p53 staining present some variations, depending on the laboratories where the research has taken place (between 21.5 and 52% with different antibodies and on a different number of cases). In the studied cases, the percentage of p53 positive cells was of 27.5%. The p53 protein over-expression can be useful in establishing the mammary carcinomas prognosis, only if it is analyzed in connection with other factors, thus improving the information provided by them: therefore it contributes to the identification of the patients with an increased risk of disease progression.

P273

Transient ischemic attack after oral L-thyroxine loading test: a case report

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Many causes of levothyroxine malabsorption have been described in the literature such as celiac disease, giardiasis, severe liver cirrhosis and drug interactions. A 27-year-old woman admitted to our institution with overt hypothyroidism symptoms. She had total thyroidectomy for thyroid papiller carcinoma and was using 400 μg/day levothyroxine (LT_4). Her serum free thyroxine (fT_4), free triiodothyronine (fT_3) and thyrotropin stimulating hormone (TSH) levels were 0.4 ng/dl, 1.0 pg/ml, and 98.2 μIU/ml, respectively. No change was occurred in her serum levels of TSH, fT_3 and fT_4 after the addition of 25 μg/day liothyronine treatment to her therapy. After excluding the other known causes of levothyroxine malabsorption, a single oral test dose of 1000 μg levothyroxine was administered to the patient to investigate possible absorption defect of levothyroxine. There were no differences in her serum TSH and fT_4 levels within 6 h. However at the 5 h of the test, we observed amorosis fugax, aphasia and hemiparesis on the right side of her body. Her cranial CT and MR imaginations were totally normal. She was diagnosed as transient ischemic attack (TIA) and was started to treat with antiedema therapy and antiagregant therapy. The neurological symptoms resolved completely within 18 h and the treatment was discontinued after 5 days. In this case, we suggested that levothyroxine malabsorption is due to prolonged hypothyroidism, which was leaded to impaired intestinal absorption by accumulation of glycosaminoglycans. Since we did not find any change in serum fT_4 level during LT_4 loading test, we assumed TIA was occurred coincidentally. Clinicians should be careful during high dose L-thyroxine loading test especially in patients with prolonged hypothyroidism with the increased risk of atherosclerosis.

P274

Hypopituitarism revealed after repetitive hyponatremia as complication of hemorrhagic fever

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Six years after hemorrhagic fever with renal syndrome (HFRS) 73 years old man was admitted in hospital because of hyponatremia (Na 129 mmol/l) and abdominal pain. Before HFRS he was treated for pancreatitis, hypertension, ulcerative colitis and gallstones. In the year 2002 he was admitted with fiver, vomiting, diarrhoea, headache and blurred vision. Serologic immunoflourescence testing was positive for Hantaan (Puumala) virus. During the hospital course haemodialysis was necessary and disseminated intravascular coagulation was present. Despite renal recovery he described loss of appetite and weight, tiredness, occasional constipation, bradycardia and cold intolerance. Slowly he lost libido and axillary hair. In 6 years he was eight times hospitalized. Urethral stricture and sclerosis colli vesicae urinariae was operated (2002, 2004). Blood in stool with diarrhoea, later he was treated for constipation. Because of syncope and bradycardia was implanted pace maker. In the year 2005 he was again on infection depp. With disorientation, fever, prostration and hypotension. Virus pneumonia was suspected. He prepared himself for colonoscopy and was admitted in hospital in the year 2006 because of hypoglycaemia (glucose 2.2 mmol/l) and hyponatremia. Two days after discharged he was admitted in neurological depp. because of vertigo and diplopia. In four of this eight hospitalisation patient was hyponatremic (lowest value 125 mmol/l) what was corrected during hospital treatment.

On last hospitalisation endocrine functions was examined. Hypothyrosis (TSH 0.297 mIU/l, FT_4 6.68, $FT_3 < 0.4$ pmol/l), adrenal insufficiency (morning cortisol < 20, short ACTH stimulation test: cortisol 53 121 nmol/l) and hypogonadotropic hypogonadism (FSH 0.5, LH < 0.1 mIU/ml, testosterone 0.4 nmol/l) were found out. Prolactin level was low-normal. Brain computer tomography was normal. Hypopituitarism was established and replacement therapy was begun.

Hypopituitarism is rarely considered after HFRS. We should suspect it, even after many years.

P275

Paget's disease of sacrum: a case report

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Background

Paget's disease may affect as many as 3% of adults older than 40 years of age; it is often asymptomatic and usually progresses slowly. Paget's disease affects men

and women almost equally, but men tend to be more symptomatic. The disease is usually not clinically apparent until age 50–60 years. It usually progresses slowly and does not develop in new sites. Many different bones can be affected, and the lesions can vary from single, monostotic lesions to involvement of almost the entire skeleton. The pelvis, femur, spine, skull, and tibia are most commonly involved, whereas hands and feet are rarely affected. Paget's disease of sacrum is rare. A monostotic lesion in the sacrum is reported.
Introduction
A 58 years old man was referred to us for diabetes mellitus treatment. The routine chemistry screen showed an elevated serum alkaline phosphatase concentration. Serum Ca and P levels were normal. Thyroid hormones showed normal values. PTH level has elevated and 1,25 (OH)2D3 level has decreased. The urinary deoksipiridinyum level was elevated to 7.8 nMDPD/mMKr and serum osteokalsin was 3.4 in normal range. A history of low back pain was noticed. A plain X-ray and MRI scan of pelvis reported a paget disease that located at sacrum. Bone scintigraphy demostrated strong accumulation of 99mTc on the sacrum. With the diagnosis of monostotic Paget's disease of sacrum, treatment with bisphosphonate was started.
Conclusion
This case was unusual in term of clinical presentation and location.

P276

Possible effects of IGF-1 and IGF-3 in the development of growth failure, type 2 diabetes mellitus and severe insulin resistance in a case of Seckel syndrome

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Seckel syndrome (SS) is described as the prototype of the primordial bird-headed type of dwarfism (Seckel 1960). It represents a spectrum of multisystem abnormalities. We present a case of SS and discuss the possible effects of IGF-1 and IGF-3 in the development of growth failure, type 2 Diabetes Mellitus (DM) and severe insulin resistance (IR).
Case
A 21-year-old female was referred to our clinic because of growth retardation and amenorrhea. At birth, she was small for gestational age, she walked at the age of 7 years. She was amenorrheic after menarch at 18-year-old. Physical examination: BP 120/80 mmHg, central obesity (15 kg, 94 cm, BMI 17 kg/m^2, W/H 1.3), microcephaly, bird-headed appearance, mental retardation, acanthosis nigricans (AN). Ophthalmoscopy: non-prolipherative diabetic retinopathy. Biochemical analyses: FPG: 332 mg/dl, TG: 1046 mg/dl, T-C: 270 mg/dl, HDL-C: 22 mg/dl, elevated liver function test, HOMA-IR index: 17.7, microalbuminuria: 137 mg/d, CCr 142 ml/min. GH, IGF-1, cortisol levels were normal. IGF-3 and LH levels were elevated. Insulin tolerance test showed markedly increased GH and cortisol levels. Her bone age was higher than 18. Pelvic US: multiple anechoic cysts in ovaries and markedly increased endometrium thickness. She was diagnosed as SS with early onset type 2 DM and severe IR. To our knowledge, DM is not common in SS, only 3 cases of early unset of type 2 DM with progressive ataxia were reported by Bangstad in 1988. Insulin resistance is an important etiologic factor in the pathogenesis of DM, however, more important factor in our case is that IGF-1 resistance leading to both growth failure and IR. Despite sufficient GH, normal IGF-1 and elevated IGFBP3, the growth failure implicated an IGF-1 resistance. Moreover, AN in IR results from increased bioavailibity of IGF-1. Finally, IR constitutes an important risk for an increased risk for CVD and endometrium ca in her life.

P277

Polyuria as a main feature of parathyroid crisis due to parathyroid glands hyperplasia

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Aim
To present a case of severe primary hyperparathyroidism (PHPT) manifested predominantly with polyuria.
Case
A 53 years old caucasian female presented to the emergency room with polyuria (5-6 l), and increasing during last 4 weeks dehydration, weight loss (10 kg), weakness, confusion. Diabetes mellitus and insipidus had been excluded. After hypercalcemia (3.6–3.9 mmol\l), Ca++ to 2.0 mmol\l and PTH of 616 pg\ml (11–62) were revealed acute PHPT was diagnosed. There were no bone changes and nephrolythiasis. Creatinine 65 mcmol\l (44–80), urine specific gravidity 1003–1015. D-dimers level $>$20 mcg\ml (0–0.5) witnessed about disseminated intravascular coagulation (DIC). Rehydration, calcitonin, magnesium, potassium, glucocorticoids, heparin were administered. USG: hyperplasia of inferior parathyroid glands (PG), adenoma not excluded. Transcutaneous biopsy: PG hyperplasia. Disease severity dictated necessity of surgical PG exploration without delay. Hyperplasia of all PGs was revealed, especially right inferior ($2.5\times2.0\times1.4$ cm). Three PG an half of left superior PG were surgically removed. Intraoperative PTH measurements: transient 3-times increase of PTG level during parathyroidectomy (result of surgical trauma) with following decrease to the end of operation to 63 pg\ml, and to 16.5 pg\ml 3 h after operation.
Histopathology
Hyperplasia of PG chief cells, most pronounced in right inferior PG. Hypocalcemia (1.5 mmol\l) without Chwostek sign developed only to 5th day after parathyroidectomy and necessary treatment followed. PTH level preserved on 16.5 pg\ml.
Conclusion
Acute PHPT due to PG hyperplasia manifesting with polyuria is relatively rare and may be successfully urgently treated (being accompanied by DIC) without complicated diagnostic procedures.

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Cushing syndrome caused by topical corticosteroids coexistent with pituitary incidentaloma – case report

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Iatrogenic Cushing syndrome caused by the use of steroid medications is common because of the widespread use of these medications for the treatment of many diseases, however development of Cushing syndrome from the topical corticosteroids is very rare in adults.
A 45-year-old men (BMI: 44 kg/m^2), with a history of psoriasis, developed manifestations of Cushing syndrome, which included weight gain, central obesity, moon face, facial plethora, buffalo hump, red-purple striae, proximal muscle weakness and hypertension. It was discovered that over the past several years the patient had been applying to his total body skin surface ointment containing steroids which was combined with the use of occlusive dressings.
Laboratory studies were consistent with suppression of the hypothalamic–pituitary–adrenal (HPA) axis. Plasma and urinary cortisol levels and plasma ACTH concentration were undetectable. A computed tomography (CT) scan of the adrenal glands was normal. A pituitary magnetic resonance imaging (MRI) scan showed a 2 mm tumor. We diagnosed this tumor as a pituitary incidentaloma. The discontinuation of the use of topical steroids was recommended. The 15-months follow-up revealed the gradual improvement of the clinical symptoms and laboratory tests. The plasma and urinary cortisol and plasma ACTH were within the normal range.
Treatment even with topical steroids could be dangerous for patients because of development of cushingoid symptoms. We described the case of patient with iatrogenic Cushing syndrome coexistent with pituitary incidentaloma.

P279

Long-term follow-up of a 46XX case with congenital adrenal hyperplasia and male gender identity

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Congenital adrenal hyperplasia (CAH) owing to 21 hydroxylase deficiency (21OHD) is an inherited autosomal disorder characterized by diminished glucocorticoid and aldosteron biosynthesis. Partial 21OHD leads to the classical simple virilizing form, characterized by prenatal virilization of external genitalia in female fetuses without salt wasting. Ambiguous genitalia in a genetically female infant is frequently due to CAH. The uncertainty about the sex of a newborn is often incomprehensible to most parents. Undiagnosed females can be grown as males and they are faced to multiple clinical, hormonal and metabolic abnormalities.
Case
A 33-year-old man was referred because of ambiguous genitalia. His history was unremarkable except micturation while sitting. He was married. Physical examination: He had gynecoid body habitus (147 cm, 66 kg, W/H 0.78). He was bearded. He had hypospadias and micro penis or clitoris hyperthrophy. We found out that when he was 9-year-old, he was recorded in our hospital registrations as having ambiguous genitalia with female internal genitalia. The records marked a strict male gender identity by a psycological evaluation and refusal of therapeutic managements by his parents. After 24 years, we reevaluted the case. The karyotype was 46XX. Testosteron and 17OHPG was markedly elevated. Abdominal CT showed ovaries, uterus and bilateral adrenal hyperplasia with right sided mass (30×30 mm). Biopsy was performed. Pathologic examination was consistent with adrenocortical adenoma. He/she was diagnosed as CAH due to 21OHD. Because of his/her strict male gender identity and his marrige, his/her decision was to live as a man. Accordingly, she underwent hysterectomy, oopherectomy, cliteral phrenilum construction operations. She was given glucocorticoids and androgen replacement therapy. During follow-up, 5 years later, adrenocortical adenoma was enlarged up to 8 cm. Unilateral adrenalectomy was performed. This case underlines the importance of diagnosis of CAH during infancy/childhood and lifelong follow-up which is a difficult but important task for physicians.

P280

An unusual presentation of autoimmune polyendocrine syndrome: a case report

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In new classification of autoimmune polyendocrine syndromes (APS), APS-3 has defined as combination of autoimmune thyroid disease and other autoimmune disease except hypoparathyroidism and Addison's disease. The present report describes a patient with APS-3 and pseudomalapsorbtion of levothyroxine (LT4). Twenty-three year old woman was referred to our center with hypothyroidism due to Hashimoto's thyroiditis (HT) despite the usage of high dose LT4 treatment. HT was diagnosed firstly eight years ago and she had been treated with 125 μ/day LT4. In March 2007, thrombocytopenia with bleeding was developed and she was diagnosed as idiopathic thrombocytopenic purpura. Subsequently, in August 2007, she was admitted to hospital with palpitation and diagnosed as silent myocardial infarction. Serum IgG anticardiolipin antibodies were found positive but no further evaluation was made at that time. In October 2007, splenectomy was performed because her thrombocytopenia was refractory to the medical treatment. After splenectomy, she was maintained complete remission but euthyroidism could not be achieved despite escalating doses of oral LT4. After referral to our center, patient characteristics, nutritional habits, drug interference, gastrointestinal diseases and the other known reasons of LT4 malabsorption were evaluated but no pathology was detected. Oral LT4 load test was performed with 400μg of LT4 to investigate pseudomalabsorption. A peak increment of 3.8 μg/dl in serum total T4 levels was observed and pseudomalabsorption of LT4 was proven after the test. Serum IgG anticardiolipin antibody measurements were repeated and found positive. Primary antiphospholipid syndrome (PAPS) was diagnosed after evaluation of PAPS's criteria.
To the best of our knowledge, this is the first report of combination PAPS and HT as newly called APS-3C. At the same time she had pseudomalabsorption. Oral LT4 load test should be considered in suspicion of pseudomalabsorption in such cases before proceeding to extensive evaluations for malabsorption syndromes.

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Amiodarone-induced thyrotoxicosis – case report

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Introduction
Amiodarone-induced thyrotoxicosis(AIT) is a condition fraught with difficulties from the diagnostic and therapeutic standpoints. It can be developed precociously or years after the beginning amiodarone uptake and after its suspension. AIT is subdivided in three different forms. Type 1 is developed in subjects with underlying thyroid disease, being caused by an exacerbation by iodine load of thyroid autonomous function. Type 2 is a form of destructive thyroiditis and the majority of the cases is developed in normal thyroid glands. Mixed forms of AIT may also be observed.
Case report
Man, 60 years-old, revealing history of tetralogy of *Fallot* surgically corrected and atrial fibrillation since 2005, under therapeutic with amiodarone(200 mg/day), digoxin and hypocoagulation therapy since diagnosis. He was hospitalized due to a bradyarrhythmia, submitted to a definitive pacemaker implant, suspended amiodarone therapy and started the study for future heart transplant. He re-started atrial fibrillation and amiodarone was reintroduced(400 mg/day). Six days after, thyroid function test results were compatible with thyrotoxicosis(TSH − 0.01 UI/ml; FT_4 − 1.88 ng/dl;FT_3-1.82 pg/ml) with negative antithyroid antibodies and TSH receptor antibodies. The colour flow Doppler sonography showed a normal thyroid gland with normal vascularity and the 24-h thyroid radioactive iodine uptake value was 1%. Results obtained from complementary clinical examinations point out evidences of ATI type 2. Nevertheless, due to the non-normalised thyroid function controlled by hydrocortisone, the patient started combination treatment with propiciltiouracil(100 mg/day) and prednisolone. This therapy was effective in reducing the serum concentration of thyroid hormones.
Discussion
The type of AIT could not be assessed in most of the reported cases.

P282

Breast metastases by medullary thyroid carcinoma: case report with an update

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Introduction
Medullary thyroid carcinoma (MTC) commonly metastasizes to cervical lymph nodes, liver, lungs and bone. Metastatic lesions in the breast have been previously reported but they are extremely rare and to our knowledge, this is the first case in literature described on a male patient. We report a case of a 49-year-old man with a 23-year history of sporadic MTC with bone, liver and lung metastases treated with somatostatin analogues for 6 years, who developed a painful breast metastasis.
Case report
A 49 year-old man was diagnosed in 1985 of a MTC. Total thyroidectomy was performed and from then onwards he has been followed up in our hospital for this purpose. In 1992 he received external radiotherapy for a neck mass. In 1994 he presented bone and lung metastases. In 2002 as Calcitonin levels increased (55 000 pg/ml) he started with incoercible diarrhea. Treatment with Lanreotide was initiated and a marked control of the diarrhea was achieved. Since 2004, when liver metastases appeared, he has had a stable control of the disease, until last year, when he presented painful bilateral gynecomastia and a 1 cm left breast mass on ultrasonography. Fine needle aspiration biopsy revealed a metastatic MCT. Although his disease was widely spread, because of the pain, he underwent left mastectomy. The pathologic examination of the specimen revealed metastatic MTC with positive immunologic staining for Calcitonin.
Conclusions
(1) This case illustrates a rare site for thyroid metastases and differs form other cases reported, in the patients gender. Breast metastases should be considered a diagnostic possibility in patients with MTC presenting with breast lesions.
(2) Cytological features and a positive immunocytochemistry for Calcitonin can be useful to confirm the diagnosis of metastatic MTC.
(3) Somatostatin analogues have played an important role on controlling the diarrhea and the course of our patient's disease.

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Nesidioblastosis in a patient with gastrinoma

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Introduction
Islet hyperplasia and nesidioblastosis are described in the non neoplastic pancreas of patients with gastrinoma. It is not definately established whether hypergastrinemia can influence these changes. Moreover, rarely insulinoma and gastrinoma occur in the same patient but cases of single tumours cosecreting both insulin and gastrin have been described. Otherwise, the syndrome of hyperinsulinemic hypoglycaemia with nesidioblastosis after Roux-en-Y gastric bypass has been previously reported and it is controversial.
Case report
We report on a 53-year-old woman suffering from multiple endocrine neoplasia type 1 (MEN1) confirmed by menin gene mutation analysis. MEN1 disease started with gastrinoma followed by primary hyperparathyroidism. Gastrinoma was located in gastrinoma triangle and presented gastric implants. She underwent tumor resection and a Roux-en-Y gastrectomy. Of 17 months later she began with symptoms of neuroglycopenia owing to endogenous hyperinsulinemia. Suspecting multiple microinsulinomas, a subtotal pancreatectomy was performed. Histopathologic examination revealed nesidioblastosis and several microadenomas. None of them expressed gastrin nor insulin but they expressed glucagon.
Conclusions
This case illustrates a rare cause of hypoglyacemia in a patient with gastrinoma. Although different causes can produce hypoglycaemia in these patients, in our case, the possibility of it being a cosecretor tumor or a neosidioblastosis secondary to hypergastrinemia is unlikely since there were no signs of persistant disease by the time hypoglycaemia started. However, it could be related to nesidioblastosis after Roux-en-Y gastrectomy, as it has been reported after bariatric surgery.

P284

Common variable immune deficiency as a rare cause of osteoporosis

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A 27 year-old-man was admitted to our hospital with pain in the back. Bone mineral density evaluation revealed severe osteoporosis at L1–L4 vertebrae, as well as on femur, too. Secondary osteoporosis reasons like thyrotoxicosis, glucocorticoid therapy, hypercortisolemia, hypercalciuria, hyperparathyroidism were excluded. Laboratory examination of the patient revealed to a hypoglobulinemia. Further evaluation of the immunoglobulin levels were in concordance with panhypoglobulinemia. The patients vit D level was also low. The patient had been diagnosed as to having a 'Common variable Immune Deficieny'. Common variable immunodefiency (CVID) is characterized with B-cell and T-cell dysfunction and hypogammaglobulinemia. Recurrent bacterial infections, diminished Ig levels and impaired antibody production are frequent observed problems of CVID. The most common infections are recurrent otitis media, chronic sinusitis and recurrent pneumonia sometimes leading to bronchiectasia. Almost half of the patients experience problems in the gastrointestinal tract and these may be associated with malabsorption due to chronic diarrhoea. Malabsorption may be observed among CVID patients. Our patients' low vit D level was attributed to malabsorption. Osteoporosis at a young age especially in young men should be always extensively evaluated and accompanying hypoglobulinemia maybe a clue for CVID.

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Treatment of metastatic medullary thyroid carcinoma with sorafenib

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Medullary thyroid carcinoma is an uncommon malignancy of hereditary and sporadic presentation. Mutations in the RET-protooncogene are involved in the pathogenesis of $>50\%$ of the sporadic cases. Currently, there is no effective treatment for metastatic medullary thyroid carcinoma. The aim was to present a case of metastatic medullary thyroid carcinoma that was treated by the administration of sorafenib, a multiple kinase inhibitor.
A patient, female aged 38 years with no family history of MEN syndrome or familial medullary thyroid cancer, presented with a diffuse enlargement of the thyroid gland. The patient underwent total thyroidectomy and left modified radical neck dissection, revealing a left lobe medullary carcinoma invading the right lobe with transcapsular extension and extensive lymph node invasion. Two years later she presented with persistent disease and underwent a radical neck dissection and neck radiotherapy. Four years later an ^{111}In-octreotide scintigram performed showed signs of possible somatostatin receptor positive lung and bone metastatic disease. Four consecutive doses of 150 mCi ^{111}In-octreotide were administered. Calcitonin levels were 12 851 pg/ml (normal values <13 pg/ml). Two years later she presented with extensive metastatic disease, calcitonin levels being 14 914 pg/ml. She was started on sorafenib 400 mg orally twice daily. A month later calcitonin levels were 7322 pg/ml, 2 months later being 8302 pg/ml. The patient developed a malar rash, malaise and transient diarrhea. Surgical resection is the mainstay of treatment for medullary thyroid carcinoma. However, once the carcinoma becomes unresectable there is no effective treatment. Easily administered, active and tolerable agents, such as sorafenib, are clinically relevant when they offer disease regression or prolonged disease stabilization. Further studies are needed to examine the effect of sorafenib in metastatic medullary thyroid carcinoma.

P286

Gasser ganglion as pituitary tumor – case report

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Introduction
The Gasser Ganglion is a large semi lunar-shaped ganglion of the trigeminal nerve. It contains the cells of origin of the most sensory fibers of the fifth cranial nerve.
Aim
Our purpose is to describe a case where Gasser ganglion has an anatomic variant (near the sella turcica), mimicking a hypophyseal tumor.
Case presentation
Of 17 years female patient has a history of bradispaniomenorrhea (menses at 30–50 days) since the last year with no galacthorrea. She associates increased serum prolactin (816 μUI/ml, with normal level below 492), and 6 months later 65.31 ng/ml (normal limit below 24). No other causes of hyperprolactinemia were detected except from a pituitary hypodense tumor of 0.6 by 0.52 cm, having a density of 41 Haussfield Units, as revealed by computed tomography (CT). But also right retrosellar it was discovered another hypodense area, on the median line, which seemed to be an evidential gasserian ganglion. The diagnosis of microprolactinoma was considered and treatment with dopamine agonists as bromocriptin 7.5 mg per day was recommended. 6 months later, the patient tried to stop the medication by her own initiative. The prolactin raised to 117.43 ng/ml (normal below 24 ng/ml). The CT scan showed mainly the same dimensions of the tumor, but a higher density (92HU). The therapy was initiated again.
Conclusion
The anatomic disturbance of the Gasser ganglion represents a differential diagnosis for pituitary or retrosellar masses as microprolactinoma in our case.

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Progression of endocrine hypofunction: a case of polyglandular autoimmune syndrome type 2

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A 52-year-old, previously well Indian lady was admitted in May 2008 with pneumonia. Concurrently, she was diagnosed with diabetes mellitus (blood

glucose 20 mmol/l). Her BMI was 19 kg/m^2 and she was initially commenced onto sulphonylurea therapy. She had a further admission in July 2008 with dizzy episodes, falls, postural hypotension (systolic postural drop 75 mmHg) and erratic blood glucose levels (range 1.1–30 mmol/l). Blood ketones were 3.6 mmol/l without acidosis.

C-peptide (702 pmol/l) was present and islet cell antibodies were negative, but anti-GAD antibodies were positive. The diagnosis was modified to Latent Autoimmune Diabetes in Adults (LADA) and insulin was initiated. An initial short synacthen testing (SST) revealed low basal cortisol but adequate rise post synacthen. Basal ACTH and plasma renin activity were not elevated (8.3 ng/l and 1.17 nmol/l per h respectively), although adrenal antibodies were positive. Fludrocortisone therapy led to some clinical improvement. Further testing revealed subclinical hypothyroidism (free T_4 14 pmol/l, thyroid stimulating hormone 13 mu/l) with strongly positive thyroid peroxidise antibodies ($>$910 u/ml). She was commenced on levothyroxine. During further admissions with unresolving dizziness and nausea, further SSTs revealed an increasingly suboptimal response to synacthen. In summary, we present a case of autoimmune polyglandular syndrome type 2 in development, characterised by autoimmune Addison's disease in combination with autoimmune hypothyroidism and autoimmune diabetes. It is particularly interesting to observe the progression of endocrine hypofunction over time. Furthermore, we hypothesise that the absence of expected rise in plasma renin activity is due to diabetic hyporeninaemia secondary to renal juxtaglomerular glycosylation.

Adrenal investigations		July 2008	September 2008	October 2008
Plasma rennin (nmol/l per h)		1.17		
Aldosterone (pmol/l)		738		
ACTH (ng/l)		8.3		
SST cortisol (nmol/l)	0 mins	117	248	112
	30 mins	519	486	407
	60 mins	681	632	518

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Retroesophageal parathyroid adenoma – scintigraphic and intra-operative scintimetric localization of an ectopic parathyroid adenoma with Tc-99m tetrofosmin: a case report

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Background

Primary hyperparathyroidism (PHPT) is nowadays an asymptomatic disease characterized by mild hypercalcemia and elevated parathormone (PTH) levels. PHPT is caused by parathyroid adenoma in 80–85% of patiens, up to 20% are located ectopically and therefore an ultrasound investigation is not helpful. SPECT sestamibi or tetrofosmin scintigraphy of the neck and thorax is considered to be the optimal method for the evaluation of ectopic parathyroid adenoma.

Case presentation

We report a case of 62-year old female patient with history of left thyroid lobectomy in 2003 presenting with back pain. Biochemical investigations confirmed primary hyperparathyroidism with reduced bone density (T score: -1.6) Ultrasound examination was unsuccesful. Tc-tetrofosmin parathyroid scan and SPECT of the neck and thorax showed uptake of radiotracer in retro-esophageal space. In february 2008 patient underwent primary operation without success. Biochemically sinificant hypercalcemia (Ca 3 mmol/l per N: 2.25–2.75 mmol/l/, Ca^{2+} 1.53 mmol/l per N: 0.9–1.3 mmol/l/) with elevated intact PTH level (206 pg/ml per N: 9–72 pg/ml/) persisted until the radionavigated neck exploration. Perioperative histological study confirmed parathyroid adenoma. Definitive histology revealed a $32\times15\times10$ mm parathyroid adenoma. After surgery the patient was normocalcemic with a normal intact PTH levels.

Conclusion

We report a role of preoperative scintigraphic and intraoperative scintimetric localisation and confirmation of an ectopic parathyroid adenoma and their positive impact on the parathyroidectomy success.

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Glucocorticoid resistance syndrome: treatment with ketoconazole, an efficient therapy solution – case report

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Background

Glucocorticoid resistance syndrome is a rare, familial or sporadic disease, caused by genetic mutations of the glucocorticoid receptor or at any other level of the signaling pathway. It is characterized by general, partial, target-tissue insensitivity to glucocorticoids, leading to activation of the hypothalamo–hypophysis–adrenal axis resulting in compensatory increased levels of cortisol, but also increased concentrations of adrenal products with mineralocorticoid and androgenic activity by stimulating the adrenal with the excessive ACTH concentration. The clinical presentation is chronic fatigue, anxiety, symptoms and signs of mineralocorticoid excess (hypertension, hypokalemic alkalosis) and of androgen overproduction (acne, hirsutism, infertility, precocious puberty). The usual treatment consists in administration of high doses of Dexamethasone.

Case report

We present three cases (2F, 1M, 21–28 years old) with glucocorticoid resistance syndrome. The two female patients presented severe hirsutism, menstrual irregularities and anxiety; all patients presented early-appeared hypertension and obesity. All patients had high plasma cortisol levels, normal to high ACTH levels, increased 24-hour urinary excretion of steroid-metabolites (17OHCS, 17CS), elevated androgen concentrations, normal circadian pattern of cortisol and resistance of HPA axis to dexamethasone suppression. All patients received treatment with ketoconazole 800 mg/day and were evaluated at 3 months, then every 6 months. All patients had a favorable clinical evolution with remission of hypertension, amelioration of hirsutism, weight-reduction and also normalized biological parameters. The male patient presented mild hepatocytolysis, as a side effect of the drug, that remitted after reducing the dose to 400 mg/day.

Conclusions

We consider that treatment with ketoconazole in glucocorticoid resistance syndrome is an efficient and safe alternative to therapy, with favorable effects on clinical and biological features of the disease.

P290

Intramuscular testosterone undecanoate – the experience of 11 years

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A reliable form of androgen substitution therapy in terms of favorable kinetics and tolerance as well as effective restoration of androgenicity is paramount in hypogonadal men. A feasible modality is the intramuscular injection of the long-acting ester testosterone undecanoate (TU).

We report data from 183 patients (99 with primary, 70 with secondary hypogonadism and 14 with late-onset hypogonadism) aged 15–70 years (mean 37 ± 12 years) receiving altogether 2135 intramuscular injections of 1000 mg of TU during a maximal treatment time of 11 years.

The medication was well tolerated and local irritation of the injection site was moderate and did not exceed a duration of 3 days. Serum trough levels of testosterone were generally within the low normal range, indicating sufficient substitution. Individual dosing intervals ranged from 10 to 14 weeks. In accordance, patients reported restoration of sexual functions and convenient changes in mood patterns, e.g. gain of vigor and loss of depressiveness. In contrast to short-acting testosterone esters, sensation of fluctuations in androgen concentrations was rarely reported. Hematocrit was significantly elevated under treatment but remained within the normal range, except for 13 measurements (maximal value 54.4%). PSA concentrations did not exceed 4.0 μg/l, except for one measurement (5.5 μg/l) in a case of later confirmed prostatitis. Bone density generally improved in all patients.

In summary, intramuscular injections of testosterone undecanoate represent a feasible, safe and well tolerated modality of androgen substitution in hypogonadal men of a wide age-range, also on the basis of more than one decade of experience.

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The R106C mutation of the V2 vasopressor receptor gene (AVPR2) causing X linked congenital nephrogenic diabetes insipidus is responsive to short term desmopressin challenge

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Background and aims
Patients with AVPR2 gene mutations present nephrogenic diabetes insipidus (NDI) resulting to a severe deficit in urine concentration despite high levels of circulating Antidiuretic Hormone (ADH). The mutation in codon 106 of the AVPR2 gene leading to the substitution of arginine by cysteine (R106C) is known to produce a mild disease while *in vitro* characterization revealed a complete loss of function. We report the case of a 24-year-old male member of an Algerian family with the R106C mutation. His work and social life was severely affected by a polyuria of 6 l of urine per day. He presented hydro ureteronephrosis and hypotonic enlarged neurogenic bladder needing 7 to 8 intermittent characterizations on a daily basis. He was referred to our clinic for a desmopressin (dDAVP) urine concentration test.
Materials and methods
Blood and urine samples were collected hourly for a period of 6 h. The patient received 2 dDAVP (4 μg) subcutaneous injections at *60 and **180 min. Baseline ADH values were at 27 pg/ml (Reference values: 1.9–2.1).
Results

Plasma osmolality (mOsm/l)	306*	306	298**	308	303	306
Urine osmolality (mOsm/l)	183*	287	288**	293	363	384
Free water clearance (ml/min)	0.8*	0.12	0.05**	0.06	−0.21	−0.20
Secreted AMPc (pmol/ml GFR)	5.5*	5.7	2.8**	9.1	1.5	1.5
Creatinine Clearance (ml/mn/1.73m^2)	80.3*	120.9	97.8**	76.4	103.9	94.8

The rise in urine osmolality, the negativation of free water clearance, the rise in nephrogenic AMPc shows *in vivo* that the R106C mutation is responsive to dDAVP. Hence assuming a mean urine osmolality of 300 mOsm/l under dDAVP treatment the urine output could be limited to less than 3 l.
Conclusion
This case emphasizes that the R106C mutation can present with urological complications and that dDAVP test is useful to predict patient response to treatment in the case of partial NDI.

P292

Clinical analysis of 150 patients with pituitary insufficiency (20 years experience)

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Background
Pituitary insufficiency is an uncommon endocrine disorder (incidence 2–4 per 100 000 per year), which clinical symptoms depend on the degree of hormone depletion and the rapidity of onset.
Aim
To determine the clinical presentation, aetiology and clinical forms in 150 patients diagnosed with pituitary insufficiency.
Material and methods
Retrospective study of clinical records of 150 patients with pituitary insufficiency diagnosed during a 20 years period. Data regarding clinical, biological and radiological work-up were recorded.
Results
Of 115 (76.6%) patients were females and 35 (23.4%) were males; clinical signs at presentation: weakness (96%), amenorrhea (53.3%), loss of pubic hair (53.3%), loss of axillary hair (50.6%), dizziness (37.3%), hypotension (14%), headache (12%), visual disturbances (10.6%); aetiology: ischemia (47.3%), pituitary tumour (18.6%), pituitary surgery or radiation (18%), head trauma (6%), hypothalamic disorders (5.3%), idiopathic (3.33%), infections (1.33%); clinical forms: panhypopituitarism 40 subjects (26.6%), anterior pituitary insufficiency 87 subjects (58.6%), partial deficiency of anterior pituitary hormones 23 subjects (14.6%).
Conclusions
Female patients are more affected by pituitary insufficiency than male patients; most common clinical sign was weakness followed by amenorrhea and loss of pubic hair; ischemia and pituitary tumors represented the major causes of pituitary insufficiency; major clinical form was complete deficiency of anterior pituitary hormones; anamnesis and clinical examination completed by hormonal assessment and radiological investigations are essential for an accurate diagnosis.

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Prevalence and peculiarity of arterial hypertension treatment in acromegaly patients

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Occurrence of arterial hypertension (AH) in acromegaly (A) is the significant risk factor for sudden death. The study group included 232 patients with A aged 47–67 year (mean age 54). The duration of active phase of A was 7–17 years (median 11), GH levels were 23–19.2 ng/ml (mean 6.5), IGF-1 were 223–568 ng/ml (mean 354). AH was found in 186 patients (80.2%): 1st degree – 27%, 2nd – 32%, 3rd – 41%. Of 24-hour arterial blood pressure monitoring showed primary night increase of blood pressure (BP) in 45% patients. Mean levels of systolic and diastolic BP is strongly correlated with the duration of an active phase of A ($r=0.57$ and $r=0.68$) and GH level ($r=0.51$ and $r=0.58$) ($P<0.0001$). Remission in patients with an age of A onset <45 year leads to normalization of BP ($P<0.005$), but this was not found in patients with an onset of A $>$ 45-year-old. ($P=0.24$). To estimate the efficiency and safety of cardioselective beta-adrenoblockers in patients with A, we created the study group included 19 patients with an onset of disease $>$ 45-year-old (53-62-year-old (mean age 59-year-old)). All of them had high BP levels despite the remission of A and the management of antihypertensive therapy (ACE inhibitors – 95%; diuretics – 53%; dihydropyridine calcium-channel blockers – 37%). Criteria of exclusion was occurrence of coronary heart disease, heart failure, rhythm and conductivity disorders in the past. Of 13 patients received Bisoprolol and 6 patients – Nebivolol.
Results
In 32% patients treatment with beta-adrenoblockers led to severe conductivity disorders or sick sinus syndrome that is higher than prevalence of all recorded adverse reaction of Bisoprolol (11.2%).
Conclusion
Prevalence of AH in patients with A is 1.5-fold higher than in general population. The treatment of AH with beta-adrenoblockers in patients with myocardial dysfunction require caution, careful screening and ECG monitoring.

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The Ramadan: do Muslim diabetes patients adhere to their religion or to the doctor?

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Muslims with diabetes are confronted with a dilemma during the annually fasting period: the Ramadan. The Islam foresees a delay of fasting for sick people, but Muslim patients with diabetes do not feel themselves as sick people. How they cope with it?
Muslim diabetes patients are contacted in the outpatient department of three hospitals in Antwerp. Well educated students in diet education with a Muslim tradition, question the patients about their food consumption, treatment and attitudes concerning the Ramadan.
In this ongoing study 64 questionnaires are collected and analysed: 42 females and 22 men. The mean age is 55 years. The median duration of their disease is 10 years and 80% remains more than 10 years in Belgium.

Two third (64%) of the questioned patients follows the Ramadan completely and 5% partially, while 31% do not follow the fasting. Fifty of the 64 interviewed patients know that they can be exempted from fasting because of diabetes. But only 21/50 uses this exemption. Fifteen asked for medical advice: four follows the suggestions of the doctor, but 11 did not. Thirty of the 64 patients took their medication twice a day, while two stops all medication intakes during Ramadan. Sixteen patients remembered that their doctor took the initiative to talk about diabetes and the Ramadan: seven appreciated it very much, while nine do not feel it was necessary. Sixteen patients had no talk with their doctor about the Ramadan, but they would appreciate it. On the other hand, 13 patients would not appreciate if their doctor would talk about the Ramadan and diabetes.
The majority of Muslim diabetes patients in Belgium have the intention to follow the Ramadan. They change their usual food intake and medication schedules according to the rules of the Muslim tradition. Medical advice is seldom asked, but initiative of doctors will be appreciated.

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Adherence of internists and family physicians to SEMT guidelines for type 2 diabetes mellitus in Turkey

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Background
Clinical practice guidelines on diabetes mellitus (DM) have been developed in 2006 by The Society of Endocrinology and Metabolism of Turkish (SEMT). The ongoing ADMIRE Project is designed to evaluate the effect of implementation of various activities to increase the awareness of physicians on the adherence to SEMT guidelines. The first phase results of the project is presented.
Methods
Of 180 physicians evaluated previous 12 months' medical records of their type 2 DM patients with special emphasis on whether the patients were followed consistently with SEMT guidelines. This report depends on the analysis of 6032 visits of 1790 patients.
Results
Of 62% of patients were women, mean duration of DM 7.7 years and mean age 58.7 years. Of 60% of the patients had any chronic and ¼ of any acute complication within previous year. Of 96% was under pharmacotherapy (61% OAD,15% insulin and 20% OAD + insulin). The rate of SMBG was 40% at first, and increased to 51% by the end. Overall 30% of the patients was not in full compliance with SEMT guidelines in any visit within the previous year (DM symptoms 6%, acute 12% and chronic complications 10%, CV risk factors 6% and family history of DM 15%). In only 8% of patients, physical examination was performed in full compliance with SEMT guidelines at least once (height 48%, weight 40%, waist 74%, BP 11%, thyroid 35%, neurologic 46%, feet 35% and eye 57%). In only 18% of patients, laboratory evaluation was performed in full compliance with SEMT guidelines at least once (lipid profile 6%, creatinine 15%, microalbuminuria 72% and ECG 21%). Only 16% of patients were at A1C target ($\leq$6.5%) at first visit, this gradually increased to 23% (proportions at target at first FBG 14%, PPBG 10% and increased to 29 and 17% by the end of the year). Mean A1C decreased from 8.5% at first visit to 7.6%, FBG from 190 mg/dl to 153 mg/dl, and PPBG from 236 mg/dl to 195 mg/dl during the previous year. Proportion of patients with good glycemic control was lower in females ($P=0.042$), with longer duration of DM ($P=0.38$), and the number of chronic complications ($P=0.002$). Adherence to physical and laboratory recommendations was associated with good glycemic control.
Comment
The level of medical care offered to patients with DM in Turkey is not sufficient. Physicians should be specifically trained to increase the level of adherence to guidelines during their clinical practice.

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Subclinical hypothyroidism as a cardiovascular risk factor

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Controversy remains as to the risk of cardiovascular disease (CHD) associated with subclinical hypothyroidism (SCH). Substantial evidence indicates altered cholesterol and lipoprotein metabolism in SCH when serum TSH is above 10 mUI/l. It is apparent that an enhanced CV risk could apply to these patients, amplified by the co-presence of other risk factors such as endothelial dysfunction and elevated C-reactive protein.
We assessed the association between SCH and CHD. We investigated 69 patients, 35 with SCH and 34 euthyroid subjects, with baseline TSH measurements and 24 months follow-up data to determine whether SCH was associated with coronary heart disease; the prevalence of CHD in subjects with subclinical thyroid dysfunction was evaluated. Subjects with SCH had a significantly higher prevalence of CHD than euthyroid subjects OR, 2.2, 95% CI, 1.2–3.8, TSH:7.0–9.9 mUI/l, *P*: 0.02; OR, 3.2, 95% CI, 1.8–8.9, TSH over 10 mUI/l, *P*: 0.01. In multivariate analysis, the risk of CHD was higher among those with high TSH levels (TSH: 7.0–9.9 mUI/l, HR, 2.49, 95% CI, 1.17–5.3, *P*: 0.02, TSH over 10 mUI/l, HR, 3.27, 95% CI, 1.59–6.34, $P<0.01$). Among the 56 participants without CHD at baseline, the HR for incident CHD events was higher among those with TSH over 7.0 mUI/l (TSH: 7.0–9.9 mUI/l, HR, 1.6, 95% CI, 1.0–2.6, *P*: 0.04; TSH over 10 mUI/l, HT, 2.5, 95% CI, 1.3–5.3, $P<0.01$). The increased risk of coronary heart disease events remained significant after adjustment for standard cardiovascular risk factors. Subclincal hypothyroidism is associated with an increased risk of CHD events among adults with a TSH level of 7.0 mUI/l or greater. Subclinical hypothyroidism may be an independent risk factor for coronary heart disease but investigation is warranted to assess whether SCH causes/worsens CHD.

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Sustained response to interferon α in a patient with an advanced metastatic serotonin secreting endocrine tumour – case report

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This 52-year-old lady presented in 2001 at a GI clinic complaining of occasional abdominal cramps, which could be severe and prolonged. Her symptoms were not associated with diarrhoea or constipation. Weight loss of 4.5 kg over 4 months was noted. Coeliac disease was excluded and a diagnosis of severe irritable bowel was made.
In January 2002 she returned to the clinic with further weight loss (total 8 kg), cyclical symptoms of diarrhoea lasting 3–5 days and occasional flushing. Neuroendocrine tumour markers were measures. Urinary 5HIAA was grossly elevated at 637 (RR10–47), 5HT 12.05(RR0.30–1.30), pancreastatin (PST) > 1000 ng/l (RR<50) and neurokinin A (NKA), an independent indicator of poor prognosis was 350 ng/l (RR<20). CT and Octreotide scintigraphy showed extensive hepatic metastases with para-aortic and iliac lymphadenopathy. No primary tumour was identified. The surgical team considered hepatic disease to be inoperable.
Treatment with somatostatin analogues was commenced. Symptoms continued, urinary 5HIAA remained grossly elevated and PST and NKA continued to rise dramatically. The somatostatin analogue dose was increases on two occasions with no improvement. Interferon α, concomitant with somatostatin analogues, was commenced, 1.5 MU 3 times weekly increasing to 9 MU 3 times weekly. Within 2 months symptoms eased and this regime was continued. By 6 months symptoms had abated. Urinary 5HIAA settled around the upper limit of normal and 5HT returned within the reference range after 18 months. Circulating NKA was secured below 100 ng/l within a year and has been maintained 40–80 ng/l thereafter. Both PST and Chromogranin A have remained >10 fold reference range. Scans show stable/reduced disease.
Due to symptoms of migraine and fatigue the dose of interferon has been reduced from time to time and the drug has been withdrawn for short periods. This has resulted in an immediate rise in NKA. Survival, post diagnosis now approaches 7 years.

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Primary hyperparathyroidism in the eastern black sea region of Turkey: a description of 101 cases

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Background
The most common etiology of hypercalcemia is primary hyperparathyroidism (PHPT). PHPT is a disorder of calcium, phosphorus and bone metabolism

secondary to uncontrolled increased parathyroid hormone secretion. PHPT has a variable clinical expression. Symptomatic PHPT is still the predominant form of the disease in many parts of the World, especially in developing countries.
Methods
We summarized the clinical presentation, biochemical and radiological features, and operative findings from the case records of the last 16 (1992–2008) years including 101 patients at a tertiary care centre in the Eastern Black Sea region of Turkey who had documented PHPT.
Results
The female:male ratio was 4.9:1 with ages ranging from 19 to 84 years (mean ± S.D., 56.0 ± 13.54 years). Renal symptoms were the major symptoms in 61 patients (60.3%) followed by gastrointestinal symptoms in 59 patients (58.4%) and weakness/fatigue in 54 patients (53.4%). Common clinical manifestations included nocturia (40.5%), bone pain (36.6%), constipation (32.6%), polyuria (31.6%). Renal stone was present in 31 patients (30.6%). Hypertension was observed in 43 patients (42.5%). Only two patients were asymptomatic. In seven patients, serum calcium level were in normal range while serum calcium levels were higher than normal in other patients. Mean intact PTH level (±S.D.) was 461.27 ± 553.14 pg/ml. In direct bone X-rays examination had 49 patients (48.5%) had salt-pepper appearance of cranium, 37 patients (36.6%) had subperiosteal resorption, four patients (3.9%) had bone cysts and Brown tumor and six patients (5.9%) had pathologic fractures. Seventy patients (69.3%) undergone were operation.
Conclusion
Almost all of the patients presented with late symtoms and complications of PHPT. Serum calcium and phosphorus were the best screening tests for the diagnosis of PHPT in this series. The diagnosis should be further confirmed determining the intact PTH level.

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Nuroendocrine tumours of unusual localization

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Neuroendocrine tumours (NETs) are rare neoplasm arising from dispersed endocrine system. Their incidence is estimated to be five cases per 100 000 population, although the true incidence may be higher as the consequence of often oligosymptomatic course of disease and indolent behaviour of NETs. The most typical localization of NETs is gastrointestinal tract and bronchi. However they may develop in every organ of the body containing neuroendocrine cells. The diagnosis of NET is usually made based on symptoms related to (1) the endocrine functuion of the tumour and the type of the secreted biologically active molecules and (2) local invasion. NETs of atypical presentation are most often found because of the local symptoms or incidentally. The possibility that type of the tumour is the distant metastasis of the more common type of NETs should always be considered. Iit is obligatory in every case of rare NET (including rare localization within gastrointestinal tract, i.e. gallbladder or Meckel's diverticulum) to search for other possible primary lesion, particularly by scintigraphic methods. The aim of the study is to present NETs of rare localization from the material of The Chair and Department of the Endocrinology, CM UJ. In NET database of Chair and Department of Endocrinology of the Medical College of the Jagiellonian Univeristy, comprising 244 patients mostly from south-eastern part of Poland, 11 tumours of unusual origin has been registered so far. They are: 4 NET of Vater's ampulla, 2 NET of gall bladder, 1 NET of Meckel's diverticulum, 1 ovarian NET, 1 epiglottic NET, 1 thymic NET and 1 NET arising from sphenoid sinus. All of them were diagnosed because of the local symptoms. Early excision of the lesion resulted in achieving disease regression. Based on that cases descriptions authors will discuss diagnostic and therapeutic pathway in NETs of rare localization.

P300

Successful pregnancy in a patient with Carney's complex, primary pigmented nodular adrenocortical disease and biochemical cortisol excess

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Primary pigmented nodular adrenocortical disease (PPNAD) is a rare cause of ACTH independent adrenal Cushing's syndrome. A 32-year-old female with atrial myxomas was found to have Carney's complex (PRKAR1A mutation negative). Screening showed biochemical Cushing's syndrome (24 h urine free cortisol 918 nmol – nr 100–400) and the characteristic paradoxical increase in UFC during the 48 h dexamethasone suppression test was confirmed: 24 h UFC pre-dex 340 and 316, low dose dex 472 and 583, high dose dex 1261 and 1699 nmol. CT adrenals showed bilateral nodularity. She had impaired 75 g glucose tolerance (2 h glucose 10.8 mmol/l), HbA1c 5.5% (nr 4.4–6.4%). Clinically BMI 22 kg/m^2, normotensive, mild skin thinning over hands and forearms and mild facial plethora. Prior to planning IVF pregnancy bilateral adrenalectomy was recommended, but declined. Untreated Cushing's in pregnancy is associated with hypertension (68%), diabetes (25%), pre-eclampsia (14%), septicaemia and wound infection (2%) as well as adverse effects on the fetus, although the natural history of pregnancy with PPNAD unknown. The presence of a mechanical mitral valve (following resection of two large atrial myxomas) raised additional concerns. Close monitoring was maintained throughout pregnancy. Late fetal growth deceleration prompted delivery by caesarean section at 36 weeks (live female 2010 g), the only other complications being haematoma and blood loss relating to anticoagulation. Both are well 1 yr later. Glucose tolerance and blood pressure were normal throughout, with no clinical or biochemical features of disease progression. 24 h UFC near term was 457 nmol (normal for pregnancy). We are aware of only one published case of PPNAD in pregnancy which reports that hypercortisolism was exacerbated. *In-vitro* studies revealed dose-dependent stimulation of cortisol production by oestradiol. The patient reported here emphasises that the pregnancy outcome of patients with PPNAD is not universally poor and hints at phenotypic heterogeneity possibly related to genetic heterogeneity in this rare condition.

P301

Hungry bone syndrome following thyroidectomy for thyrotoxicosis: case report

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Hungry bone syndrome is common in tertiary hyperparathyroidism after parathyroidectomy. Less frequently, it has been described after thyroidectomy in patients with hyperthyroidism. We hereby report a case of hungry bone syndrome in a patient who suffers with Graves' disease.
A 44-year-old woman with a history of refractory Graves' disease presented with 4 weeks history of shortness of breath, palpitations and peri-oral paraesthesia following total thyroidectomy. She had previously been treated with high doses of carbimazole for 18 months followed by radioactive iodine. She remained thyrotoxic and after refusing further radioactive therapy underwent total thyroidectomy. Following post-operative hypocalcaemia she was discharged on Adcal 1.5 g TDS, Alfacalcidol 0.5 mg OD and levothyroxine 100 mcg. On the day of admission, laboratory results revealed a profound hypocalcaemia (total calcium 1.1 mmol/l; ionized calcium 0.53 mmol/l) and hyperphosphataemia (2.66 mmol/l) with undetectable levels of parathyroid hormone. Echocardiogram showed tachycardia with prolonged QT of 0.55 s. She was treated with intravenous calcium infusion which restored her calcium levels, however within 12 h her calcium levels fell rapidly, causing a tonic-clonic seizure. After initial stabilization with intravenous calcium administration, oral calcium carbonate and calcitriol were required. She continued to require intravenous calcium infusion every 2 to 3 days and her oral calcitriol supplementation was increased gradually to 16 mg daily over a period of four weeks. There was an unusually high need for calcium combined with a low calcium excretion in the urine. Bone-specific ALP continued to rise in the first few weeks indicating increased bone reconstruction. Following prolonged hospital stay, she was discharged on high doses of vitamin D and elemental calcium. Six months postoperatively it was possible to gradually reduce her calcitriol to 5 mg daily with 2 g calcium supplement. This case highlights hungry bone syndrome in a case of severe and prolonged thyrotoxicosis treated with total thyroidectomy. This condition can be monitored by the use of calcium profile investigations, including bone-specific ALP.

P302

Paraneoplastic Cushing's syndrome due to prostate cancer: a rare occurrence

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We present the case of a 72-year-old gentleman was admitted to our hospital with atrial fibrillation secondary to severe hypokalemia of 1.8 mmol/l, but normal serum sodium and creatinine. He had prostate cancer with extensive liver and bone metastases. After potassium supplementation 180–200 mmol/day for 10 days serum levels persisted between 2.3 and 2.9 mmol/l. During this short period he developed early Cushingoid features, jaundice and diabetes. After overnignt dexamethasone 1 mg, his serum cortisol was 1988 nmol/l with corresponding ACTH of 434 ng/l (0–46). Free cortisol in 24 h urine was >2942 nmol/l (<350). We treated him with metyrapone which normalised potassium levels at 3.7 mmol/l within 4 days enabling us to stop supplements. Unfortunately he died from metastatic prostate cancer 6 weeks after initial presentation.
Cushing's syndrome as a paraneoplastic manifestation of prostate cancer is rare and prognosis after its onset is poor, survival ranging from a few days to 3.5 months in various case reports. We used metyrapone as it is short acting, has few adverse effects and is effective in doses from 250 mg to 6 g daily thus lending itself to precise titration. Prolonged therapy with metyrapone can cause production of adrenal androgens which could be detrimental in a patient with prostate cancer. However it is unlikely to be significant in this setting where the life expectancy is very short.
There is one previous case report of metyrapone being successfully used as single agent to control steroid excess in the setting of prostate cancer. Metyrapone is underused probably because of restricted availability and unfamiliarity of oncologists with the drug.

P303

Large intrathoracic goiter mimicking lung cancer

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Herein, we present a case of a 68-year-old woman who had hyperthyroidism and a large mass lesion in the upper portion of her right lung. The patient had a history of previous subtotal thyroidectomy 40 years before. A few years after the operation she noticed some enlargement on her neck. Two years before the admission she was evaluated for some unrelated complaint and a large mass was discovered on her right lung on X-ray examination and she was considered to have a lung cancer at first sight. Tomographic examination revealed a big mediastinal mass of 120×100 mm compressing and deplacing the adjacent structures on the right upper lung region. Biopsy of the lesion was in concordance with thyroid tissue. A scintigraphic evaluation revealed a big thyroid tissue extending from the right thyroid lobe and was in concordance with a large intrathorasic goiter. Her TSH <0.005 μU/ml (*N*: 0.27–4.2). FrT3 was 5.65 (*N*: 2.0–4.4), and FrT4 was 2.45 ng/ml (*N*: 0.93–1.7) and she was thyrotoxic. Despite long-term antithyroid medication at maximal doses she remained in hyperthyroid status. Before surgery plasmapheresis was performed several times in order to achieve an euthyroid status.

P304

Double primary ovarian malignancies in a patient with bipolar disease complicating Swyer syndrome

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Background
Swyer syndrome is a type of pure gonadal dysgenesis 46 XY caryotype in phenotypically female patients. A rare case of Swyer syndrome complicated by dysgerminoma and Retiform Sertoli-Leydig cell's tumor is reported.
Case presentation
A 20-year-old phenotypically female patient presented to our department with primary amenorrhea. She was 1.68 m and weighed 82 kg (BMI 28). She presented with minimal breast development, sparse axillary and pubic hair and normal female external genitalia. Medical history included bipolar depression with two severe suicidal attempts. Endocrinological evaluation demonstrated hypergonadotropic hypogonadism. Abdominal imaging revealed hypoplastic uterus and streak gonads. Chromosomal analysis was performed and the karyorype proved to be 46 XY. The patient underwent diagnostic laparoscopy; this confirmed the ultrasonographic diagnosis and bilateral gonadectomy was performed.
Results
The histopathologic examination revealed dysgerminoma in the left gonad and Retiform Sertoli-Leydig cells tumor in the right gonad. Surgical staging was performed via bilateral pelvic lymphadenectomy accompanied by omentectomy. Peritoneal random biopsies and histology did not reveal metastatic disease. Two sessions of carboplatin chemotherapy were administered and treatment with estrogen was prescribed. Three months later the patient presented with deterioration of her psychiatric condition with frequent episodes of mania in spite of medical therapy.
Conclusion
Because the occurrence of malignancy in dysgenetic gonads is high, early diagnosis and prophylactic removal of the dysgenetic gonads is essential. Hormonal replacement therapy should be given with caution in patients with underlying bipolar disease because of the possible destabilizing effects of estrogens.

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Three cases with inappropriate TSH syndrome

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Patients with inappropriate TSH syndrome have elevated serum free thyroxine (FT4) and free triiodothyronine (FT3) concentrations and normal or slightly elevated serum thyroid stimulating hormone (TSH) level. Inappropriate TSH syndrome include generalized resistance to thyroid hormone (GRTH), pituitary resistance of thyroid hormone (PRTH) and TSHoma.
We report here three cases that the first patient has GRTH, the second has TSHoma and third patient has PRTH. Patient with GRTH has goitre and normal TSH and high serum levels of FT3 and FT4. The patients with GRTH and PRTH have sufficient TSH respond to TRH stimulation test. After T3 suppression test, patient with GRTH has unelevated SHBG but ferritin level was elevated. Whereas patient with PRTH has elevated SHBG and elevated ferritin levels. The patient with GRTH and the patient with PRTH has not pituitary adenoma on pituitary MRI. Patient with TSHoma has inappropriate TSH secretion, pituitary adenoma on pituitary MRI and elevated α subunit/TSH molar. The patient with TSHoma has insufficient TSH respond to TRH stimulation test.
As a result; if the relation between T3, T4 and TSH can not be explained, GRTH, PRTH and TSHoma diseases should be kept in mind and the necessity tests for differential diagnosis should be made for inappropriate TSH syndrome.

P306

Stromal luteoma of the ovary: case report

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Introduction
The stromal luteoma of the ovary is a rare tumour occurring mostly in post menopausal women. Endocrine symptoms and sometimes virilizing signs may be observed, although abnormal vaginal bleeding is the most frequent manifestation. This tumour is surrounded by ovarian stroma being entirely composed of luteinized cells devoid of crystals of Reinke. Hyperthecosis of ovarian stroma is often observed. Its evolution is always benign.
Case report
Women, 64 years old, revealing history of hypertension, obesity, primary infertility and obstructive sleep apnoea. The patient was sent to the outpatient clinic for hirsutism, alopecia androgenetic for the last three years and reduced

libido. Her physical examination revealed hirsutism (score >6, Ferriman-Gallwey scale) and frank virilization. She had a normal gynecological examination. Her serum testosterone and 17-hidroxiprogesterone levels were increased with normal serum androstenedione and DHEA levels. The abdominal–pelvis axial computerized tomography and the pelvis magnetic resonance imaging showed a solid nodule, with 16–19 mm of diameter in the left ovary, confirmed by endovaginal ultrassound. The patient underwent a bilateral oophorectomy and the histology revealed a stromal luteoma of the ovary. After surgery, the patient revealed clinical improvement and rapid normalized the androgen levels.

Discussion

The diagnosis of virilizing tumours of the ovary is often difficult and challenge, especially in small tumours, not detectable in gynaecological examination. In a women with virilization signs is essential a careful gynaecological examination, the measure of serum androgens and the axial computorized tomography of adrenal and ovary to exclude an androgen production tumour. Nevertheless, the transvaginal ultrasound is the most sensitive method for the detection of an ovarian tumour.

P307

Coincidence of primary hyperaldosteronism with thyrotoxic nodular goiter presenting as hypokalemic periodic paralysis: complicating or mimicking one another?

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Thyrotoxicosis and primary hyperaldosteronism both cause hypokalemic periodic paralysis. A 51-year-old woman, with a history of 3 episodes of transient muscle weakness, was admitted to the emergency unit with complaint of the weakness of legs. Her medical history included hypertension for 10 years. A nodule approximately 3 cm in diameter was palpated in the left anterior neck. Decreased strength (2/5) and deep tendon reflexes in lower extremities symmetrically with normal sensory examination were detected. Initial laboratory findings were significant for a potassium of 1.5 mEq/l and sodium of 148 mmol/l. Thyroid function tests were compatible with primary hyperthyroidism with a hyperactive nodule in scintigraphy. The patient was prescribed propylthiouracil. Her potassium was replaced. She completely regained muscle strength. A diagnosis of thyrotoxic hypokalemic periodic paralysis was supposed. Nevertheless, a decrease in potassium level was observed in each time, immediately when replacement of potassium was stopped. A high level of aldosterone 51.6 ng/dl with supressed renin 0.2 ng/ml per hour, and the high ratio of aldosterone to renin (258) were compatible with the diagnosis of primary hyperaldosteronism. Plasma aldosteron was found to be 66.8 ng/dl after saline infusion test. Imaging of surrenal glands showed an adrenal mass on the left side. The diagnosis of hyperaldosteronism was supposed. Spironolactone, 200 mg/day, was started gradually. On the second week of therapy the patient became normokalemic without support of oral potassium perchloride. Spironolactone 200 mg/day and amlodipin 10 mg/day was enough to control her blood pressure. Whether thyrotoxicosis or hyperaldosteronism triggered hypokalemic periodic paralysis in this patient is a matter of debate. Two cases of thyrotoxicosis and primary aldosteronism complicating with hypokalemic periodic paralysis have been introduced to literature to date. In conclusion, adrenal function should be considered in a patient with hypertension and hypokalemia whatever the presentation of cases are.

P308

Hypergonadotropic hypogonadism due to vanishing testis syndrome: case report

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Introduction

Anorchia or vanishing testis syndrome is defined as the absence of testicular tissue in genetic and phenotypic males. To establish this diagnosis certain criteria must be present, namely non palpable testis during examination under anaesthesia and blind ending spermatic vessels visualized within the retroperitoneu, or the spermatic vessels and vas deferens exiting a closed internal inguinal ring. Bilateral congenital anorchia affects one in 20 000 males.

Case report

Man, 21 years old, has been referred to the outpatient clinic when he was 13 years old, due to non palpable bilateral testis. Serum testosterone level was low (<10 ng/dl) with increased levels of LH(29.26 mUI/ml) and FSH (120.48 mUI/ml). The chromosomal analysis discloses a 46, XY karyotype. He underwent a surgical exploration laparoscopy, where no testis were found, followed by a bilateral inguinal exploration that showed the spermatic vessels and vas deferens exiting a closed internal inguinal ring. The diagnosis of vanishing testis syndrome was then established and where implanted testicular prostheses. The remnants were removed and underwent pathologic examination, which identified the presence of tissue compatible with vas deferens; no viable germ cell elements were identified. The patient is nowadays under androgen replacement therapy.

Discussion

There is controversy regarding the optimal management of the testicular remnant associated with the vanishing testis syndrome. Some urologists suggest surgical exploration, either via laparoscopy or an inguinal/scrotal approach, whereas others believe these procedures are unnecessary. These different opinions are based on the differences in the reported incidence of viable germ cell elements within these remnants and the subsequent concern for future malignant degeneration.

P309

A case of giant-cell jaw tumour and primary hyperparathyroidism

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Patient DE, a 49-year-old woman, was operated in 2005 for two giant-cell tumours of the mandible, and in 2008 for another tumour with the same localisation and histology. Inferior cervical ultrasound after the second surgery revealed a parathyroid adenoma of $20\times27\times15$ mm behind the lower pole of the right thyroid lobe, confirmed by Tc tetrofosmin scintigraphy. The patient had metabolic features suggestive for primary hyperparathyroidism: calcium – 10.9 mg/dl (normal range – 8.5–10.2), phosphate – 1.6 mg/dl, (2.4–4.1), calciuria – 376 mg/24 h, (50–250), in the presence of very high serum levels of parathyroid hormone – 1311 pg/ml, (10–69). DXA-evaluated BMD was in the osteoporotic range at the spine (*T* score of – 3.3), but not at the hip and radius level. The patient was submitted to parathyroid surgery. The lower right parathyroid gland contained a parathyroid adenoma with oxyphylic cells. The surgeon excised a second lesion at the opposite side, suspected to be a second parathyroid adenoma, but proving by histology to be a thyroid adenoma. PTH levels remained however increased one month after surgery (124.5 pg/ml) with abnormal metabolic profile, suggesting remnant hyperparathyroidism and implicitly the presence of at least one other parathyroid adenoma. A new surgical intervention was therefore scheduled. Jaw brown tumours with giant cells may represent the unique symptom of primary or secondary hyperparathyroidism, but may equally be a sign for the rare hyperparathyroidism–jaw tumour syndrome produced by autosomal dominant mutations of the HRPT2 gene (1q31.2), encoding parafibromine, a tumour suppressor with apoptotic effect. Although no familial aggregation was known, the presence of multiple adenomas and the histological aspect, with frequent nuclear inequalities, were suggestive for the hyperparathyroidism–jaw tumour syndrome, and investigation of the HRPT2 gene was therefore initiated. If mutation is found, genetic screening of all first degree relatives is of importance.

P310

α-Adrenergic blockade with doxazosin: case report

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Pheocromocytoma is a catecholamine-secreting tumor that arises from chromaffin cells of the adrenal medulla. In general, they are unilateral and the treatment of choise is complete surgical resection. Surgery and other medical procedures such as chemotherapy or radiotherapy may result in massive catecholamine release that can be fatal. Some form of preoperative pharmacologic preparation is indicated for all patients to control blood pressure, arrhytmia and promote intravascular volume expansion. Combined α and β-adrenergic blockade is the adopted approach by most centers. Until now, phenoxybenzamine has been the preferred α-adrenergic blocking agent. However, due to the its recent unavailability, we

used other agent, a selective α1-adrenergic blocking agent – doxazosin. The authors report three different clinical cases in which doxazosin was used.
Case 1
A 58-year-old woman with a malignant pheocromocytoma with hepatic and vertebral ganglia metastatic lesions in whom bilateral adrenal resection had previously been made. She was now submitted to tumor irradiation with therapeutic doses of ^{131}I-MIBG.
Case 2
A 65-year-old woman submitted to right adrenalectomy for a pheocromocytoma.
Case 3
A 36-year-old woman with MEN2A who had been previously submitted to bilateral adrenalectomy and radical thyroidectomy in another institution. She had a biochemical and imaging confirmed relapse of disease (left adrenal *loca* node, localized by ^{123}I-MIBG) and refused surgical management. She came for the first time to our department already pregnant – 26th week of gestation. Elective cesarean section was performed in the 38th week of gestation.
Doxazosin was a safe and effective alternative in these three cases. There is no great experience with this agent, specially during pregancy. Its more favorable side effect profile compared to fenoxybenzamine may be an advantange and will be reviewed in this presentation.

P311

Graves' disease and thymic hyperplasia: case report

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Graves' disease is characterized by the occurrence of antibodies against thyroid-stimulating hormone (TSH) receptor that stimulate the gland to produce T4 and T3. It can be accompanied by an infiltrative orbitopathy and oftalmopathy. Another seldom- regognized feature of this disease is thymic hyperplasia.
The authors report the case of a 22-year-old woman with Graves' disease (TSH receptor antibodies 178 U/l) with exuberant oftalmopathy and an incidentally discovered anterior mediastinal mass with no invasive characteristics. She began treatment with a thionamide agent (tiamizol) with great improvement of the thyrotoxic state and concomitant reduction of the thymic mass dimension. This patient was submitted to total thyroidectomy and one year later the thymic mass had totally regressed.
Although thymic hyperplasia is a common and reversible feature in Graves' disease, in most of the cases thymic enlargement is minimal and a radiologically detectable massive enlargement of the thymus is infrequently reported. The recognition of this association is very important. If the thymic mass radiological characteristics suggest benignity and if it shrinks with Graves' disease improvement, one should treat Graves' disease and radiologically watch the mass. This attitude will spare a major surgery – thymectomy.
It has been suggested that the thymus gland has TSH receptors and that might be the pathophysiological explanation for this association.

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Case report: papillary thyroid carcinoma in a patient with Pendred syndrome

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Objective
Pendred syndrome is an autosomal recessive disorder characterized by sensorineural hearing loss, goiter, and a partial defect in iodide organification. Pendred syndrome is caused by mutations in the SLC26A4 gene. Here, we report a patient with pendred syndrome and papillary thyroid carcinoma.
Case report
A 19-year-old man admitted to our clinic with swelling in his neck. Congenital hypothyroidism was diagnosed at the age of one. His twin brother and his sister had been diagnosed as congenital hypothyroidism when they were one year old. In physical examination, a visible goiter was present and the thyroid was enlarged with multiple palpable nodules. In laboratory examination serum TSH was 2.1 μIU/ml (0.4 to 4.0), free T4 was 0.87 ng/dl (0.85–1.78), free T3 was 4.21 pg/ml (1.57–4.71) and Thyroglobulin was >300 ng/ml (0–55). Ultrasonographically, there were multiple nodules in thyroid. Thyroid scintigraphy showed hypoactive nodular goitre. Perchlorate discharge test revealed increased uptake and washout. Increased discharge pointed to an organification defect as in Pendred syndrome. Fine needle aspiration biopsies were benign. Sensorineural hearing loss was not detected. Bilateral total thyroidectomy was performed because of cosmetic complaints and enlarged multinodular goitre. Postoperative histopatology was reported as papillary thyroid carcinoma of 13 mm. There was no capsular or vascular invasion. After surgery 100 mCI radioactive iodine was given to the patient and also L-thyroxin was started.
Conclusion
Goiter is the most frequent symptom of Pendred syndrome and associated with an insufficient thyroid hormone synthesis caused by a defect in iodide organification. Pendrin expression has been studied in various human benign and malign thyroid neoplasms. Thyroid carcinoma can be seen in patients with Pendred syndrome. Recurrence of benign thyroid nodules after thyroidectomy in these patients is common. Therefore, if surgical management is considered for any reason in these patients, total/near total thyroidectomy should be preferred.

P313

The study of an immunohistochemical aggresivity marker in mammary carcinomas

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Introduction
The mammary cancer is the most frequent malign tumor encountered in females, characterized by a high distant metastasis tendency. Among the potential prognosis factors, we mention the biomarkers that measure or are associated with biologic processes involved in the tumorous progression. The study analyzes the p53 protein's positivity in correlation with the mammary cancer's classical prognosis factors: the hystologic type, the histopathologic degree, the clinical stage and the status of the axial lymphonodules.
Purpose
The immunohistochemical evaluation (IHC) of an aggresivity marker in mammary cancer.
Methods
Using the immunohistochemical method of ABC Elite avidin–biotin complex staining and the p53 human anti-protein mouse monoclonal antibody, the DO 7 clone (1:500 dilution) on tissue sections fixed in 10% formaldehyde and included in paraffin, we have obtained a red staining of the tumorous cells' nuclei.
Results
Out of 40 mammary carcinomas where we have immunohistochemically determined the p53 protein, we have assessed that 29 of them proved to be negative, 6 had a moderate staining and 5 had an intense staining. Several studies estimate that the over-expression of the p53 protein is comprised between 25 and 50% of the cases.
The p53 immunoreactivity was more frequently encountered in pre-menopausal women and in invaded axial lymphonodules tumors. We remarked a strong connection between the p53 over-expression and the studied tumors' grading.
Conclusions
The results of the p53 staining present some variations, depending on the laboratories where the research has taken place (between 21.5 and 52% with different antibodies and on a different number of cases). In the studied cases, the percentage of p53 positive cells was of 27.5%. The p53 protein over-expression can be useful in establishing the mammary carcinomas prognosis, only if it is analyzed in connection with other factors, thus improving the information provided by them: therefore it contributes to the identification of the patients with an increased risk of disease progression.

P314

Schmidt's syndrome atypical case

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Thyroid pathology, as a part of Schmidt's syndrome, could be presented as a chronic autoimmune thyroiditis (95–97%) or as a Grave's disease (3–5%). Usually refractory hypothyroidism develops after a chronic autoimmune thyroiditis occurs, so the permanent thyroid hormone replacement is necessary during all life. But we are investigating a different course of that disease.

A 31-year-old woman had been sick from 2003, when Schmidt's syndrome was diagnosed by typical clinical and laboratory symptoms. Thyroid pathology was presented as a hypothyroidism after autoimmune thyroiditis, so a replacement therapy by thyroid hormones, mineralocorticoids, corticosteroids was administered. After hormones administration her general condition improved quickly and had been good until 2007; laboratory data demonstrated compensation of thyroid and adrenal insufficiency. In 2007, the patient appeared with weight loss, weakness, severe tachycardia, anxiety. Laboratory data revealed that a thyroid-stimulating hormone (TSH) serum level decreased below 0.01 mIU/l, free thyroxin – elevated to 58.8 pmol/l. L-thyroxin dose was reduced, but symptoms of thyrotoxicosis remained. After that a thyreostatic therapy with thyrozol administration was provided. The patient's condition and laboratory data were controlled each three months, so the therapy type – thyrozol or L-thyroxin, or combination – was changed according to the investigation results. In June 2008, it was decided to cancel the whole thyroid therapy, after that and until recent time the patient felt good, and the laboratory investigation during this period demonstrated euthyroidism.
It's well known that a similar disease course of thyroid pathology could occur in case of an isolated autoimmune thyroiditis, but we did not find such a clinical case description as a part of Schmidt's syndrome in literature. Thyrotoxicosis combined with the adrenal insufficiency becomes a more dangerous condition than when alone, so in Schmidt's syndrome case that condition should be diagnosed and treated in early stages.

P315

Non-insulinoma pancreatogenic hypoglycemia syndrome (NIPHS): recently described disease entity: case report

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Introduction
Case reports of individuals with non-insulinoma hyperinsulinic hypoglcaemia of organic origin has been more frequently described in literature. This syndrome has been described as a rare complication of bariatric surgeries and the term of NIPHS (non-insulinoma pancreatogenic hypoglycemia syndrome) has been proposed.
Aim
The aim of our study is to present patients case who had previously undergone the gastric surgery. Bilroth II and finally diagnosed of NIPHS.
Case description
A 45-year-old man who had previously undergone gastric surgery, was refered to our hospital for evaluation of hypoglycemia. At the beginning, the patient experienced only episodes of postprandial hypoglycaemia and subsequently episodes of fasting hypoglycaemia appeared. Diagnostic imaging including ultrasound, CT, Octreoscan and EUS were negative for the patient. Post-operative changes and chronic pancreatitis impeded the interpretation of obtained results. Farmacological treatment with Proglicem or somatostatine analogues led only to transient improvement in control of hypoglycemic status.
Because pharmacological treatment was ineffective patient undergone partial pancreatectomy.
Finally the diagnosis of NIPHS has been established based on postoperative histological diagnosis established after partial pancreatectomy.
Conclusion
1. NIPHS must always be considered in differential diagnosis of adult patients with hypoglycaemia.
2. NIPHS may occur not only in patients after bariatric surgeries but also after gastric surgeries caused by peptic ulcer.

P316

Malignant pheochromocytoma with brain metastases and coexisting meningioma: case report

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Introduction
Pheochromocytoma is usually benign neuroendocrine tumor arising from chromaffin cells. Malignant tumors which account for 5–26% mainly metastize to bones, lungs, liver, but very rarely to brain. The coexistence of pheochromocytoma with brain meningioma may hinder the diagnosis.
Aim
The aim of this study is to present the case of a malignant pheochromocytoma female with brain metastases and coexisting meningioma.
Case description
Patient, aged 60, with non-stable hypertension, after adrenalectomy in 2005 because of right adrenal tumor, $38\times32\times70$ mm in size. Histopathologic diagnosis – pheochromocytoma. In September 2006, neurologic abnormalities appeared. CT examination revealed three cerebral tumors. Craniotomy was done and tumors removed. Histopathologic examination revealed two pheochromocytoma metastases in left frontal and parietal cerebral regions and one meningioma in temporal region. Brain radiotherapy was administrated – total dose of 20 Gy. Concentrations of urinary metanephrines and serum chromogranine A were normal. 131-I MIBG scintigram detected no pathologic uptake in the body. In August 2008, abdominal CT examination showed reccurence of disease in the site after right adrenalectomy and focal lesion in the right lung.131-I MIBG scan showed abnormal uptake in the region of right adrenal gland and the head of pancreas. MR examination revealed metastases in the right cerebellar hemisphere and left frontal cerebral lobe. Biochemical tests results were normal. CVD scheme chemotherapy and brain radiotherapy (total dose of 20 Gy) were administrated The patient remains under oncological and endocrinological observation.
Conclusions
1. Pheochromocytoma is rarely malignant tumor and brain metastases are its atypical localization.
2. Due to unequivocal histolpathologic standards, malignancy is diagnosed by the presence of metastases.
3. Patients treated for pheochromacytoma require constant endocrinological and oncological observation.
4. Pheochromocytoma with coexisting meningioma may impede the diagnosis of brain metastases.

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Postmenopausal hyperandrogenism: report of two cases

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In postmenopausal women with clinical hyperandrogenism Cushing's syndrome and adrenal/ovarian neoplasms must be excluded, but radiological and biochemical studies are not always useful for localizing the source of androgens.
Case 1
A 75-year-old woman developed alopecia and progressive hair growth on the chest and abdomen over 4 months. Five years before a successful parathyroidectomy had been carried out. Hormonal evaluation: Testosterone (T): 5.84 ng/ml; DHEAs: 0.44 mg/l; Androstenedione (A): 2.76 ng/ml. Abdominal CT found no tumours. Transvaginal ultrasound of the ovaries was normal, and MRI identified an uncertain small nodule in the left parametrium. Anexectomy and bilateral ooforectomy was performed, and histology identified two mesoovaric tumours of 6 and 12 mm (right and left), both Leydig cell tumours. One month after, hormones were: T: 0.1; A: 2.0. Six months later alopecia and hirsutism were resolved.
Case 2
A 53-year-old woman referred by dyslipidemia and hypertension. She had suffered an acute myocardial infarction 3-year before. She had noticed hair growth for the last year, particularly in shoulders, acral enlargement and increasing weight. Acromegaly was ruled out by oral glucose tolerance test but revealed DM2. Hormonal evaluation: T: 2.98; DHEAs: 0.4; A: 4.8 ng/ml. Abdominal CT: 27 mm right adrenal hypointense mass and normal ovaries. Transvaginal ultrasound: ovaries slightly enlarged. After uneventful laparoscopic adrenalectomy, androgen levels remained high. Bilateral ooforectomy was performed. Histology identified ovaries of $60\times30\times20$ mm with diffuse stromal hyperplasy, and scattered groups of luteinized cells. Three months later, she had T: 0.2 and A: 1.6 ng/ml. Glycemic control improved and hirsutism reverted 6 months later.
Conclusion
We described two unusual cases. The first emphasizes the finding of bilateral Leydig's tumour, scarcely reported. The second pays attention on the association of stromal hyperthecosis, hyperandrogenism and metabolic disturbances. Finding an incidental adrenal adenoma illustrates the troublesome diagnosis in this pathology.

P318

Prader-Willi syndrome: case report

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Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are distinct neurogenetic disorders involving the imprinting mechanism at 15q11–13 region. We report on 4 years and 9 months old boy who was referred to our laboratory in order to investigate a clinical bilateral cryptorquidy. The patient was born to non-consanguineous and healthy biological parents. Informed consent for publication was obtained from the parents. After normal pregnancy, the patient was delivered by caesarean section at full term, with a birth weight of 2600 g, but his height and head circumference were unknown. When he was born, he presented bilateral cryptorquidy and also he showed feeding problems. His development progress was delayed. He walked and developed speech at 3 years old. When he was examined at the age of 4 years, his head circumference was 50 cm, height 1.05 cm and weight 41 kg. Fluorescence *in situ* hybridisation using probes for SNRPN and D15521 loci, which map inside the chromosomal region 15q11–15q13 deletion or disomy of only maternal pattern. Methylation analysis at SNRPN showed an alpha axon, which carried out by Southern blot analysis revealed an abnormal only maternal methylation pattern. Microsatellites analysis of the patient showed the presence of only one region in three heterozygous loci inside the 15q11–13 region. The main PWS typical fractures' in our patient are hyperfagia with obesity, mental deficiency and bilateral cryptorquidy. The predominant genetic defects in PWS are 15q11–13 deletions of paternal origin or only maternal chromosome disomy. In contrast to that, AS occurs in the presence of maternal deletions or paternal chromosome 15 uniparenteral disomy. In conclusion, we report this case with the objective of reinforce the necessity of analysis DNA methylation within the 15q11–13 region, which is an important tool for the correct diagnosis among children who presents with mental deficiency, bilateral cryptorquidy and obesity.

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Dwarfism and female extenal genitalia due to congenital partial hypopituitarism in a 46XY Seckel syndrome with microcephaly and multiple skeletal deformities

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Seckel syndrome (SS) is a rare disorder of severe growth retardation and craniofacial-skeletal abnormalities. In scant number of reports, neonates had intact hypothalamic–pituitary–adrenal axis before they die because of cardio-pulmonary abnormalities. We present an unique case of SS at the age of 18 years and discuss the possible explanations of his growth retardation and sex reversal.
Case
A 18-year-old female presented with short stature and primary amenorrhea. There were first degree consanguinity between parents. At birth, she was small for gestational age. She couldn't walk and speak until the ages of 6 and 10 years, respectively. Physical examination: central obesity (17 kg, 107 cm), mental retardation, microcephaly, bird head-like facial dysmorphism, corneal opasity, syndactily, female external genitalia. Her height and bone ages were consistent with 5 and 10 years-old, respectively. Hormonal analyses: GH and IGF-1 were low; GH and cortisol responses to insulin tolerance test showed GH deficiency and normal cortisol response. Testosterone and estrodiol levels were low with inadequately low levels of LH. Testosterone response to LH-RH was markedly increased. Chromosomal analysis: 46XY (SRY+). Pelvic US revealed a blinded vagina and absence of gonads and internal genitalia. Brain MRI: cerebral and cerebellar atrophy and cortical dysplasia. He was diagnosed with SS with partial hypopituitarism. We evaluated that the dwarfism was related to congenital GH deficiency. He had also gonadotropin deficiency. In early fetal life, normal male karyotype directed the development of gonads to testis as Müllerian duct development was inhibited. However, because of LH deficiency, gonads might be regressed. Testosterone is essential in developing male external genitalia, its deficiency causes sex reversal as in our case. Moreover, the existence of testis supported by LH-RH test, is another assignment for us to locate. Our case had a CNS developmental pathology, with endocrine insufficiency unlike reported cases.

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A case of adrenomyeloneuropathy and Addison disease

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Adrenoleukodystrophy has a prevalence rate of 1:20 000 and is a cause of adrenal insufficiency in association with demyelination within the nervous system due to a failure of β-oxidation of fatty acids within peroxisomes due to reduced activity of very long chain acyl-CoA synthetase. Several forms are recognized; a childhood cerebral form (30 to 40% cases), adult adrenomyeloneuropathy (40% cases), and Addison's disease only (7% cases). Adrenomyeloneuropathy, by contrast, presents later in life with the gradual development of spastic paresis and peripheral neuropathy. Here, we describe an illustrative case of adrenomyeloneuropathy and discuss the clinical presentation, diagnosis and management.
Case
The patient was healthy until the age of 15. He had progressive gait disturbance and urinary incontinance after he had progressive neurological abnormalities. He was diagnosed as adrenal insufficiency and neurologic sequela related with meningitis at the age of 18. In his first admission to our clinic at the age of 25, he showed acute *adrenal insufficiency*. After he recovered, we evaluated muscle strength as weak (3–4/5) and deep tendon reflexes as hyperactive in the lower extremities while normal in the upper extremities. Babinski signs were elicited bilaterally. Accordingly, these neurologic findings excluded sequela related to meningitis. He had urinary incontinence, erectly disfunction with preserved libido and ejaculation. The patient's ACTH levels were elevated despite low levels of serum cortisol. Abdominal CT demonstrated adrenal atrophy. T2-weighted cerebral MRI showed a high signal intensity lesion in the occipital subcortical area. *EMG* demonstrated *sensorimotor demyelinating* polyneuropathy. Clinical features and laboratory findings confirmed the diagnosis of AMN. This case indicates the importance of neurological findings in Addison disease not to overlook this rare pathology, adrenomyeloneuropathy.

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A primary differentiated carcinoma of ectopic mediastinal thyroid: report of two cases

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Thyroglossal ductal cyst is the most common congenital cervical pathology. Abnormalities in the embryologic development and migration of the thyroid gland can result in this ectopic thyroid tissue, which may occur in the midline in any position from the base of the tongue to the mediastinum. They are rarely seen in adults. Malignant transformation of the cyst is quite rare and is encountered mostly in adults.
We retrospectively reviewed two cases of thyroglossal duct carcinoma diagnosed in the central hospital of the army of Algiers from 1990 to 2008. The two patients are females, a 64 and 43 year-old with ectopic thyroid tissue in the mediastinum associated to a multinodular goiter. A surgical excision plus thyroidectomy was performed and histology showed in one case a follicular carcinoma and in the other a papillary carcinoma. The ectopic thyroid tissue was clearly separate from the thyroid gland and there was not malignant tissue in the thyroid gland. Prophylactic radioactive iodine treatment was done. The two patients are still in follow-up and no pathology was detected 3 years later.
This type of carcinoma is usually an incidental finding and has a good prognosis, with only rare instances of metastasis close follow-up is need for recurrence and distal metastasis may be possible.

P322

Triple X syndrome (47, XXX) with infertility and obesity

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Background
Triple-X (Trisomy X) is found in approximately 1 per 1000 females. Mental retardation is the most common feature in triple-X females. Women with Triple X usually are fertile, but they sometimes get the menopause earlier than other women. They are not generally phenotypically abnormal. Although reproductive organs, pubertal development, and fertility are normal in most cases. Some are first identified in infertility clinics, others in institutions for the mentally retarded, but probably many of them remain undiagnosed.
Case
A 31-year-old woman was referred to the endocrinology clinic of our hospital due to amenorrhea for 1 years. She had been married for four years and with no children. She first menstruated at the age of seventeen and then menstruated at 2–3 months intervals (oligomenorrhea). She is 155 cm in height and 80 kg in weight and BMI: 33 kg/m^2. She had no eunuchoid body habitus. System and genital examination were phenotypically normal. However vulva and vagen atrophic, labium minuses were hypoplasic. She has average intelligence. Laboratory findings were shown hypergonadotropic hypoganadism: concordant with (FSH 32.05 mIU/ml, LH 13.58 mIU/ml, Estradiol 24.35 pg/ml) other anterior pituitary hormones were normal. Uterus size was found normal in pelvic ultrasonography. She had streaked gonads on both sides. In the analysis of chromosomes, 47, XXX formation (Triple-X syndrome) was detected.
Conclusion
While Triple-X females are usually fertile and thin, this syndrome should be taken into consideration in the cases of fertility and obesity.

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Treatment of a case of metastatic thyroid cancer with sorafenib

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Although the prognosis of thyroid cancer is in general quite favorable when standard management paradigms are applied, some patients do much less well. Radioactive iodine refractory, recurrent or metastatic disease is prognostically more worrisome. Sorafenib, a multitargeted small molecule kinase inhibitor, including the VEGF receptor and BRAF kinase, has been evaluated in patients with thyroid cancer. The aim of the study was to present a case of metastatic thyroid cancer and the clinical course after the administration of sorafenib.
A patient, male, aged 54 years, had been operated upon for a multifocal papillary thyroid carcinoma in the left lobe of the gland 12 years ago. The patient had not received radioiodine treatment after surgery. During the preoperative evaluation for coronary bypass surgery lung lesions were discovered. On biopsy the lung lesions proved to be metastatic disease from the papillary thyroid carcinoma. Consequently, radioactive iodine 150 mCi was administered, followed by two other 150 mCi doses of radioactive iodine. Evaluation with a positron emission tomography scan with ^{18}F-fluorodeoxyglucose revealed widespread metastatic disease in the lungs, the neck and a metastatic area in the liver. The patient was administered sorafenib 400 mg twice daily. Thyroglobulin levels decreased immediately from 226 ng/ml before to 45 ng/ml after treatment and the decrease was sustained. The patient developed transient diarrhea lasting a few days, tiredness and face paleness.
A case of a patient with metastatic thyroid cancer is presented who received sorafenib showing signs of a possible beneficial effect. The sustained decrease in thyroglobulin levels in this patient is a sign of a potential beneficial effect of sorafenib in metastatic thyroid carcinoma. However, an evaluation of objective tumor regression and larger trials are needed for the complete evaluation of the effect of sorafenib on the clinical course in patients with metastatic thyroid cancer.

P324

Insulin autoimmune syndrome in a patient with type 2 diabetes: a case report

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Introduction
Insulin autoimmune syndrome (IAS) is a clinical condition characterized by the presence of autoantibodies to insulin or insulin receptors in patients not previously treated with insulin. This syndrome has been reported mainly in Asia, and it is a rare cause of hypoglycemia in Caucasians. So far there are no reports of IAS in patients with type 2 diabetes mellitus never treated with insulin.
Case report
The authors describe a 59-year-old Caucasian male, diagnosed type 2 diabetes 5 years previously, treated with metformin and gliclazide, referred for hypoglycemic episodes in fasting and late postprandial period. Initial laboratory evaluation revealed fasting glucose = 123 mg/dl, HbA1c = 7.1%, fasting insulin = 1173.3 uUI/ml (normal range: 5–20 uUI/ml), fasting proinsulin = 89.6 pmol/l (normal range: < 9.4 pmol/l), C-peptide = 8.64 ng/ml (normal range: 0.9–7.1 ng/ml), anti-insulin antibodies = 201.1 U/ml (positive if > 0.5), antinuclear antibodies > 1/160, and negativity for anti-IA2, anti-GAD and anti-ICA antibodies. Further serologic and hormonal examination was unremarkable. During a 72 h fasting test, the patient had glucose levels between 51 mg/dl (at 6 h fast) and 153 mg/dl associated with insulin levels of 2496 uUI/ml at the beginning and 705 uUI/ml at the end of the test. Imaging study using angio-CT-scan did not show any morphological pancreatic abnormality. HLA class II typing revealed the presence of DRB1*04, DRB1*03 e DQB1* alleles. After one year of follow-up, our patient, treated with metformin, has less frequent symptomatic hypoglycemia. Nevertheless, continuous glucose monitoring for 72 h, revealed repeated fasting hypoglycemia, with minimum level of 40 mg/dl, and postprandial hyperglycemia. Recent laboratory examination showed fasting insulin = 233.8 uUI/ml, C-peptide = 4.44 ng/ml, HbA1c = 7.9% and anti-insulin antibodies = 157.2 U/ml.
Conclusion
This is a rare case of IAS in a patient with type 2 diabetes mellitus not previously exposed to insulin. The development of these antibodies may be related to a genetic susceptibility, since HLA-DRB1*04 has been reported in strong association with this syndrome.

P325

Malignant struma ovarii: a case report

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Background
Struma ovarii is a rare form of the ovarian germ cell tumors composed predominantly of mature thyroid tissue. It occurs mostly in the fifth decade. About 5–10% of these tumors are malignant. We present the case of a 20-year-old-woman with papillary thyroid carcinoma arising in struma ovarii.
Case report
A 20-year-old woman presented to her gynecologist with pelvic pain. A right ovarian tumor was discovered at ultrasound examination. The patient was treated by complete right ovariectomy – histopathology revealed papillary thyroid carcinoma arising in struma ovarii (malignant struma ovarii). The patient underwent total thyroidectomy – the thyroid was found to be normal on histology. After operations the patient received ablative radioiodine treatment (200 mCi 131-I). An I-131 posttherapeutic whole-body radioiodine scintigraphy was performed and showed uptake in bone metastases. Thyroid hormone therapy using suppresive doses was introduced after radioiodine ablation. Thyroglobulin level is monitored. Next doses of radioiodine has been scheduled.
Discussion
The treatment of malignant struma ovarii remains controversial. We think that the management of malignant struma ovarii could be the same as in case of thyroid carcinoma, so after surgical removal of ovarian neoplasm, we recommend thyroidectomy, radiotherapy with 131-I and levothyroxine suppressive therapy. Long-term follow-up for the detection of metastases or tumor recurrence by serial serum thyroglobulin measurements and 131-I scan may be required in patients with this rare tumor.

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Cushing's syndrome in a patient with bilateral adrenal masses and pituitary incidentaloma: case report

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Introduction
Incidentaloma is a randomly discovered tumor with no apparent secretor activity. The term is mainly used for hypophysis and adrenals. There are relatively few situations where a patient has more than one such mass.
Aim
To present the case of a patient with three tumors: a pituitary mass and bilateral adrenal masses, with clinical manifestations of Cushing's syndrome.
Case report
A 37-year-old female patient who had for the last three years weight gain (15 kg), abdominal stria, ecchimosis, asthenia, insomnia, emotional lability, hypercholesterolemia, arterial hypertension (maximum 230/120 mmHg), type 2 diabetes mellitus. The hormonal profile showed hypercorticism: basal plasma cortisol of 38.6 ng/ml (normal <22 μg/dl). The low-dose dexamethasone suppression test showed no suppression, neither did the high-dose dexamethasone test (plasma cortisol 30.86 ng/ml). The basal level of ACTH was 8.08 ng/ml. The computed tomography (CT) scan showed a pituitary tumor of 0.7 by 0.3 cm. The adrenal CT revealed bilateral masses of 3.2 by 2.4 cm on the right, and 1.2 by 0.85 cm on the left. The right adrenalectomy was performed, considered as the cause of the Cushing's syndrome. Immediately after surgery, acute adrenal insufficiency was diagnosed and treated with hemisuccinate of hydrocortisone and electrolytic solutions. The patient recovered but for the next 2 years she had adrenal insufficiency, which was properly substituted. The CT scan showed the reduced dimensions of the left adrenal tumor (0.69 by 0.51 cm) and constant diameters of the pituitary tumor, as they were both incidentalomas.
Conclusion
The difficulties in a patient with Cushing's syndrome with bilateral adrenal masses and also hypophyseal tumor come from the low specificity of the dexamethasone suppression test. Nevertheless, the triple tumor phenotype is extremely rare but the correct therapeutically management was confirmed by the presence of post surgery adrenal insufficiency and the unchanged diameters of the other two tumors.

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Cerebrospinal fluid rhinorrhoea following dopamine agonist therapy for large macroprolactinoma

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Introduction
Dopamine agonists have been routinely used in the treatment of prolactinomas since 1971. Increasingly, cabergoline is used as first-line treatment because of its tolerability and improved patient compliance. CSF rhinorrhoea is a rare but recognised adverse effect of rapid tumour shrinkage following dopamine agonist therapy. We report a case of cabergoline induced CSF rhinorrhoea in a young man with macroprolactinoma.
Case
A 26-year-old student presented with a 6 year history of bilateral spontaneous galactorrhoea and three months left visual field impairment. Initial investigation showed a markedly elevated serum prolactin of 215 000 μ/l and normal basal cortisol, IGF-1, testosterone, gonadotropin and thyroid hormone levels. MRI pituitary showed a large macroadenoma ($5.5\times3.6\times3.1$ cm) with downward extension in to sphenoidal sinus. Perimetry was essentially normal. He was commenced on cabergoline 0.25 mg thrice weekly increased after a week to 0.5 mg three times a week. Four weeks later he developed CSF rhinorrhoea and cabergoline was abruptly discontinued. Repeat MRI pituitary demonstrated considerable tumour shrinkage. He underwent surgical repair of CSF leak following which cabergoline was restarted. He has subsequently remained well without recurrence of CSF leak.
Conclusion
CSF rhinorrhoea following rapid tumour shrinkage after dopamine agonist therapy is rare and there is no consensus regarding patient management. Patients with large prolactinomas on dopamine agonist therapy should be warned of this potential problem and monitored closely.

Comparative Endocrinology

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Characterization of a vasoactive intestinal peptide receptor type 2 (VPAC2R) in an early jawed vertebrate, sturgeon (*Acipenser schrenckii*)

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Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) are important neuropeptides that are structurally related. They have been found to exert many physiological and pathophysiological effects through the activation of three specific receptors: PAC1R, VPAC1R, and VPAC2R. In tetrapods, PACAP and VIP are potent agonists to VPAC2R. In teleosts, we have previously identified a PHIR in goldfish which shares high level of sequence similarity with tetrapod VPAC2Rs. However, this PHIR does not interact with PACAP or VIP, while fish PHI was able to activate this receptor. Here, we report the identification a full-length VPAC2R from an Actinopterygian, sturgeon (*Acipenser schrenckii*). This receptor contains 427 amino acid residues. In phylogenetic analysis, the receptor clusters with tetrapod VPAC2Rs and teleost PHIRs. Tissue distribution analysis by real-time PCR showed high levels of expression of this receptor is in gut and liver. Interestingly, after stable transfection into CHO-K1 cells, this receptor could be stimulated by human VIP, but not goldfish PHV or human PACAP as shown in functional cAMP assays. These data suggested that the ability of VPAC2R to bind PACAP was not found in sturgeon, a representative of an early jawed vertebrate (Gnathostomata), nor goldfish. This function of the receptor could be evolved after the teleost/tetrapod split and therefore is present only in the tetrapod lineage.

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Influence of orchidectomy and testosterone replacement on adrenal cortex activity in the Saharan gerbil *Gerbillus tarabuli*

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Orchidectomy performed during breeding season (winter) in the adult male gerbil, *Gerbillus tarabuli*, live trapped in its natural biotope in Béni Abbès area (30°7 N., 2°10 W.) in algerian Sahara desert, induced 50 days later, important weight, histological and hormonal changes on the adrenal gland.
Adrenal weight increases by 69.7% ($P=0.06$) and adrenal cortex height by 48.6% ($P=0.00$) with hypertrophy of reticularis zona ($+124\%$, $P=0.00$) while glomerulosa and fasciculata zona exhibited only small height variations. Important increases were also observed in the cell height and nuclear diameter of fasciculata cells (respectively 40.2 and 10.8%; $P=0.00$) and reticularis cells (respectively 23.0 and 6.6%; $P=0.00$). Moreover, connective tissue was well developed in the inner reticularis of castrated gerbils suggesting activation of extracellular matrix.
Orchidectomy induced also increases of lipidic droplets especially in the reticularis zona when adrenal and plasma contents of cortisol exhibited non significant decreases (-6.9%; $P=0.78$ and -30.4%; $P=0.26$ respectively).
Testosterone replacement, carried out by twice daily injection of enanthate testosterone (75 μg diluted in 40 μl sesame's oil) during 7 days on 50 days castrated animals, restored adrenal weight as well as all histological parameters but didn't restore adrenal and plasma contents of cortisol which showed an even more important reduction.
These results showed clearly an inhibitory effect of testosterone on the activity of the adrenal gland in this Saharan rodent. Testosterone could act either directly or by means of its endogenous regulators, such as pituitary ACTH. So, testosterone could be involved, at least in part, in the determinism of the annual variations of the adrenocortical activity and contribute to the adaptation of this species to its arid environment requiring important metabolic adjustments.

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Comparative analyses between the glycoprotein-hormone receptors and the orphan leucine-rich repeat containing G-protein coupled receptor 4 (LGR4)

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In recent decades intensive studies on the glycoprotein-hormone receptors (GPHRs) and their respective hormones have provided a number of molecular

insights into the relationship between the structure and function of these proteins. This knowledge includes an understanding of hormone binding, of naturally occurring mutations nad mechanisms of signal transduction and G-protein binding processes.
Together with the relaxin family peptide receptors (RxFP) the GPHRs belong to the Leucine-rich repeat containing receptors (LGRs), a subgroup of class A G-protein coupled receptors (GPCR). The orphan Leucine-rich repeat containing receptors 4–6 (LGRs 4–6) are partially more homologous to the GPHRs by amino acid sequences than the RxFP receptors 1–2 (formerly LGR 7 and 8, respectively). In contrast to a growing number of data for the RXFP receptors, the functional and physiological role of the orphan LGRs 4–6 has not yet been determined. We analysed and provide here sequence-structure similarities between the homologous GPHRs and LGR4 regarding potential ligand binding sites and structural determinants of intramolecular signal transduction.
Additionally, interesting new findings concerning the ancient glycoprotein-hormone (GPH) thyrostimulin have been published previously. Thyrostimulin is an agonist for the TSHR. Utilizing the knowledge about structure–function relationships in GPHRs and their hormones, we initially built homology models of thyrostimulin. In comparison to GPH/GPHR complexes these models not only help to describe properties of thyrostimulin more precisely, but also to draw conclusions regarding potential modes of hormone binding.
In summary, here we attempt to extract new molecular information concerning proteins with unknown but potentially important function in physiological processes by comparative analyses between homologous family members.

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Diabetes insipidus prevalence in the Republic of Uzbekistan according to national register

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Aim of the research
To study diabetes insipidus (DI) prevalence in the Republic of Uzbekistan (RUZ) according to National Register.
Materials and methods
Computerized database has developed which covers all 12 regions of RUZ and Republic of Karakalpakstan. Database includes 1822 patients with diabetes insipidus.
Results
According to the register there are 1822 patients with DI which occurs in men and women with similar rate – 968 (53.2%) and 854 (46.8%) respectively. The study of disease form has shown that in most cases central form of DI has seen – 896 (49.2%) patients, whereas idiopathic, renal, gestational and inherited forms have seen in 829 (45.5%), 87 (4.7%), 7 (0.4%) and 3 (0.2%) cases respectively. We revealed, that DI caused by stresses in 20.4% (372 patients) whereas neuroviral infection, hypothalamus and pituitary region tumors, heritage and pregnancy take place as a risk factors in 16.2% (295 patients), 4.8% (87 patients), 7 (0.4%) and 0.9% (16 patients) respectively. Idiopathic diabetes insipidus has diagnosed in overwhelming majority of patients – 48.9%. Among the ethiopathogenetic factors we should distinguish postoperative DI which occurs in 7 (0.4%) while postablative, after born trauma DI, autoimmune endocrinopathy, hypothalamus and sellar region tumors seen in 5 (0.3%), 22 (1.2%), 38 (2.1%) and 67 (3.7%) patients respectively. Manifestation features of DI were following: headaches in 905 (49%) whereas dizziness, dry mouth, polyuria, vision disturbances, dysmenorrhea, heartaches and fatigue in 698 (37.7%), 1331 (71.9%), 1325 (72.6%), 1279 (69.1%), 63 (3.4%), 11 (0.54%), 207 (11.2%) and 829 (44.8%) patients respectively. Also, we registered complications such as cardiovascular disease in 62 (3.4%) cases while dehydration, psychomotor excitement, coma, dysmenorrheal and renal disease in 60 (3.%), 47 (2.6%), 18 (0.1%), 67 (3.7%) and 164 (9.0%) cases respectively.
Conclusion
(1) Algorithm for the management of DI should include CT or MRI imaging of hypothalamus and pituitary region, (2) Register for DI allows studying epidemiology of the disorder as well as its evaluation and develop new treatment regimens, and improve prevention of DI in regional endocrine dispensaries and implement in everyday practice.

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Cortisol and biochemical changes in pregnant women

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Pregnancy is characterized by profound changes in woman's hormonal and metabolic status. Cortisol has been postulated to play a central role in the physiological changes associated with pregnancy. The extends of this effect in the three stages of pregnancy remain to be fully investigated. The current study investigates the level of serum cortisol in the three stages of pregnancy and correlates this with reproductive hormones and electrolyte balance. These changes were further compared with non pregnant controls. Two hundred volunteer women were recruited from our Lady Catholic Hospital, Oluyoro Oke Ofa, Ibadan, Nigeria. These subjects consist of 50 women in each of the three trimester and 50 non-pregnant, none lactating, apparently healthy, aged matched controls. Serum cortisol, progesterone and prolactin were measured by ELIZA using commercial kits other biochemical assay where done using conventional methods. The results shows significant progressive increase in the BMI of pregnant women compared with controls. The diastolic pressure was significantly increased only in the first trimester. While glucose and protein levels were significantly depressed, total cholesterol concentration increased progressively in pregnant women. Serum cortisol concentration increased significantly as early as first trimester, reach the pick in second trimester and came down in third trimester. This increase was accompanied by increase in progesterone and prolactin. Increase in serum cortisol correlate positively with increase in serum chloride and inversely with decreased serum potassium and bicarbonate. This study shows clearly that cortisol plays a central role in the biochemical changes in pregnancy. The increase in serum cortisol is a possible indicator of emotional stress and physiological challenges in pregnancy and also, possibly risk signal. The concurrent increase in progesterone and prolactin are compensating mechanism in response to these challenges. It may therefore be of clinical relevant to monitor the serum cortisol levels and some of the compensating/associated variables, especially in threatening pregnancy.

Diabetes and Cardiovascular

P333

Characterization of a young population of type 1 diabetics

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Introduction
Type 1 diabetes is one the most common chronic diseases found in children and youngsters.
Objectives
Characterization of a sample of young type 1 diabetic patients, treated with multiple daily injections of insulin.
Patients and methods
Analysis of patients files with ages between 11 and 26 years observed on diabetology consultation during the first semester of 2008, with diagnosis of diabetes for at least 6 months. Parameters evaluated: gender, age, diagnosis age, diabetes duration, A1C, BMI, self-monitoring of blood glucose, insulin scheme, carbohydrates counting (CC) and complications. It was considered effective self-monitoring of blood glucose (ESMBG) when effectuated $\geq$4 times a day. For classification of glucose control (good/bad) were used the ADA criteria.
Results
It was analyzed the data of 108 patients, 55.6% male, 44.4% female; age 20.18$\pm$3.11 years; age at diagnosis 10.69$\pm$4.91 years; duration of diabetes 9.28$\pm$5.18 years. Actual A1C 8.13$\pm$1.58%; A1C on the last year 8.01$\pm$1.43%; BMI 24.12$\pm$3.40 kg/m^2; ESMBG 67.14%. Everyone was treated with intensive insulinotherapy. About 51.85% did CC. About 30.93% presented dyslipidemia and 18.52% hypertension; nephropathy and retinopathy were presented in 7.41 and 2.78% respectively.
It wasn't verified significative statistical relationship between A1C and age, gender, duration of disease, diagnosis age, BMI, CC, dyslipidemia, nephropathy and retinopathy. It was verified significative statistical relationship between A1C e ESMBG ($P=0.004$). Patients with a bad glycemic control presented higher incidence of hypertension ($P=0.027$; OR=5); Hypertensive patients presented a higher incidence of nephropathy ($P=0.001$; OR=9.4).
Conclusions
One should enhance the difficulty of obtaining the therapeutic objectives for the referred ages. ESMBG is fundamental for obtaining such objectives. Bad glucose control was associated to a higher incidence of hypertension which was associated to a higher incidence of nephropathy.

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How diabetic children's families inject them insulin at home

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Background
Diabetes mellitus type one is a common metabolic disorder among pediatrics. Diabetic children's families should be able to measure the blood sugar of their children and inject them insulin correctly.
Materials & methods
Thirty-six families which had diabetic children were recruited to this study and were asked about the way they were injecting insulin to their children.
Results
Forty-eight percent of the clients were injecting insulin at the scheduled time according to their physicians' order. Thirty percent had injections with the correct angle and in appropriate sites. And only 14% were changing the injection sites daily.
Conclusion
According to the results, diabetic children's families still need education on how to inject insulin to their children.

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HLA DQB1 and HLA DQA1 genotypes prevalence in children and adolescence with new-onset diabetes type 1 in the lower Silesia region

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The first degree relatives of patients with diabetes type 1 have increased genetic risk of developing clinical disease. In the population of Polish diabetic subjects the most frequent alleles are HLA- DRB1*04, DRB1*03, DQA1 and DQB1. The 'high-risk genotype' may differ among populations.
The aim of this paper was to analyze prevalence of genetic risk factors in patients with new-onset diabetes in Lower Silesia and to compare the data with Polish population and Caucasian one.
The study involved 58 patients with new-onset diabetes type 1, 34 boys and 24 girls, 11 months to 18 years of age, admitted to the Department of Endocrinology and Diabetology for Children and Adolescence, Wrocław Medical University, from June 2007 to May 2008. HLA DQB1 and HLA DQA1 genes were analyzed: SNP polymorphism of HLA DR gene locus was assessed using ABI 3130 gene analyzer with GENEscan software.

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Comparative molecular analysis of TRAIL ligand and receptor expression profiles in cyclophosphamide versus streptozotocin-induced diabetes in non-obese diabetic (NOD) mice

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Background
NOD mice are the most frequently preferred animal models in type 1 diabetes (T1D) research. They develop spontaneous disease in 24 to 30 months. T1D can also chemically be induced in NOD mice for a faster disease progression. Two commonly used diabetes-inducing agents are Streptozotocin (STZ), which destructs pancreatic beta cells mainly through DNA fragmentation, and Cyclophosphamide (CY), which acts on suppressor T cells. TNF-Related Apoptosis-Inducing Ligand (TRAIL) has recently been implicated in T1D development. Although its exact role is unknown, blockage of TRAIL sensitized animals to T1D development. Here, we aimed to examine the effects and diabetes-inducing profiles of Streptozotocin and Cyclophosphamide in NOD mice, while comparatively analysing alterations in TRAIL ligand and receptor expression profiles.
Materials and methods
Diabetes development was accelerated in mice by IP injection of 200 mg/kg CY or 150 mg/kg STZ. Blood sugar measurements were used to monitor development of diabetes. Pancreatic tissues were collected at days 0, 1, 2, 4, 7, 14, 21, and 28. Alterations in TRAIL ligand and receptor expression profiles were detected by immunohistochemistry.
Results
STZ produced a faster T1D profile, as reflected by blood sugar levels of 250 mg/dl and over at day 4, accompanied by a 10% weight loss. Nearly 90% of mice were diabetic at day seven. CY-induced NOD mice, on the other hand, did not develop any signs of diabetes until after day 10. Both agents were generally well-tolerated in mice at the mentioned doses. STZ- or CY- induced pre-diabetic to severly diabetic mice revealed significant alterations in TRAIL ligand and receptor expression patterns.
Conclusion
Both agents induced prominent manifestation of T1D, although at different time intervals. Comparative analysis of alterations in TRAIL ligand and receptor expression patterns revealed an important insight into the molecular pathogenesis of T1D.

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Association of IL-4 promoter polymorphisms in Taiwanese patients with type 2 diabetes mellitus

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Type 2 diabetes mellitus (T2DM) is a common endocrine disease. Many factors can lead to the onset of T2DM, however, host genetic factors and environmental factors are the focus of discussion. The aim of this study is to investigate the putative correlation between the promoter polymorphisms of interleukin-4 (IL-4), one of the immune-regulatory type 2 helper T cell cytokines, and T2DM. Genomic DNA from 425 Taiwanese T2DM patients and 148 non-diabetic control study subjects were extracted, and their IL-4 promoter polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism. Both of the distribution of IL-4 C-589T ($P=0.013$) and C-34T ($P=0.05$) genotypes were significantly different between type 2 diabetic patients and control subjects. Significant association between IL-4 C-589T alleles ($P=0.002$) and T2DM, as well as C-34T alleles and T2DM ($P=0.024$), was also identified. Additionally, we found a statistically significant association between homologous IL-4 -589 C/C genotypes and lower circulatory high density lipoprotein (HDL-C) levels using multiple linear regression analysis with adjustment for subjects' age, sex and diabetic status. Our results suggested that IL-4 promoter polymorphisms are associated with T2DM. To the best of our knowledge, this is the first report of the significant association between IL-4 promoter polymorphisms and type 2 diabetes mellitus as well as the IL-4 homologous C/C genotypes and the lower circulatory HDL-C level.

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Saxagliptin added to a thiazolidinedione, metformin or a sulphonylurea improves glycaemic control in patients with inadequately controlled type 2 diabetes mellitus

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Saxagliptin (SAXA) is a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme. The efficacy and safety of SAXA 5 mg add-on therapy to a thiazolidinedione (TZD), metformin (MET), or an intermediate dose of glibenclamide (GLY), was investigated in patients with inadequately controlled ($HbA_{1c}>7.0\%$) type 2 diabetes mellitus (T2DM) in three randomised, double-blind trials (CV181-013, CV181-014 and CV181-040, respectively). Following a placebo run-in period, patients (aged 18–77 years) were randomised to receive SAXA 5 mg or placebo once daily plus their stable TZD, MET or GLY dose. Blinded up-titration of GLY was allowed in the GLY-only arm to a maximum daily dose of 15 mg. All studies' primary endpoint was HbA_{1c} change from baseline. Changes in fasting plasma glucose (FPG) and postprandial glucose (PPG) were also measured. Baseline characteristics within each study were well balanced across treatment groups. At Week 24, SAXA 5 mg add-on treatment provided significant ($P<0.01$) reductions from baseline in HbA_{1c}, FPG and PPG area under the curve (AUC), with increased proportions of patients achieving therapeutic glycaemic response

($HbA_{1c} < 7\%$), compared with matched controls (Table). In each study, SAXA was well tolerated. SAXA add-on therapy to ongoing TZD, MET or GLY provides significant and clinically meaningful reductions in key parameters of glycaemic control and is well tolerated in patients with inadequately controlled T2DM.

	Add-on to TZD		Add-on to MET		Add-on to GLY	
	SAXA ($n=186$)	Placebo ($n=184$)	SAXA ($n=191$)	Placebo ($n=179$)	SAXA ($n=253$)	Placebo ($n=267$)
HbA_{1c} (%)*	−0.9	−0.3	−0.7	+0.1	−0.6	+0.1
FPG (mmol/l)*	−1.0	−0.2	−1.2	+0.1	−0.5	+0.1
PPG AUC (mmol·min/l)*	−515	−149	−532	−183	−278	+66
HbA_{1c} <7% (%)	41.8	25.6	43.5	16.6	22.8	9.1

*Changes from baseline. All $P<0.01$ versus placebo group.

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Change of physical activity, diet habits and risk of diabetes after lifestyle intervention. Diabetes in Europe: prevention using lifestyle, physical activity, and nutritional intervention: the DePlan Project Krakow

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Aim
Aim of the study was to assess the effectiveness of the structured lifestyle intervention in diabetes type 2 high risk people.
Methods
The De-Plan Project participants were selected based on FINDRISK over 14. Exclusion criteria were known or OGTT diabetes. Intervention completed by 175 participants consisted of 10 group sessions on lifestyle changes, diet and physical activity education, and 6 telephone motivation sessions and voluntary physical activity sessions 1–2 a week. Fasting and OGTT blood glucose, blood pressure, fasting lipids, weight, dietary and physical activity habits were assessed twice, before and after one year of intervention.
Results
Fasting and after OGTT glicaemia lowered in 38.9 and 51.4% participants. Fasting cholesterol, HDL and triglicerydes lowered in 53.7, 44.6 and 50.3% of intervened respectively. SBP and DBP decreased in 26.9 and 33.7% participants. Weight was lowered in 63.4% of study participants, 24.6% participants lost more than 5% of initial body weight ($P<0.05$). Changes in physical activity and nutritional patterns are given in the Table below.

	All		Weight loss ≥5%	
	Before	After	Before	After
Physical activity increased during last year	7.4*	25.7*	4.7*	39.5*
Diminished total amount of fat in diet	25.1*	52.6*	20.9*	65.1*
Changed fat to unsaturated	29.1*	61.7*	37.2*	72.1*
Increased consumption of fruits and vegetables	24.0*	51.4*	30.2*	53.5*

*$P<0.05$.

After the intervention FINDRISK diminished in all study participants from 18.31 to 15.95 ($P<0.05$) and in those with weight loss ≥5% from 18.23 to 16.29 ($P<0.05$).

Conclusions
Intervention on lifestyle changes, diet and physical activity education is possible and may produce diminished risk of type 2 diabetes.

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Limits in using brain natriuretic peptide (BNP) as a biomarker of acute right ventricular dysfunction in pulmonary embolism

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Background
Risk stratification could be lifesaving in acute pulmonary embolism (PE). Echocardiographic (ECHO) acute right ventricular dysfunction (RVD) is the actual 'gold standard' in risk assessment of patients with PE. We previously demonstrated that plasma BNP levels were significantly higher in patients with PE and acute RVD on ECHO versus patients with normal right ventricular (RV) function on ECHO.
Aim and objective
Evaluation of the limits of plasma BNP in signalling acute RVD in patients with PE.
Methods
Seventy patients with confirmed PE were prospectively investigated: 42 men (60%), mean age 52.5 ± 8.8. Plasma BNP levels were measured on admission using a quantitative fluorescence immunoassay (Triage BNP). ECHO evaluation of the RV function was performed in the first hour after admission. Study protocol was approved by local Ethical Committee. Patients were divided in two groups: group 1 – with acute RVD on ECHO, $n=24$ patients (34.3%); group 2 – without acute RVD on ECHO, $n=46$ patients (65.7%).
Statistics
SPSS 16.0; MedCalc 9.6.
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% Confidence Interval C.I. 0.77–0.94), $P<0.0001$. The cut-off level of plasma BNP = 50 pg/ml showed the best sensitivity = 0.84 (95% C.I. 0.79–0.88) and specificity = 0.80 (95% C.I. 0.75–0.85) in the same time in identifying acute RVD. Eight patients from group 1, with acute RVD on ECHO, all admitted soon (<12 h) after the onset of their PE symptoms, and all experiencing at least one syncopal episode had BNP under the cut-off level.
Conclusions
Plasma BNP under the cut-off level of 50 pg/ml obtained by a unique assay could not exclude even a severe pulmonary embolism and should be interpreted with caution, especially in patients with significant and recent onset (<12 h) pulmonary embolism symptoms.

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Determination of oxidized LDL and anti oxidized LDL antibody in patients with type 2 diabetes

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Background
Oxidized low-density lipoprotein (Ox-LDL) is a key factor in the development of arteriosclerosis. It can cause endothelial dysfunction and augment lipid accumulation within the arterial wall. Increased oxidative stress in diabetes contributes to this process. Ox-LDL is a highly immunogenic molecule and it is not clear whether oxidized LDL antibodies (OLAB) are pathogenic or protective in arteriosclerosis? The aim of this study was to evaluate Ox-LDL and its antibody in type 2 diabetes and healthy subjects.
Methods
This nested case–control study included 81 type 2 diabetic patients and 69 non-diabetic healthy persons aged 40 to 65 years. Controls were sex and BMI matched with diabetic patients. Patients with history of cigarette smoking, antioxidant or

antihyperlipidemic use, coronary heart disease, hypertension, and renal impairment were excluded. We measured serum level of Ox-LDL (two monoclonal antibody of Mercodia co.) and OLAB by ELISA. Lipid profile, serum electrolytes, and HbA1C (HPLC) were also determined. Ox-LDL and its antibody were compared between diabetic patients and controls and the correlation with lipid profile, HbA1C and BMI were assessed.
Results
Serum Ox-LDL concentration and Ox-LDL to LDL ratio were distinctively higher in controls (15.7 ± 6.9 vs 11.8 ± 5.6, $P < 0.005$). Ox-LDL concentrations were correlated with LDL-C ($rs = 0.36$, $P < 0.0005$) and total cholesterol ($rs = 0.31$, $P < 0.0005$) in both groups but not with age and HbA1c. In diabetic patients Ox-LDL and its antibody were positively correlated ($rs = 0.26$, $P < 0.05$). Obese diabetic patients (BMI > 30) had higher Ox-LDL concentrations compared to diabetic patients with BMI less than 30.
Conclusion
In diabetic patients, Ox-LDL level is lower than non-diabetics and is correlated with its antibodies. Based on previous findings we suppose that the pattern of LDL oxidation enhances Ox-LDL recognition by macrophage via specific legends. This results in low serum Ox-LDL concentrations in diabetes.

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Relationship between lipid oxidation and insulin resistance in type 2 diabetes mellitus

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The endothelial dysfunction and atherosclerosis. Recent studies have demonstrated that the amount of oxidized LDL (Ox-LDL) in plasma is correlated with IR in non-diabetic population. The intent of this study was to evaluate correlation of Ox-LDL and IR in diabetic patients, and to compare it with normal individuals.
Methods
A total of 150 individuals aged 40 to 65 years were studied including 81 type 2 diabetic patients and 69 non-diabetic sex and BMI matched healthy persons. Demographic characteristics and anthropometric data of participants were recorded. Levels of circulating Ox-LDL were measured with 2 monoclonal antibody-based competitions ELISA. Oxidized LDL antibodies (OLAB), glucose, insulin, and HbA1C were also determined in fasting blood samples. Insulin resistance was estimated according to homeostasis model assessment of insulin resistance (HOMA-IR).
Results
After considering all the relevant factors including age, duration of diabetes, BMI, systolic and diastolic blood pressure, serum lipids, and HOMA-IR, regression analysis demonstrated that there was a significant correlation of Ox-LDL with cholesterol and HOMA-IR in all participants ($r = 0.39$, $P < 0.005$). This was also true for diabetic patients but in non-diabetic group Ox-LDL was only correlated with cholesterol. OLAB had weak but significant correlation with BMI in both diabetic and non diabetic groups($r = 0.23$ and 0.24 respectively, $P < 0.05$).
Conclusion
Our results suggest an association between insulin resistance and increased LDL oxidation independent of the effect of other contributing factors.

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Role of soluble fas/fas ligand pathway and osteoprotegerin levels in patients with diabetic foot

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Aim
Diabetic foot is a devastating complication of diabetes mellitus. Many factors such as neuropathy, vascular injury and infection contribute in the development of diabetic foot. Programmed cell death is a pathway that causes a tendency for the development of atherosclerosis. Whereas Fas/Fas ligand pathway induces apoptosis, osteoprotegerin (OPG) causes calcification in vascular area and also effects apoptotic pathway. In the present study, we aimed to investigate the role of Fas/Fas ligand and OPG in the pathogenesis of diabetic foot.
Materials and methods
Thirty-eight patients with type-2 diabetes and diabetic foot, 25 patients with type-2 diabetes but without diabetic foot and 25 healthy control subjects were enrolled in the study. Diabetic foot lesions are scored according to Wagner classification. Soluble Fas, Fas ligand and OPG levels were measured in serum samples by ELISA method.
Results
OPG, sFas and sFas ligand levels were found significantly higher in diabetic foot group than the patients without diabetic foot, and controls ($P < 0.05$, $P < 0.001$, $P < 0.001$ respectively). Patients with advanced diabetic foot lesions (Wagner stage 4–5) had higher sFas and sFas ligand levels ($P < 0.01$, $P < 0.01$ respectively). Although OPG levels were also higher in this group, the difference did not reach any statistical significance. In diabetic foot group, OPG was correlated with sFas ligand ($P < 0.01$, $r = 0.52$), sFas ($P < 0.05$, $r = 0.42$), CRP levels ($P < 0.05$, $r = 0.41$) and leukocyte count ($P < 0.05$, $r = 0.33$), and Fas levels were correlated with CRP levels ($P < 0.01$, $r = 0.46$) ve leukocyte count ($P < 0.01$, $r = 0.46$).
Discussion
In this study, we assessed that the apoptotic pathway in the development of diabetic foot increases by means of the Fas/Fas ligand, and that the OPG levels are associated with the apoptosis in diabetic foot. We consider that the development of new effective treatment strategies against apoptosis will play an important role in the future management of diabetic foot lesions.

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Abstract withdrawn.

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Evaluation of coronary artery disorders in diabetic patients with no or atypical cardiac symptoms

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Introduction
Coronary artery disorder has been featured as the leading cause of dead in diabetics. This study was designed to assess the prevalence of silent myocardial ischemia in asymptomatic patients with diabetes.
Methods and patients
One hundred and thirty asymptomatic type 2 diabetic patients were enrolled in the study. A questioner was filled including patients' demographic information and routin laboratory tests. HsCRP was measured for all patients. All patients underwent transtorasic echocardiography Exercise test was done for those without prolifrative retinopathy and severe degenerative joint disease. Patients with positive or strongly positive ETT were directly referred for angiography. Gated myocardial perfusion SPECT was performed in 108 patients with negative or mild positive ETT.
Results
The mean age of the patients was 51.8 ± 7.3 years and the mean weight was 72.9 ± 9.6 kg and average abdominal circumference and BMI was 94.9 ± 9.3 and 27.9 ± 9.8 respectively. The mean time interval since diabetes diagnosis was 7.8 years. Total number of 43 patients had silent ischemia according to ETT and GSPECT findings. Two patients (1.5%) had positive ETT and 10 patients (17.7%) had strongly positive ETT. GSPECT revealed ischemia in 31 patients. The size of ischemia was small in most patients. Traditional and emerging risk factors werenot significantly different between patients with or without ischemia.
Conclusion
Silent ischemia was relatively prevalent among our patients. Traditional and emerging risk factors were not able to predict silent ischemia. Designing a new guideline for earlier screening of diabetic patients seems to be helpful.
Keywords: Cronary artery disease, Diabetes, Cardiac symptoms.

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Impaired glucose regulation and arterial stiffness

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Background
Glucose intolerance produces structural and functional changes in the arterial wall which contribute to the excess cardiovascular morbidity and mortality. The present study investigated association between glucose tolerance status and arterial stiffness in subjects with normal and impaired glucose regulation (IGR).
Methods
The study group consisted of 283 Caucasian subjects, including 111 subjects with normal glucose tolerance (NGT), 61 subjects classed as impaired fasting glucose (IFG) according of the new fasting blood glucose (FBG) cutoff point of 100 mg/d and 111 patients with type 2 diabetes mellitus. All patients were evaluated for glucose, HbA1C, insulin, lipid profile, hs-CRP, HOMA-IR. PWV and AI were performed as a noninvasive recording of the two artery sites pressure waveform using SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia).
Results
PWV values increased significantly and consistently with deterioration of glucose tolerance status from NGT to IFG and DM. AI and central arterial pressure differed significantly between groups and increased from group 1 to group 3 in a continuous fashion. Arterial stiffness parameters remain significantly higher in both IFG and DM groups compared to subjects with NGT after adjustment for cardiovascular risk factors and concomitant medications. The positive correlations between FBG and arterial stiffness parameters were found in all groups.
Conclusions
Arterial stiffness parameters varied significantly across subgroups of patients with different degree of IGR, such that more alterations in glucose homeostasis were consistently associated with an increased arterial stiffness. Deteriorating glucose tolerance was associated with an increased PWV, AI and central aortic pressure even after correction for cardiovascular confounders.

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Association between polymorphisms in the promoter of heme oxygenase-1 and vascular complications in type 2 diabetic patients

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Heme oxygenase-1 (HO-1) catalyzes the conversion of heme to carbon monoxide, free iron, and biliverdin, which is then changed into bilirubin. These substances have been recently demonstrated to have antiatherogenic and antioxidative properties. The GT-repeat polymorphism is reported to be an independent risk factor for restenosis after coronary stenting and T (−413). A polymorphism increased the activity of HO-1 promoter, leading to reduce the incidence of ischemic heart disease in Japanese population. The aim of the present study was to investigate association between polymorphisms in the promoter of HO-1 and the prevalence of vascular complications in type 2 diabetic (T2DM) patients.
We genotyped rs2071746 (−413T/A) and rs3761439 (−1135G/A) in the promoter region of HO-1 in 601 T2DM patients. Clinical and biochemical parameters were measured and random urine albumin and creatine ratio or 24 h urine analysis were performed to diagnose diabetic nephropathy. The extent of atherosclerosis was determined by the measuring intima-media thickness (IMT) of carotid artery with B-mode ultrasound.
The TT genotype of rs2071746 was associated with high prevalence of nephropathy (odds ratio (OR) = 1.58, 95% CI = 1.09–2.29, $P = 0.016$) comparing to the AA + AT genotypes. Patients with AA allele of rs3761439 had increased IMT than subjects with AG + GG alleles (0.883 vs 0.761 mm, $P = 0.002$). The OR of the TT genotype for the presence of carotid plaques in lower BMI (<25) groups was 1.746 (AA + AT versus TT, CI = 1.008–3.026, $P = 0.043$).
The T(−413)A and G(−1135)A polymorphisms in the promoter of HO-1 have associations with the risk of diabetic nephropathy and subclinical atherosclerosis in T2DM patients, respectively.

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Paradoxical effects of GABA on glucose-stimulated insulin secretion from isolated islets in rat

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Introduction
The islets of pancreas contain relatively high levels of Gamma-amino butyric acid (GABA). This study was designed to determine the role of the GABA and $GABA_B$ receptor on glucose-stimulated insulin secretion of isolated islets in rats.
Materials and methods
The Collagenase digestion technique was used to isolate the islets from male Wistar rats and insulin secretion was assessed in islets exposed to glucose (8.3, 16.7 mM) in presence and absence of GABA (25, 50, and 100 μM), a $GABA_B$ agonist, baclofen (10, 20, and 50 μM) and $GABA_B$ antagonist, saclofen (50 and 100 μM); islets were incubated in Krebs–Ringer solution at 37 °C in the presence of different drugs. Following this insulin secretion was measured by the ELISA method and reported as mean ± S.E.M. μU/islet per minute. One-way analysis of variance was used for comparing means between groups.
Results
When 50 μM GABA was added 45 min before glucose, insulin secretion was found to be increased during 60 min incubation time; however adding GABA and glucose simultaneously caused a significant decrease in insulin secretion. Baclofen had no significant effect on glucose-induced insulin secretion, whereas 100 μM Saclofen significantly increased glucose (16.7 mM) stimulated insulin secretion (91 ± 8.8 vs 67.7 ± 2.58 μU/islet per 60 min, $P < 0.05$).
Conclusion
GABA could have both stimulatory and inhibitory effects on glucose-stimulated insulin secretion, depending on the time of exposure.

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Inspiratory muscle strength is correlated with carnitine levels in type 2 diabetes

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Background
Plasma carnitine insufficiency has been caused muscle weakness. Carnitine levels and pulmonary functions were lower in patients with diabetes.
Objective
To determine whether pulmonary functions are correlated with carnitine levels in patients with type 2 diabetes.
Setting
Internal medicine outpatient clinic of a university hospital.
Methods
Forty-nine patients with type 2 diabetes and 34 healthy controls were evaluated. Pulmonary functions and carnitine concentrations were studied.
Results
Carnitine levels were lower in type 2 diabetes group than control group, (52.56 ± 12.38 and 78.96 ± 10.66 ηmol/ml, respectively, $P < 0.0001$). Pulmonary functions were not significantly differed between groups. Carnitine levels were not correlated with age, duration of diabetes, fasting blood glucose levels and, glycemic control (%HbA1c) in patients with type 2 diabetes. However, carnitine levels in patients group were correlated with % forced vital capacity (%FVC) ($r = 0.35$, $P = 0.016$), % forced expiratory volume in one second (%FEV1) ($r = 0.318$, $P = 0.029$), FEV1/FVC ($r = 0.302$, $P = 0.039$), inspiratory muscle strength (PImax) ($r = 0.407$, $P = 0.023$) and, %PImax ($r = 0.423$, $P = 0.018$). Multiple regression analysis including above parameters reveals %PImax was significantly associated with carnitine levels ($\beta = 0.431$, $P = 0.019$).
Conclusions
The present study suggests that low carnitine levels may be associated with lower %PImax in type 2 diabetes.

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Mildronate positively affects compensation of diabetes in streptozotocin rats and alters iNOS gene expression in rat tissues

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Mildronate, a γ-butyrobetaine analogue is actually used as an antiischemic drug. It was also shown to have effect on mechanisms of glucose utilization.
Methods
Diabetes mellitus in Wistar rats was induced by injection of streptozotocin (50 mg/kg). Experimental rats were treatment with Mildronate (100 mg/kg daily, *per os*) for 6 weeks. Weight, blood glucose concentration, blood triglyceride concentration, blood ketone body concentration, glycated hemoglobin percent (HbA1c%), glucose tolerance were monitored throughout the experiment. iNOS gene expression was evaluated by qRT-PCR in heart, muscle, liver and kidney of diabetic animals.
Results
In diabetic rats, Mildronate treatment caused a significant decrease in mean blood glucose concentration after 4 weeks of treatment (streptozotocin group – 40.27 ± 3.34 mmol/l, streptozotocin + Mildronate group – 29.82 ± 2.12 mmol/l). Mildronate produced positive effect on triglyceride level in diabetic rats: after 4, 5 and 6 weeks of treatment streptozotocin + Mildronate group showed lower triglyceride levels, than streptozotocin group (after 4 weeks – 1.29 ± 0.10 vs 1.91 ± 0.26 mmol/l; after 5 weeks – 1.04 ± 0.03 vs 1.23 ± 0.08 mmol/l; after 6 weeks – 1.12 ± 0.09 vs 1.77 ± 0.30 mmol/l). Mildronate was able to slow down significantly the rise of HbA1c% in treated diabetic group (after 6 weeks of treatment HbA1c% in streptozotocin group – 9.66 ± 0.21%, in streptozotocin + Mildronate group – 8.75 ± 0.33%). Oral glucose tolerance test after 4 treatment weeks revealed significantly better glucose tolerance in streptozotocin + Mildronate group at 120 min after glucose ingestion. iNOS gene expression was altered by Mildronate treatment in liver, muscle and heart. This might indicate on stimulatory effect of Mildronate on insulin-independent glucose transport in tissues of diabetic rats.
Conclusion
Mildronate improves carbohydrate metabolism in experimental diabetes mellitus model, possibly via insulin-independent mechanisms.

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Topical Atorvastatine may be beneficial in the treatment of the wounds in diabetic rats

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Background
Currently, it is reported that statins may be useful in the treatment of DM foot ulceration[1]. The aim of the study was to evaluate treatment with topical atorvastatin of the wounds of streptozotocine induced diabetic rats.
Materials and methods
Fifteen × fifteen mm sized wound were created in streptozotocin-induced rats. A total of twenty-eight diabetic rats were studied in 4 groups (n: 28). Any treatment was not administered in the first group. Second, third, fourth groups were performed 1:1 mixture of lanolin and vaseline; lanoline-vaseline plus 1% atorvastatine and lanoline-vaseline plus 5% atorvastatine, respectively. On seventh and fourteenth days, state of the wound healing was observed and the percent of wound healing was determined by being measured its size. The statistical analyses were carried out by Oneway Anova Tukey HSD test.
Results
On the fourteenth day, the rates of wound healing in first, second, third and fourth groups were 14, 40, 96.59 and 96.51%, respectively. Accordingly, in the multiple comparisons; the rates of wound healing were found to be significantly higher in the diabetic rat groups administering 1 and 5% atorvastatine compared with those administering mixture of lanolin-vaseline and untreatment (for comparison each one $P < 0.001$).
Conclusion
Topical atorvastatine therapy may be useful on the *wound* healing in *diabetic rats*. Further clinic and experimental studies are needed to confirm detail these results.

P352

Coronary heart disease risk reduction in obese patients submitted to adjustable gastric band

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Background
Obesity is related to an increased cardiovascular risk, namely coronary heart disease risk (CHDr). Type 2 diabetes (T2D) is frequently associated with obesity and, by itself, is an important CHDr factor. Interventions that induce weight loss would be expected to attenuate CHDr in obese patients.
Aims
To evaluate variation in cardiovascular risk profile one year after adjustable gastric banding (AGB), in obese patients (with and without T2D); to correlate variation in CHDr estimate with variation in anthropometric parameters after surgery.
Methods
We studied 80 obese patients (11 men) that were characterized for BMI, waist circumference (Wc) and blood pressure levels; a fasting blood sample was collected for glucose, total cholesterol (t-cholesterol) and HDL-c. Smoking habits and previous diagnosis of diabetes was checked. We used the Framingham risk equation for CHDr assessment. Patients were submitted to AGB and all parameters were reassessed 12 months after.
Results
Before surgery, patients were characterized by mean age = 43 ± 10 years, weight = 124.6 ± 20.7 kg, BMI = 48.2 ± 6.3 kg/m^2, Wc = 126.4 ± 13.8 cm, systolic blood pressure (SBP) = 135 ± 21 mmHg, diastolic blood pressure (DBP) = 87 ± 11 mmHg, t-cholesterol = 193.8 ± 38.7 mg/dl, HDL-c = 50.2 ± 12.2 mg/dl and Framingham score = 6.5 ± 6.1%. Twelve patients were smokers and 26 were diabetic. Twelve months after AGB there was a significant decrease in BMI ($P < 0.001$), Wc ($P < 0.001$), DBP ($P < 0.001$), fasting glucose ($P < 0.001$), HbA1c ($P < 0.001$), t-Cholesterol ($P = 0.043$), T2D prevalence ($P < 0.001$) and Framingham score ($P = 0.022$) and a significant increase in HDL-c ($P = 0.009$). No significant correlation was present between Framingham score and anthropometric variations. There was no significant difference in anthropometric or in Framingham score variations between patients with and without T2D.
Conclusions
There is an important amelioration of several cardiovascular risk factors and of CHDr estimate in obese patients one year after AGB. No major difference in CHDr variation is observed between diabetic and non-diabetic patients. The reduction observed in CHDr is independent from the direct effect of fat mass loss.

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Atherogenic and anti-atherogenic risk markers in subjects with obesity and/or type 2 diabetes

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Introduction
A subclinical inflammatory state in obesity may be the link between diabetes mellitus (DM) and atherogenesis. This study aimed to investigate the relationship between pro atherogenic factors (C reactive protein (CRP), interleukine 6 (IL-6), vascular adhesion molecular type 1 (VCAM-1)), and anti-atherogenic factor adiponectin in subjects with obesity and DM.
Methods
One hundred and twelve subjects (51 men, 103 women) were enrolled: group DM (lean subject with DM, $n = 21$), group OB (obese nondiabetic subjects, $n = 52$, BMI ≥ 27 kg/m^2) and group OB + DM (obese diabetic subjects, $n = 49$). A health group of 32 subjects with normal BMI was the control group (CG). CRP, IL-6, VCAM-1, adiponectin, glucose and lipid profile were determined in blood. Body composition was estimated by BIA.
Results
Obese subjects had higher CRP (OB = 6.6 ± 4.3 and OB + DM = 6.0 ± 4.0 versus GC = 1.7 ± 2.0, $P < 0.001$), VCAM-1 (OB = 518 ± 122.5 versus CG = 453.8 ± 119, $P < 0.001$), and lower adiponectin (OB = 0.87 ± 0.17; OB + DM = 0.88 ± 0.19 versus GC = 1.04 ± 0.19, $P < 0.001$) and HDL (OB + DM = 46.4 ± 0.2 and OB = 48 ± 0.4 versus CG = 55 ± 3.0, $P < 0.001$). DM subjects had lower adiponectin (DM = 0.91 ± 0.21 and OB + DM = 0.88 ± 0.19 versus CG = 1.04 ± 0.19, $P < 0.001$) and higher VCAM-1 (DM = 570 ± 132 and OB + DM = 572 ± 158 versus GC = 453.8 ± 119, $P < 0.001$), TG (DM = 157 ± 45 and OB + DM = 176 ± 11.9 versus GC = 83 ± 40 $P < 0.001$) and total cholesterol (DM = 230 ± 37 and OB + DM = 220 ± 36 versus GC = 206 ± 37, $P < 0.001$). IL-6 was not

significantly different between groups. Multivariate analysis adjusted for sex and age showed that adiponectin was inversely and independently associated with BMI ($r=-0.300$, $P=0.003$) and directly with HDL ($r=0.299$, $P=0.024$) in the whole population. Adiponectin was inversely and independently associated with TG only in OB+DM group ($r=-0.224$, $P=0.028$). CRP was independently associated with visceral fat and total body fat ($r=0.610$, $P=0.0001$) in DM groups. The VCAM1 was not correlated with any variable.
Conclusion
We found significant increases on pro atherogenic markers CRP and VCAM-1 in obese subjects, as well as decreases on protective factors adiponectin and HDL, independent of the presence of type 2 diabetes.

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Optimal fasting plasma glucose level for diagnosis of diabetes in a Singaporean population

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Aim
Diabetes mellitus is defined by the World Health Organization (WHO) as fasting plasma glucose (FPG) $\geq$7.0 mmol/l (mM) or 2-hour post-load glucose (2HPG) $\geq$11.1 mM in the 75-gram oral glucose tolerance test (OGTT). However, reported FPG cut-off levels that correspond to this 2HPG level are below 7.0 mM in Asian studies. Our study thus aims to find the optimal FPG cut-off that corresponds to 2HPG of 11.1 mM in an Asian population in Singapore.
Methods
Seven hundred and eighty-seven subjects were screened for diabetes with a 75-gram OGTT at the outpatient clinics of a Singapore hospital from 2001 to 2007. Plasma glucose levels were measured using a Beckmann-Coulter analyser. Regression models and receiver operating characteristic (ROC) curves in SPSS 16.0 were used to define the optimal FPG cut-off. Stratified analyses were performed for age and sex.
Results
The mean age of our patients (393 males, 49.9%) was 50.0$\pm$15.4 years (range 14–93). Their average FPG was 6.5$\pm$2.8 mM (range 3.0–24.5), and mean 2HPG was 11.0$\pm$5.6 mM (range 3.2–36.4). Exponential regression models were the best fit (higher R^2 value than linear, quadratic and logarithmic) for the whole population. The FPG level corresponding to 2HPG 11.1 mM using the exponential model was 6.1 mM. Patients aged 50 years and above had lower FPG cut-off (6.1 mM) corresponding to 2HPG 11.1 mM than younger patients (6.2 mM). The FPG cut-off derived from ROC analysis of the whole population was 6.0 mM (sensitivity 81.5%, specificity 82.0%, area under curve 0.90). Both age groups had FPG cut-off of 6.0 mM in the ROC analyses. Men and women had similar FPG cut-offs in both quadratic models and ROC analyses.
Conclusion
The FPG cut-off for diagnosis of diabetes in our Asian population is lower than the current WHO criteria.

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Endogenous estrogen levels are associated with endothelial function in males independently of lipid levels

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Introduction-aim
It has been suggested that estrogen may play an important role in the regulation of endothelium-dependent vasodilatation in both sexes. Especially in men, estradiol administration has been shown to improve endothelial function; however such reports are conflicting. The aim of our study was to examine the relation of endogenous sex hormone levels with markers of early atherosclerosis in a cohort of apparently healthy individuals.
Methods
One hundred and forty-three males (age 46.25$\pm$9.56 BMI 20.4 – 43.3, median 26.36 kg/m^2) attending a preventive medicine program were examined for unrecognised features of the metabolic syndrome. Early markers of atherosclerosis such as endothelium dependent vasodilatation (flow-mediated-dilatation, FMD) and intima media thickness (IMT) of the common carotid artery were recorded. BMI, waist and hip circumference and arterial pressure were also recorded. Estradiol, testosterone, SHBG, free testosterone, insulin, as well as glucose and lipid levels were measured.
Results
Higher estrogen levels were associated with lower cholesterol levels ($r=-0.1963$, $P=0.047$) and higher BMI ($r=0.2790$, $P=0.004$). Estradiol levels were positively correlated with FMD ($r=0.2016$, $P=0.041$). FMD was negatively associated with total cholesterol ($r=-0.2056$, $P=0.022$), low density lipoproteins ($r=-0.2322$, $r=0.009$) and triglycerides levels ($r=-0.1796$, $P=0.046$). Multivariate analysis showed that the association of estrogen levels with FMD was independent of lipid levels ($r=0.292$, $P=0.041$). No significant association of estradiol levels with the IMT of the common carotid artery was found. Free and bioavailable testosterone were negatively associated with the IMT of the left carotid artery only ($P<0.03$).
Conclusions
Estrogen levels are associated with FMD, showing a protective effect, in apparently healthy, slightly overweight, male subjects. This appears to be a direct effect of endogenous estrogen on cardiovascular health independent of lipid levels. Circulating androgen may be favorable for structural changes such as the IMT thickness of carotid artery.

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Glucose intolerance and risk of cardiovascular disease: results of the 7.6 year follow-up of the Tehran lipid and glucose study (TLGS)

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Background
To determine the risk of cardiovascular disease (CVD) in an Iranian population according to glucose tolerance status.
Methods and results
The study population consisted of 1752 men and 2273 women aged $\geq$40 years without CVD. After a median follow up of 7.6 years, 340 CVD events occurred (197 in men and 143 in women). Women generally had more Framingham risk score (FRS) than men (12.7 vs 11.9, $P<0.001$) and there was no difference between the FRS of newly diagnosed diabetes mellitus (NDM) and known diabetes mellitus (KDM) in both genders. Applying Cox proportional hazard modeling, after controlling risk factors, hazard ratios (HRs) and 95%confidence intervals for CVD in women with KDM and NDM were 3.88 (2.40 to 6.27) and 2.34 (1.39 to 3.95) and the corresponding values for men were 1.72 (1.00–2.95) and 1.52 (1.01–2.31) respectively. In age adjusted model, impaired fasting glucose or impaired glucose tolerance (IFG/IGT) was associated with 56% increased risk for CVD only in women (HR: 1.56, 95% CI 1.00 to 2.45). The multivariate HR for abnormal glucose metabolism (KDM, NDM and IFG/IGT) was significant in women 1.8 (1.2 to 2.7) but not in men 1 (0.7 to 1.4). Adjustment with FRS instead of risk factors did not change our results.
Conclusion
All diabetics should receive intensive primary prevention for CVD regardless of risk factors and whether they are NDM or KDM, with further emphasis on female with abnormal glucose metabolism.

P357

Pharmacokinetics of the dipeptidyl peptidase-4 inhibitor saxagliptin in subjects with renal impairment

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Background/aims
Saxagliptin is a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme. Saxagliptin is cleared by both metabolism and renal excretion. The aims of this study were to determine the effect of renal impairment (RI) and haemodialysis (HD) on the pharmacokinetics of saxagliptin.

Methods
This open-label, parallel-group study was conducted in subjects with normal renal function (glomerular filtration rate (GFR) > 80 ml/min), mild (50–80 ml/min), moderate (30–50 ml/min) and severe (< 30 ml/min) RI, and end-stage renal disease (ESRD) requiring HD (8 subjects/category) in clinical pharmacology units. Subjects were administered single oral doses of saxagliptin (10 mg), and on a separate occasion received intravenous iohexol for a secondary GFR assessment (not HD subjects). A 4-h HD session was started 2 h after saxagliptin dosing in ESRD subjects. Serial blood and cumulative urine samples for pharmacokinetic assessments of saxagliptin, its major active metabolite (BMS-510849), and iohexol were collected.
Results
Iohexol systemic clearance correlated well with estimated GFR. The degree of RI did not affect the C_{max} of saxagliptin or its major metabolite. In mild RI subjects, the overall mean systemic exposure (AUC_∞) values of saxagliptin and its major metabolite were 1.2- and 1.7-fold higher, respectively, than mean AUC_∞ values in subjects with normal renal function. The saxagliptin and metabolite AUC_∞ values in moderate RI subjects were 1.4- and 2.9-fold higher, respectively, than subjects with normal renal function. The corresponding values in severe RI subjects were 2.1- and 4.5-fold higher, respectively. A 4-h HD session removed 23% of the saxagliptin dose.
Conclusion
AUC_∞ values for saxagliptin and, to a greater extent, its major metabolite were correlated with the degree of RI, whereas C_{max} values were not well-correlated. Saxagliptin and its metabolite were cleared by haemodialysis.

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Biphasic insulin aspart 30/70 improves glycaemic control in patients with type 2 diabetes: clinical practice experience from Indian subgroup of the IMPROVE study™

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Aims & objectives
IMPROVE™ is a 6-month, multi-national, prospective, observational study, assessing the safety and efficacy of biphasic insulin aspart 30/70 (BiAsp 30) in patients with type 2 diabetes.
Methods
We present the efficacy data from the Indian cohort of IMPROVE study. A total of 17 995 subjects entered the study and 16 942 subjects completed the study. BiAsp 30 was initiated and dose was adjusted at the physician's discretion, reflecting everyday practice. Efficacy analysis set (EAS) includes 16 322 subjects. Efficacy was assessed by change in mean HbA1c, proportion of subjects achieving HbA1c targets (≤6.5%, <7%, target set by treating physicians), change in FBG, change in PPBG and change in FBG variability following 26 weeks of treatment.
Results
In comparison to baseline, there were significant reductions in mean HbA1c (n=15 624, 9.33 to 7.33%, $P<0.001$), FBG (n=14 935, 191 to 120 mg/dl, $P<0.001$), FBG variability (n=59, 21 to 11 mg/dl, $P<0.001$) and 2-hour post breakfast PPBG (n=10 319, 277 to 171 mg/dl, $P<0.001$) at the end of 26 weeks treatment. Significant improvement was also seen in proportion of subjects achieving HbA1c ≤6.5% (19%), HbA1c<7% (40%) and HbA1c target set by treating physicians (32%).
Conclusions
In this real life practice study, BiAsp 30 effectively improved glycemic parameters. Initiation of BiAsp 30 treatment significantly reduced mean HbA1c, FBG, 2-hour PPBG and FBG variability. There were also significant improvements in proportions of subjects achieving HbA1c targets. In conclusion, BiAsp 30 is an effective option for treating type 2 diabetes in Indian subjects.

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Saxagliptin either as add-on therapy to metformin or as initial combination therapy with metformin improves glycaemic control in patients with type 2 diabetes

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The efficacy and safety of saxagliptin – a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme – was investigated in two double-blind, randomised trials (CV181-014/Study 1 and CV181-039/Study 2), either as add-on therapy in patients with type 2 diabetes mellitus (T2DM) inadequately controlled by metformin alone (HbA_{1c} 7.0–10.0%) or as initial combination therapy with metformin in drug-naïve T2DM patients (HbA_{1c} 8.0–12.0%), respectively.
Following a placebo run-in, patients with inadequately controlled T2DM (n=743) on metformin, in Study 1, were randomised to receive once-daily saxagliptin 2.5, 5.0 or 10.0 mg, or placebo, plus their stable metformin dose, and drug-naïve patients (n=1306), in Study 2, were randomised to receive saxagliptin/metformin 5/500 mg (S5/MET), 10/500 mg (S10/MET), saxagliptin 10 mg or metformin 500 mg once-daily. In the S5/MET, S10/MET and metformin alone treatment groups of Study 2, metformin was up-titrated incrementally (Weeks 1–5) to a maximum of 2000 mg/day. Both studies' primary endpoint was HbA_{1c} change from baseline. Treatment groups were well balanced for baseline characteristics within each study. At week 24, significant ($P<0.0001$) reductions in adjusted-mean HbA_{1c} change from baseline were observed in Study 1 for saxagliptin 2.5, 5.0 and 10.0 mg (−0.59, −0.69 and −0.58%, respectively), compared with placebo (0.13%), and in Study 2 for S5/MET (−2.53%) and S10/MET (−2.49%), compared with saxagliptin (−1.69%) or metformin (−1.99%) alone. In each study, saxagliptin plus metformin provided significant ($P<0.001$) reductions in fasting plasma glucose and postprandial glucose, increased proportions of patients with therapeutic glycaemic response ($HbA_{1c}<7\%$), and was well tolerated with no increased incidence of hypoglycaemia compared with matched controls. Saxagliptin add-on or initial combination therapy with metformin provides significant and clinically meaningful reductions in key parameters of glycaemic control and is well tolerated in patients with inadequately controlled T2DM.

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Effects of estrogen therapy on cardiovascular risk factor in transsexuals

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Estrogens play a protective role on the peripheral vascular endothelium in both females and males, however, no studies have reported on the effects of estrogens on male coronary arterial reactivity. We investigated the effects of estrogen therapy on coronary and endothelial function in a group of male to female transsexuals (M-to-F). Eight M-to-F transsexuals (group A), receiving oral estrogen therapy for 72 months were studied in comparison with 23 age-matched healthy controls subdivided into 3 groups: 8 women (group B), 7 men without cardiovascular risk factors (group C), and 8 men (group D) with a comparable cardiovascular risk of transsexuals. Coronary flow reserve (CFR), was assessed to investigate the coronary endothelium. Flow mediated dilation (FMD) was assessed on brachial artery after flow increase and after nitroglycerine administration. Cardiovascular risk factors (smoke, blood pressure, lipid profile) were recorded.
The average number of risk factors was significantly greater in transsexuals and group D than in groups B and C. Systolic blood pressure was higher in group A an D than in B. Total and HDL cholesterol levels were similar in all groups but LDL cholesterol and triglyceride levels were higher in group A and D than in group B and C. CFR was higher in transsexuals compared with group D, but significantly reduced compared with groups B and C.
FMD in transsexuals was higher, although not significantly, than in group D, whereas it was significantly reduced compared with group B and C. Response to nitroglycerine was similar in transsexuals and in group D, and significantly reduced compared to groups B and C.
Estrogen therapy in M-to-F transsexuals is not harmful in terms of peripheral vascular function and coronary endothelial function. A tendency toward small improvement of endothelial risk factors with respect to the general population seems to be produced by the treatment.

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Carotid intima media thickness and serum osteoprotegerin and RANKL levels in diabetic and prediabetic patients

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While well defined for their roles in new bone formation, osteoprotegerin and RANK-ligand (RANKL) are also being questioned for their possible association

with the atherosclerotic process since the similarity between the pathogenic process of atherosclerotic plaque calcification and new bone formation is recently under observation. In our study we aimed to find whether there is an association between OPG and RANKL levels and carotid intima media thickness (taken as a measure of atherosclerosis) in diabetic and prediabetic subjects.
We evaluated 78 subjects (17 male). Twenty of them were type 2 diabetic, 16 had impaired glucose tolerance (IGT), 19 had impaired fasting glucose (IFG) and 23 were healthy controls. None of the subjects had a known cardiovascular or cerebrovascular disease neither suffered micro- macrovascular complications of diabetes. Anthropometric measurements are taken in all subjects, serum OPG and RANKL levels are measured as well as serum lipids and lipoprotein a, C reactive protein, homocysteine and insulin. Carotid intima media thickness is measured by ultrasonography.
Overall, RANKL and OPG levels did not differ between groups. There was a positive correlation between OPG and mean carotid intima media thickness in IFG group only ($P<0.05$; $r=0.47$). OPG is positively correlated with insulin levels in type 2 diabetic patients ($P<0.05$; $r=0.51$). RANKL levels were positively correlated with triglyceride levels in healthy controls ($P<0.05$; $r=0.42$) In multivariate analysis, we failed to find an independent parameter related to carotid intima media thickness in each group.
OPG level is positively correlated with mean carotid intima media thickness in subjects with IFG. This effect disappears when confounding factors are taken into account. We believe that the relationship between OPG and RANKL levels and atherosclerosis needs to be studied in larger populations with or without conventional risk factors for atherosclerosis.

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Klotho gene polymorphism may be a genetic risk factor for metabolic syndrome in men

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Introduction
Klotho has an important role in insulin signaling and the development of ageing-like phenotypes in mice. Recently, the G-395A polymorphism in the promoter region of the human klotho gene has been reported to affect promoter function. It has been also shown to be an independent genetic risk factor for atherosclerotic cardiovascular disease. The aim of this study was to examine the possible role of this polymorphism in the metabolic syndrome.
Subjects and methods
The study population consisted of 32 men with metabolic syndrome aged 63.5 ± 14.8 years and 64 healthy men of similar age. The body mass index and the waist to hip ratio were recorded and blood samples were obtained after overnight fasting for biochemical tests. The G-395A polymorphism was genotyped in peripheral blood leucocytes.
Results
The G-395A genotypes were found to be in Hardy-Weinberg equilibrium in both study groups. Compared with healthy men, men with metabolic syndrome were less frequently carriers of the GG genotype and the G allele (53.1 vs 76.6%, $P=0.03$ and 73.4 vs 86.7%, $P=0.02$ respectively). Neither BMI, nor lipid profile was different among genotypes of the G-395A polymorphism in men with metabolic syndrome. However, patients carriers of the GG genotype had less frequently diabetes compared with patients with GA or AA genotype (30.8 vs 69.2%, $P=0.03$).
Conclusion
The G-395A polymorphism of the klotho gene may be involved in the pathogenesis of metabolic syndrome and glucose metabolism in men.

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Chemotherapy does not influence measures of glycaemic control in non-diabetic patients affected by acute leukaemia

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Aim
Aim of the study was the evaluation of the effects of chemotherapy on insulin secretion and insulin sensitivity in patients affected by acute leukaemia.

Materials and methods
Thirty-two non-diabetic patients (17 male, 15 female; age 51.1 ± 17.4, range 16–76 years; BMI = 25.1 ± 3.7, range 18.4–32.8 kg/m^2; 6 = 18% with a family history of diabetes mellitus) affected by acute leukaemia, 4 LLA (12.5%) and 28 LMA (87.5%), have been submitted to chemotherapy (different combination of mitoxantrone, cytarabine, vincristin, doxorubicin, etoposide and prednisone). Fasting glycaemia, insulin (IRI) and C-peptide have been evaluated before and after chemotherapy as well as HbA1c. Indices of beta cells function and insulin resistance have been calculated.
Results
The results are reported in the Table (P = NS).
Conclusion
All patients showed a basal condition of impaired fasting glucose, hyperglycaemia being frequently observed in critically-ill patients. Preliminary results of our study, however, show that chemotherapy does not influence measures of glycaemic control in non-diabetic patients affected by LLA and LMA.

	FG (mmol/l)	IRI (mU/l)	C-pep (mcg/l)	HbA1c (%)	Glyc/Ins (mmol/mU)	HOMA-IR	HOMA β cell function
Pre-chemot.	6.5 ± 0.3	19.6 ± 2.9	5.2 ± 0.7	5.0 ± 0.8	0.50 ± 0.34	6.0 ± 4.8	148 ± 115
Post-chemot.	6.5 ± 0.4	18.5 ± 1.8	4.6 ± 0.7	5.0 ± 0.4	0.41 ± 0.22	5.8 ± 4.2	130 ± 66

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Pancreatic function in β-thalassemic patients

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Background & aims
Impaired glucose tolerance and diabetes as well as cardiac dysfunction are known complications in homozygous beta-thalassemic transfused patients due to iron overload. We aimed to study pancreatic alpha and beta-cell function in regularly transfused patients with homozygous beta-thalassemia and to investigate if cardiac dysfunction may correlate with the development of impaired glucose metabolism.
Methods
An oral glucose tolerance test (OGTT) with 75 g was performed in 38 beta-thalassemic patients (17–45 years old), 28 with normal and 10 with impaired fasting glucose. All patients were receiving two blood units every 15–20 days and were on iron chelation therapy. Glucose, insulin, C-peptide and glucagon plasma levels were assayed every thirty minutes up to 2 h. Patients were divided according to the American Diabetes Association criteria into those with diabetes mellitus, impaired glucose tolerance and those with normal glucose tolerance. A division concerning cardiac dysfunction was also made according to Doppler Echocardiograph and myocardium MRI results.
Results
Diabetes mellitus was diagnosed in eight patients, and impaired glucose tolerance was observed in ten patients, giving a prevalence of total impaired glucose metabolism of 47.3% in our patient population. After OGTT, the area under insulin plasma concentrations versus time curve ($AUC^{Insulin}_{02\ h}$) was lower for patients with impaired glucose metabolism compared to those with normoglycaemia (3936 (μIU/ml) min versus 6549 (μIU/ml) min, $P<0.01$). Similarly, $AUC^{Insulin}_{02\ h}$ was lower in patients with cardiac dysfunction compared to those with normal cardiac function (3079 (μIU/ml) min versus 6189 (μIU/ml) min, $P<0.05$). The area under the curve for glucagon after OGTT was similar in normoglycemic and hyperglycemic patients.
Conclusions
The significant decrease of $AUC^{Insulin}_{02\ h}$ in thalassemic patients with impaired glucose metabolism is consistent with pancreatic beta-cell failure while alpha-cell function does not seem to be influenced. In thalassemic patients with normal fasting glucose, cardiac dysfunction may be a prognostic factor for impaired glucose metabolism.

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Characterization of type I interferon (IFN) mediated diabetes sparing activity

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We have shown that Type I IFN paradoxically inhibits autoimmune diabetes in the NOD mouse and BB rat. We assessed the structure function relationship and potential mechanism of this interferon action by determining: (1) the diabetes sparing effect of several type 1 IFNs: recombinant human IFN-A/D bgl 11 (rIFN-alpha), rIFN-alphaB/D, rIFN-alpha-consensus (CIFN), IFN-taumod1 in NOD mice (2) the effect of IFN-tau administration in IL-4 KO and IFN-gamma KO NOD mice (3) the effect of IFN-tau administration on NOD spleen cell expression of CD152 (CD40-ligand) and class 11 MHC (Ia^k) (4) the effect of various type 1 IFNs on LPS induced spleen cell production of nitric oxide *in vitro*. Both rIFN-alpha and IFN-tau (2×10^5 units IP TIW) potently inhibited the development of diabetes ($P<0.01$) while rIFN-alphaB/D and CIFN did not. IFN-tau (2×10^5 units IP TIW) inhibited the development of diabetes in IL-4 KO NOD mice while had no effect on IFN-gamma KO mice. IFN-tau (2×10^5 units IP TIW) administration decreased the expression of class 11 MHC expression on B cells and CD40L expression on T cells by 45 and 60% respectively. rIFN-alpha and rIFN-alphaB/D inhibited LPS stimulated NO by 84 and 74% respectively ($P<0.01$) while IFN-tau and CIFN had no effect. In conclusion, these data support the contention that IFN-gamma reduction has a role in mediating the diabetes sparing effect of type 1 IFN. Further, only some Type I IFNs have this inhibitory effect on the autoimmune diabetic process in mice. The inhibition of class 11 MHC binding and CD40–CD40 ligand interactions by IFN-tau may play a mediate the diabetes sparing effect. Although some type 1 IFNs inhibit the production of NO, this action may not be an important mediator for any or all type 1 IFNs since NO inhibition did not correlate with diabetes sparing activity.

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A comparison between adhesion molecules (as markers of inflammation) in identifying cardiovascular disease in postmenopausal women

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Background
There are increasing evidences that inflammation is involved in the pathophysiology of both coronary heart disease and cerebrovascular disease. Adhesion molecules have been advocated as a marker of inflammation.
Objectives
To evaluate the capacity of inflammation's markers (sICAM1, sVCAM1) to identify cardiovascular disease, comparing adhesion molecules with a standard diagnosis of cardiovascular disease.
Methods
We examined 35 postmenopausal women with mean aged of 57.91 ± 12.91 years. As risk factors have been assessed the body weight, smoking status, glicemia, and serum lipids fractions. In order to confirm or exclude cardiovascular disease we perform clinical exam, ECG and, when was necessary, echocardiography, stress test or coronary angiography. Adhesion molecule (sICAM1 and sVCAM1) were measured (in ng/ml) in stored serum samples collected, using ELISA method. Optimum sensitivity, specificity, predictive values, and area under the receiver operating characteristic (ROC) curve were evaluated.
Results
Cardiovascular disease was present in 14 (38.9%) of the cases. There were no significant differences registered regarding sICAM1's and sVCAM1's values between patients with and without cardiovascular disease (sICAM1 364.5 ± 122.90 vs 362.79 ± 116.93 ng/ml $P=$NS, respectively for sVCAM1 702.75 ± 200.39 vs 605.07 ± 172.77 ng/ml, $P=$NS). Area under the ROC curve was 0.505 for sICAM1 ($P=$NS) and 0.668 for sVCAM1 ($P=0.07$). Diagnostic cut off levels with the optimum sensitivity and specificity derived from the ROC curve were found to be: sICAM1 228.4 ng/ml (sensitivity 92.86%, specificity 19.05%) and for sVCAM1 685.59 ng/ml (sensitivity 57.14%, specificity 85.7%).
Conclusion
Although, sVCAM1 is under the influence of some factors that are not fully explained (such age, presence of endothelial dysfunction or other cardiovascular risk factors), in postmenopausal women, sVCAM1 seems to be a better identifier of women at risk for cardiovascular disease in comparison with sICAM1.

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The relationship between hemoglobin levels and endothelial functions in diabetes mellitus

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Introduction
Hemoglobin (Hb) is the main carrier and buffer of Nitric oxide. Recently, an inverse association between Hb and the endothelium dependent relaxation was observed in patients with Type 2 diabetes. Testing whether this association exists also in diabetic nephropathy is important because anemia in these patients starts at an earlier stage than in other renal disease. Also, at population level diabetes and renal dysfunction, particularly albuminuria, contribute independently to the risk of cardiovascular complications. Therefore, we investigated the association between Hb and the forearm blood flow mediated vasodilatory response to ischemia (FMD) in a group of well selected patients with diabetic nephropathy.
Methods
We enrolled 89 diabetics with proteinura who were normotensive, non-obese, non-smoker, non dyslipidemic and cardiovascular events free. None of the patients were taking metformin or drugs that interfere with the renin–angiotensin system. FMD of the brachial artery was assessed by high resolution ultrasound.
Results
The age, sex, BMI, blood pressure, HbA1c and glomerular filtration rates (GFR) were similar in patients having Hb values either above or below the median Hb values. In the multivariate analysis, higher Hb levels were associated with significantly lower FMD values ($\beta=-0.44$, $P<0.001$). Adjustment for the full set of Framingham risk factors and further adjustment for proteinuria, hsCRP, insulin, GFR and the uric acid levels did not produce a significant reduction in the strength of the association between Hb and FMD.
Discussion
According to the results diabetic nephropathy patients with higher Hb values have impaired endothelial functions independent from any other established cardiovascular risk factor. Our findings show that frank proteinuria exposes a situation wherein Hb may limit the endothelium-mediated vasoregulation in type-2 diabetes. Further studies are warranted in order to see whether these findings may explain the mechanism of increased cardiovascular event rates in patients with diabetes mellitus and diabetic proteinuria.

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Lack of association between polymorphisms at the adiponectin gene locus and type 2 diabetes as well as body mass index in Latvian population

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Association studies between genetic variants of adiponectin gene and susceptibility to type 2 diabetes (T2D) and increased body mass index (BMI) have provided contradictory results. We selected 10 SNPs (rs2241767, rs1501299, rs3777261, rs16861210, rs2241766, rs822396, rs182052, rs17300539, rs16861194, rs266729) in promoter and coding regions of adiponectin gene based on haploblock structure and previously reported association studies. Selected SNPs were screened in 170 patients with T2D and 665 controls from Genome Data Base of Latvian Population. Neither of polymorphisms were associated with T2D status when analyzed using logistic regression and adjusted for gender, age and other significant covariates. Similarly, none of analyzed variants displayed significant allelic differences in BMI levels as assessed by linear regression with correction for significant cofactors. In addition correlation between allelic frequencies of all SNP and other diseases present in cohort of individuals selected for study was analyzed. Two polymorphisms were associated with presence of acute myocardial infarction (rs1501299, $P=0.004$; rs3777261, $P=0.034$). These associations, however, lost their significance when

adjusted for multiple testing. In summary, we conclude that SNPs in adiponectin gene are unlikely to represent the risk for T2D and increased BMI in Latvian population.

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Epicardial adipose tissue thickness and serum visfatin levels in patients with new diagnosed prediabetes and type 2 diabetes mellitus

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The relation between obesity and diabetes mellitus is known for long periods and this relation is based on insulin resistance. Recent studies showed that epicardial adipose tissue thickness measured by echocardiography is the marker of the visceral adipose tissue and cardiovascular risk. Visfatin is a new described adipokine and it has been showed to have mRNA expression in epicardial adipose tissue. There is a conflict about the role of visfatin on obesity, insulin resistance and type 2 DM pathogenesis.
In this study, we aimed to determine the relation between serum visfatin levels and epicardial adipose tissue thickness in different forms of diabetic patients.
We included a total of 128 persons in the study. Thirty-six patients were diabetic (diagnosed by 75 g OGTT), 69 were prediabetic and the remaining 23 were healthy (control). Serum biochemical measurements, body anthropometric measurements, epicardial adipose tissue thickness were calculated and recorded. The results were evaluated statistically.
Our study showed that epicardial adipose tissue thickness was higher in diabetic and prediabetic groups than control group and the difference was statistically significant ($P=0.006$ in diabetic group; P: 0.009 in prediabetic group). In diabetic and prediabetic patients there was no correlation between serum visfatin and epicardial adipose tissue thickness ($P>0.05$). On the other hand, in diabetic and prediabetic patients with obesity, the epicardial adipose tissue thickness was found to be correlated with serum visfatin levels and the correlation was statistically significant ($P<0.05$).
According to our results, epicardial adipose tissue thickness is an easy and cost effective method of detecting insulin resistance in visceral obese patients. Larger series are needed to determine the threshold levels of epicardial adipose tissue thickness which shows insulin resistance. We think that epicardial adipose tissue thickness may be an effective parameter in determining treatment modalities and drug choices in type 2 DM in future.

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The relationship between body mass index-left ventricle mass index-myocardial performance index in type 2 diabetes

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Introduction
Diabetes mellitus (DM) has been associated with abnormalities of cardiac function and left ventricular hypertrophy. Diabetic individuals, particularly women, had higher heart rates, greater left ventricular wall thicknesses, greater cardiac mass than unaffected subjects. We aimed to investigate association between waist circumference–body mass index (BMI) and left ventricle mass (LVM)-left ventricle mass index (LVMI), left ventricle myocardial perfusion index (MPI) in patients with type 2 DM and without known cardiac disease.
Methods
The patients with type 2 DM were examined with tissue doppler imaging echocardiography to detect MPI.LVM was calculated by the Penn Convention formula.
LVMI was calculated. Waist circumference was measured, BMI was calculated. The exclusion criterias; known cardiac diseases, pulmonary diseases, endocrine diseases except DM, anemia, angina pectoris, dyspnea, peripheral edema, serum creatinine level >1.5 mg/dl, ejection fraction (EF) $<50\%$. The GraphPad Prisma V.3 package program was used for statistical analyses.
Results
A total of 42 patients, men (40.5%) and women (59.5%) aged 37–57 years were included. In all patients, there were significant correlations between waist circumference and LVM; between BMI and LVM; between BMI and LVMI. Looking at the results according to sex; there were significant relations between BMI and LVM (P: 0, r: 0.685), BMI and LVMI (P: 0.007, r: 0.528) in women. This parameters were not associated in men. There was significant correlation between BMI and MPI (P: 0.026, r: -0.537) in only men (Table 1).
Conclusion
BMI associated with increasing LVM and LVMI, is an important risk factor in expecially women for diabetic heart disease. The MPI, a new doppler index of global cardiac function, has limited importance in type 2 diabetes without clinical cardiac disease and is more important in men.

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Correlation between serum 25 hydroxy vitamin D3 and laboratory risk markers of cardiovascular diseases in type 2 diabetic patients

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Background
Vitamin D deficiency is prevalent worldwide. Low 25 hydroxyvitamin D3 concentration inversely associate with type 2 diabetes, metabolic syndrome, insulin resistance, and probably cardiovascular disease. The objective was the evaluation of association between vitamin D deficiency and cardiovascular risk factors among some diabetic patients.
Methods and materials
One hundred and nineteen type 2 diabetic patients in IRAN from December to March 2008 were used. Coronary, cerebrovascular and peripheral vascular diseases were confirmed by medical history review, examination and paraclinical tests. Blood biochemical parameters including laboratory risk markers of cardiovascular disease were determined by standard laboratory procedures. Serum 25 (OH) D was measured during winter. The correlation between vitamin D deficiency and cardiovascular prevalent and also laboratory variables was determined.
Results
Mean patients age was 55.3 ± 11.2 year. The mean 25 (OH) D concentration was 32.4 ± 21.6 ng/ml. Prevalence of hypovitaminous D was 26.1% among the diabetic patients. The difference with control group was not significant ($P=0.12$). In overall, 36 (30.3%) patients were positive for coronary vascular disease (CVD). The correlation between hypovitaminous D and CVD was not significant ($P=0.11$). Vitamin D deficiency had a significant relationship with body mass index ($P=0.003$), metabolic syndrome($P=0.05$), high sensitive CRP ($P=0.009$), microalbuminuria ($P=0.04$), and glumerolar filtration rate ($P=0.02$). FBS, HbA1C, lipid profiles, homocysteine, uric acid and insulin resistance was not related to vitamin D deficiency.
Conclusion
The results showed a correlation between hypovitaminous D and inflammatory markers that could be contributed in cardiovascular disease so vitamin D may be important in cardiovascular health.

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***N*-acetylcysteine is able to reduce the oxidation status and the endothelial activation after a high-glucose content meal in patients with type 2 diabetes mellitus**

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Introduction
Post-prandial hyperglycaemia seems to play a pivotal role in the pathogenesis of cardiovascular complications of diabetes mellitus, as it leads to oxidative stress which in turn causes reduced NO bioavailability that produce endothelial activation.
Aim
Aim of the study was to assure that the administration of *N*-acetylcysteine (NAC), thiolic antioxidant, is able to decrease oxidation status and endothelial activation after a high-glucose content meal.

Subjects and methods
Ten patients with type 2 diabetes mellitus (DMT2) (Group 1) and 10 normal subjects (Group 2) assumed a high-glucose content meal without (phase A) or after (phase B) the administration of NAC. Glycaemia, insulinemia, ICAM-1, VCAM-1, E-selectin, malonaldehyde (MDA) and 4-hydroxynonenal (HNE) were assessed at 0, $+30'$, $+60'$, $+90'$, $+120'$ and $+180'$.
Results
During phase A in group 1, only HNE and MDA levels increased after the meal assumption ($+60'$: 7.7 (6.2–8.5) vs 6.9 (5.6–8.0), $P<0.05$ and 4.8 (3.6–5.3) vs 4.3 (3.7–4.9), $P<0.02$ respectively); all parameters remained unchanged in group 2. During phase B, in group 1, HNE, MDA, VCAM-1 and E-selectin levels after the meal were lower than those in phase A (see Table), while no change for all variables were observed in group 2.
Conclusions
A high-glucose meal produces an increase in oxidation parameters in DMT2. NAC reduces the oxidative stress and, subsequently, reduces the endothelial activation. In conclusion, NAC could be efficacious in the slackening of the progression of vascular damage in DMT2.

	0′	+30′	+60′	+90′	+120′	+180′
HNE	6.9 (4.9–7.3)	7.2 (4.6–7.8)*	7.3 (5.1–8.1)*	7.1 (5.1–7.9)*	6.8 (5.0–7.9)*	7.0 (4.8–7.9)
MDA	3.9 (3.0–4.4)	4.2 (2.9–4.7)*	4.3 (3.0–4.7)*	4.1 (3.0–4.8)*	4.2 (2.9–4.6)*	4.1 (3.0–4.8)*
VCAM-1	1.1 (0.7–1.8)	1.2 (0.7–1.8)	1.2 (0.6–1.9)*	1.2 (0.7–1.8)*	1.1 (0.7–1.9)	1.1 (0.7–1.7)*
E-SEL	3.1 (2.6–4.3)	3.1 (2.0–3.8)*	3.1 (2.0–3.6)*	2.7 (2.0–3.7)*	2.8 (1.8–3.9)*	2.7 (2.1–3.9)*

*$P<0.05$ respect to same time in phase A.

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Hyperhomocysteinemia may be a risk factor for diabetic foot ulcer development

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Background
Diabetic foot ulcer and lower extremity amputation are leading causes of mortality and morbidity in diabetic patients. Diabetic neuropathy and peripheral vascular disease are major reasons for diabetic foot ulcer. Most of the studies showed that hyperhomocystinemia is related with microvascular and macrovascular complications in diabetic patients. Also hyperhomocysteinemia is related to poor ulcer healing. In this study, we investigated the relationship of hyperhomocysteinemia and diabetic foot ulcers.
Methods
Fifty-one patients with diabetic foot ulcers, 35 diabetes patients without foot ulcers and 60 healthy individuals enrolled in this study. Wagner classification of diabetic foot ulcers was used. Plasma homocysteine levels were measured by Fluorescence Polarization Immuno Assay.
Results
Mean age was 60.0 ± 10.5 years in diabetic foot ulcer group, 57.8 ± 9.6 years in diabetic group and 55.6 ± 6.8 years in control group ($P=0.062$). The groups were similar in terms of mean age and sex distribution. Homocystein levels were 10.67 ± 1.79 μmol/l in control group, 17.54 ± 5.64 μmol/l in diabetic foot ulcer group, 13.26 ± 4.25 μmol/l in diabetic group and was statistically different ($P<0.001$). But no difference was seen between diabetic foot ulcer group and diabetic group ($P>0.05$). Multivariate regression analysis showed that; peripheral neuropathy and hyperhomocysteinemia ($r=2.357/P=0.008$ and $r=0.171/P=0.013$ respectively) were independent risk factors for diabetic foot ulcer development.
Conclusions
According to our findings, along with peripheral neuropathy and peripheral artery disease, hyperhomocysteinemia may be a risk factor for diabetic foot ulcer development.

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Incidence of diabetic ketoacidosis during Ramadan fasting in Benghazi, Libya

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Background
Ramadan is the ninth month on the lunar calendar and for over a billion Muslims it is a holy month during which all healthy adults must observe absolute fasting from dawn to sunset. The risk of diabetic ketoacidosis is thought to be higher during Ramadan fasting due to hormonal disequilibrium.
Aim and objectives
The aim of this study was to examine the hypothesis that diabetic ketoacidosis is more frequent during Ramadan fasting.
Patients and methods
A retrospective analysis of the records of all patients admitted with DKA to all Benghazi hospitals during the lunar year 1428 Hijri (January 2007 to January 2008).
Results
Fifteen episodes occurred during Ramadan (4.6 episode/10 000 diabetic) as compare to 19.45 episodes/month (6 episode/10 000 diabetic/month) during the other lunar months ($P=0.000$), there was no significant difference in the patients' mean age (37.6 ± 10 vs 38.3 ± 19, $P=0.8$), or mortality rate (13.3 vs 14.4%, $P=0.9$) during Ramadan and other months. The commonest precipitating factor of diabetic ketoacidosis during Ramadan was infection (46.6%), followed by miss dosing (33.3%).
Conclusion
There is no increase in the incidence and mortality from DKA during Ramadan which might indicate that Ramadan fasting is not a significant risk factor for diabetic ketoacidosis.

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ECoRI polymorphism but not XBaI polymorphism of apolipoprotein B gene is associated with carotis intima media thickness in type 2 diabetic patients

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ApoB plays a central role in lipoprotein metabolism through regulation of total cholesterol and LDL-cholesterol (LDL-C) concentrations in plasma. Two restriction fragment length polymorphisms (ECoRI and XbaI) represent single base alterations in the coding region of ApoB gene. ECoRI polymorphic region of ApoB gene is due to on an amino acid change (Glu→Lys). The XbaI polymorphic region of ApoB gene results from a substitution of (A→T) in the threonine codon and does not change the amino acid sequence.
In this study, we aimed the determine ECoRI and XbaI restriction enzyme polymorphisms of ApoB gene with respect to carotis intima media thickness (CIMT) in 238 type II diabetic patients and 118 control subjects. After polymerase chain reaction with specific primers for ApoB gene, PCR products were digested (ECoRI and XbaI), electrophoresed and visualized.
No significant changes in cholesterol, TG, HDL-C, LDL-C, Apo A and ApoB levels were determined between control and type II diabetic subjects. The frequencies of XbaI polymorphism in diabetic patients were 42.5% XX, 48.4% Xx and 9.1% xx; and in control subjects 44.4% XX, 44.4% Xx and 11.2% xx ($P>0.05$). The ECoRI frequencies are 2.2% EE, 41.1% Ee and 56.7% ee in diabetic patients; 7.8% EE, 23.7% Ee and 68.4% ee in controls ($P<0.0442$). CIMT measurements were significantly increased in diabetic subjects ($P=0.0040$).
Our results suggest that there was a relation between the ECoRI polymorphic site of ApoB gene with CIMT in type II diabetic patiens. It is possible that ECoRI polymorphic site of ApoB gene leads to oxidation of LDL-C and thereby an increase in CIMT.

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Insulin-loaded lipid nanospheres surfaced with polysaccharides for oral delivery

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Insulin delivery through oral route requires protein protection against gastric environment followed by intestinal absorption. Several approaches using colloidal carriers have been proposed to achieve such attempts. Examples are nanoparticles produced from lipid materials (mono, di and triacylglycerols, waxes, fatty acids), polymeric materials (poly acid lactic/glycolic co-polymers, poly-epsilon caprolactone) or from polysaccharides (hyaluronic acid, dextran sulphate, alginate, chitosan). The present work proposes a new drug delivery system composed of cetyl palmitate nanospheres as matrix core, reinforced with polysaccharide molecules for oral insulin administration. Mean particle size, swelling behaviour and protein release profiles in simulated gastrointestinal conditions have been assessed. Increased insulin protection and modified release profile from lipid nanospheres was observed by reinforcing their matrix with alginate-chitosan and/or dextran sulphate. Surfacing the nanospheres with polysaccharide molecules could avoid insulin release at pH 1.5, protecting the protein from the acidic environment and reducing the total insulin released at pH 6.5. This effect was explained by an interaction between the permanent negatively charged groups of dextran sulphate and insulin molecules. For oral protein absorption, the ileum seems to be an ideal site for nanosphere uptake, where abundant Peyer patches exist with proteolytic enzyme activity. The paracellular pathway has also been shown to contribute to protein absorption, most polypeptide drugs diffuse through the aqueous-filled tight junctional pathway due to their hydrophilic nature.

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Influence of admission plasma glucose level on short and long-term prognosis in patients with ST-Segment elevation myocardial infarction

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High admission plasma glucose (APG) levels after ST-segment elevation acute myocardial infarction (STEMI) are common and associated with an increased risk of death in subjects with and without known diabetes.

Aim

The aim of this study is to analyse the short and long term prognostic significance of APG in patients with STEMI with and without diabetes.

Material and methods

This study included all patients registered in Coronary Unit, Department of Cardiology at Internal Clinic, Clinical Center Kragujevac from January, the 1st 2007., to June, the 30th 2007. Patient survival was measured on 28 days and one year after admission. Diabetes mellitus was defined as the use of insulin or glucose-lowering medication on admission, or a diet for diabetes documented in medical history.

Results

We studied 115 patients admitted consecutively with STEMI. The majority of patients in the study were males (69.6%).The mean age of patients was 64.25 $\pm$ 10.69 years. At the time of hospital admission average plasma glucose was 8.77 $\pm$ 2.54 mmol/l. Average APG is statistically significantly higher in patients who died one month after STEMI than who survived (10.1 $\pm$ 2.85 vs 8.45 $\pm$ 2.37 mmol/l, $P=0.006$). Average APG is statistically significantly higher in patients who died one year after STEMI than who survive (9.4 $\pm$ 2.37 vs 8.42 $\pm$ 2.57 mmol/l, $P=0.047$). Total mortality of STEMI (one-month survival) pts is 19.1%. Total mortality of STEMI (one-year survival) pts is 35.6%. There is no statistically significance in average APG in diabetic patients with STEMI who died after one month and who survived (10.09 $\pm$ 2.68 vs 10.0 $\pm$ 2.51 mmol/l, $P=0.657$), as well as those who died after one year and who survived (10.1 $\pm$ 1.92 vs 10.09 $\pm$ 2.8 mmol/l, $P=0.996$). But, there is statistically significance in average APG in nondiabetic patients with STEMI who died after one month and who survived (9.97 $\pm$ 2.97 vs 7.91 $\pm$ 2.08 mmol/l, $P=0.001$), as well as those who died after one year and who survived(9.17 $\pm$ 2.49 vs 7.84 $\pm$ 2.24 mmol/l, $P=0.013$).

Conclusion

This study demonstrates that high admission plasma glucose level is common in patients with STEMI and is associated with high risk of mortality among patients with or without diabetes mellitus. Our study showed that nondiabetic patients with high admission plasma glucose have higher risk of mortality than patients with a previous known history of diabetes mellitus.

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The effect of glycemic control on mean platelet volume in patients with type 2 diabetes mellitus

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Objective

Morphologic and functional platelet abnormalities have been previously reported in patients with type 2 diabetes mellitus (DM) and some of these abnormalities have been linked to increased cardiovascular risk. Mean platelet volume (MPV) informs not only about platelet size, but also about its functions. Larger platelets bear more thrombotic potential. Higher MPV values have been correlated with cardiovascular events in the short and long run. Previous studies have demonstrated higher MPV levels in type 2 diabetic patients in comparison with those with normal glucose metabolism. However, the effect of glycemic control on MPV has not been investigated before. This study aimed to explore the effect of glycemic control on MPV.

Subjects and methods

MPV of 94 patients with type 2 DM were determined before and after glycemic regulation. The control group consisted of 61 age and body mass index matched cases with normal glucose metabolism.

Results

MPV levels were found to be significantly higher in diabetic patients (9.7 $\pm$ 1.5 fl) than the controls (7.8 $\pm$ 0.9 fl) ($P<0.001$). Glycated hemoglobin levels before and after glycemic control were 8.8 $\pm$ 2.0 and 6.4 $\pm$ 0.5% respectively ($P<0.0001$). MPV levels were not significantly different before and after glycemic control (9.7 $\pm$ 1.5 and 9.5 $\pm$ 1.5 fl respectively, $P=0.17$) in type 2 DM patients. The two groups were also similar as far as the platelet numbers were concerned.

Conclusion

High MPV values in type 2 DM patients may reflect an intrinsic abnormality of the platelets, which might in turn conceive tendency to cardiovascular events.

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Association between APOE genotype and hsCRP in Iranian population: Tehran, lipid and glucose study

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Introduction

High sensitive CRP (hsCRP) has been reported to associate with an increased risk of cardiovascular disease. There is some evidence on apoE polymorphism in relation to the parameters of inflammation. We have investigated the association between APOE genotypes and hsCRP levels in an Iranian population.

Materials and methods

We performed a cross-sectional study of 966 subjects (419 men and 547 women) from the Tehran Lipid and Glucose Study. hsCRP level were determined and a segment of the Apo E gene was amplified by PCR and the polymorphism revealed by RFLP using HhaI restriction enzyme.

Results

The presence of the ε4 allele was significantly associated with decreased serum CRP levels after logarithmic transformation of CRP level. Increase of the CRP level was associated with increase of some risk factors of the cardiovascular disease including age, hypertension, obesity and lipid profile.

Conclusion

The previous reports about the association between hsCRP level and apoE polymorphism tested in our population and the relationship between the presence of the E4 and the decrease of the hsCRP was confirmed. However the pattern of the association is in contrast to what one might have expected, since it is the E4 allele that is most strongly associated with cardiovascular disease.

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Ca 19-9 levels in type 2 diabetes mellitus patients

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Background
Ca 19-9 is a tumor-associated antigen. In this study, we aimed to compare Ca 19-9 levels in type 2 diabetes mellitus (DM) patients and healthy control group.
Method
Two hundred and fifteen type 2 DM patients (82 male and 133 female) and 209 healthy control group (79 male, 130 female) age, sex and body mass index(BMI) matched were included in the study. Duration of diabetes, HbA1c and presence of complications were analyzed. Cases with high serum levels of Ca 19-9 (0–35 U/ml) were evaluated with abdominal MRI. A female patient with high serum Ca 19-9 was diagnosed as pancreas carcinoma and excluded from the study.
Results
Median Ca 19-9 in DM patients was 13.8 (0–302.8) and 7.53 (0.4–46.97) in control group and difference was statistically significant ($P<0.001$). Number of cases with high serum Ca 19-9 levels in patients and control group were 45 and 2, respectively. The difference was again statistically significant ($P<0.001$). Considering all cases, Ca 19-9 levels were similar in both females and males ($P=0.794$). In DM patients, Ca 19-9 did not correlate with BMI, duration of diabetes or number of complications, however, it was found to be positively correlated with HbA1c levels ($\rho=0.17$, $P=0.015$). Ca 19-9 did not change with presence of nephropathy, retinopathy, neuropathy and number of complications ($P=0.778$, $P=0.258$, $P=0.241$ and $P=0.457$, respectively).
Conclusions
Chronic pancreatitis is a risk factor for pancreatic cancer, and the same is also true for diabetes. Ca 19-9 is used in the diagnosis of pancreatic cancer but also a marker of pancreatic tissue damage that might be caused by diabetes. Therefore it is necessary to define the normal range of Ca 19-9 in type 2 diabetic patients in order to eliminate additional approaches. Therefore, diabetic patients have to be followed up for pancreatic cancer.

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Plasminogen activator inhibitor 1 and atherosclerosis in diabetes mellitus type 2

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Background and aims
Plasminogen activator inhibitor -1 (PAI-1) increases in diabetes and this might contribute to decreased fibrinolisis and accelerated atherosclerosis. PAI-1 is also contributor to the development of acute myocardial infarction. Measurement of intimal medial thickening (IMT) is indicator of presence and extent of coronary artery disease. Aim of the study was to find association between PAI-1 marker of decreased fibrinolisis and atherosclerosis by measuring IMT of carotid artery in patients with type 2 diabetes.
Materials and methods
Investigation was performed in 49 patients with diabetes mellitus type 2 (53.83 ± 8.38 years; 24 f/25 m, BMI: 27.82 ± 4.79 kg/m^2) and 30 healthy controls (46.87 ± 11.42 years; 12 f/18 m, BMI: 26.01 ± 2.39 kg/m^2). PAI-1 was measured by spectrophotometric method using commercialy kit (Behring). The IMT in common carotid arteries was measured on a longitudinal scan of the common carotid arteries at a point 10 mm proximal from the beginning of the dilatation of the bifurcation bulb. We defined the IMT as mean IMT of the near and far walls at the point of measurement.
Results
Compared to healthy controls, patients with type 2 diabetes mellitus showed higher concentrations of PAI-1 (3.03 ± 1.02 vs 2.67 ± 0.53 μ/ml; $P>0.05$). In the group of patients type 2 diabetes we found significant positive correlation between PAI-1 and IMT ($\rho=0.535$; $P<0.01$), while in group of nondiabetics there was no statisticly significant correlation ($\rho=0.030$; $P>0.05$).
Conclusions
In conclusion, we have demonstrated that the level of PAI-1 in diabetes mellitus type 2 correlates with the degree of IMT and also that PAI-1 is an useful marker for detecting early atherosclerosis in patients with type 2 diabetes.

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Combined treatment in childhood diabetes could influence remission period

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Background
The most striking change in diabetes over recent years has been the convergence of previously distinctive phenotypes. Recently diagnosed children are younger, taller and with greater BM. Pediatric endocrinologists are facing with children presenting mixed signs of both diabetes types. So called hybrid diabetes, or type 1 ½ has insulin resistance in type 1 phenotype and vice versa autoimmunity in obese children.
Patients and methods
Ten children (6 girls and 4 boys), with recently diagnosed diabetes and on insulin treatment, aged mean 12.45 years (range 5.5–16) with mean BMI of 22 kg/m^2 and GHbA1c of 9.66% at admission, two weeks later additionally received Metformin as insulin senzitizer and apoptosis reducing agent. Including criteria were basal C peptide over 0.2 pmol/l and preserved pulsatility of insulin secretion.
Results
Treated children entered faster in remission period and according to daily glycemic profiles insulin was gradually excluded. Mean insulin free period was 1.6 years (range 0.2–3.5) with mean GHbA1C of 6.37%. Only 3 of them needed small insulin doses 2, 4 and 12 IU of intermediate acting insulin, according to age and BM.
Conclusion
Diabetes in childhood is changing its well known face. In this hybrid diabetes insulin sensitizer combined with insulin in early period could have favorable effect on metabolic control and duration of remission period.

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Impact of age, gender, DM duration and BMI on metabolic control of T2DM patients in Jordan

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Aim
To assess the impact of age, sex, duration of diabetes and body mass index confounders on diabetes control in a Jordanian cohort of T2DM at endocrine clinic at KHMC.
Patients and methods
Patients randomly selected over 18 months from outpatient clinic at KHMC in Amman-Jordan. Diabetes control assessed by mean of latest 3 HbA1c and FBS. Patients were divided in 2 groups according to age (<55 years vs >55 years), gender, duration (<10 years vs >10 years) and BMI (normal, overweight, and obese according to WHO criteria). Statistical analysis is performed using SPSS11.5.Good diabetes control is assessed according to ADA criteria.
Results
A total of 405 patients were selected (223 males, 182 females). 115 patients (28.4%) were having a good control with HbA1c <7%. About 25.6% of males and 31.9% of females were having good control ($P=0.161$).
There was no difference in diabetes control of those <55 years ($n=183$) versus those >55 years ($n=222$) (29 vs 28%; $P=0.812$). Females in both age groups were having none statistically significant better control than males. Of those of DM duration <10 year ($n=242$), 34.7% were having good control versus only 19% for those >10 year duration ($n=163$) (P value $=0.001$, RR $=1.83$ (1.27–2.62), OR $=2.26$ (1.38–3.73)). Females were again having a better diabetic control in both duration groups, $P=0.024$.
There was no difference in diabetic control in all BMI categories studied. Thirty percent of overweight patients were having good control versus <20% in normal and obese patients.
The mean HBA1c in males was 8.1 ± 1.7 vs 7.9 ± 1.5% in females. For the group <10 year, HBA1c was 7.75 ± 1.5 vs 8.32 ± 1.54% in those >10 year. The mean HBA1C of the group aged<55 years was 8.1 ± 1.8 vs 7.9 ± 1.5 in those >55 years of age.
Conclusion
In the Jordanian cohort with T2DM, the diabetic control was modest at 28.4%. Females were having a better diabetic control at all categories. There is no difference in diabetic control when genders, BMI or age confounders were studied.

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Retinol binding protein-4 is associated with TNF-α and not insulin resistance in subjects with type 2 diabetes mellitus and coronary heart disease

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We studied the association between RBP4 and various markers related to insulin resistance and diabetic complications as well as inflammatory markers in Saudi population suffering from type 2 diabetes and coronary heart disease. Patients with type 2 diabetes were divided into 3 groups according to the type of treatment and involvement of coronary artery disease. Serum TNF-α, insulin, CRP, resistin, leptin and adiponectin were analysed in all samples. RBP4 plasma levels increased significantly in the group of diabetic subjects treated with oral hypoglycemic agents and diabetic patients with coronary heart disease (30.2 ± 11.8; 33.4 ± 13.6 respectively), while there was no significant change in the other group for diabetic subjects on low-carbohydrate diet (25.1 ± 10.9) compared to control group (22.6 ± 9.5). RPB4 levels were positively correlated with TNF-α in the group of diabetic subjects on oral hypoglycemic agents and diabetic patients with coronary heart disease ($r^2 = 0.245$, $P < 0.05$; $r^2 = 0.448$, $P < 0.05$ respectively). No correlations were found between RBP4 level and insulin resistance in all studied groups. Our findings suggest that serum RBP4 levels is associated with pro-inflammatory cytokine (TNF-α) and is not associated with insulin resistance among patients with type 2 diabetes and coronary heart disease.

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Impact of metabolic syndrome, diabetes and prediabetes on cardiovascular events: Tehran Lipid and Glucose Study

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Background and aims
To compare and contrast the cardiovascular disease (CVD) risk associated with the metabolic syndrome (MetS) and dysglycemia, independent of each other, we evaluated the 6.7-year incidence risk of CVD and coronary heart disease (CHD).
Methods and results
In an Iranian population, 4018 subjects ≥ 40 years with no history of CVD at baseline, were followed up for new CHD and CVD events. Incidence rates and hazard ratio (HR) were estimated by the presence or absence of MetS, dysglycemia, and by the various traits of MetS. Considering the glycemic status, the ability of MetS in prediction of CVD after adjustment with age, sex,CVD risk factors, and components of MetS, was assessed. The prevalence of MetS, impaired fasting glucose or impaired glucose tolerance (IFG/IGT) and diabetes were 51.4, 27.3, and 18.7%, respectively. The prevalence of MetS in IFG/IGT and diabetes was 67.7 and 85.2%. Among the components of the MetS, only hypertension had a significant HR of 5.2 (95% CI, 1.9–14.0) for incident CVD. After full adjustment, diabetes remained as a significant predictor of incident CVD/CHD, regardless of the presence of MetS. IFG/IGT predicted outcomes only in the presence of MetS for CVD (HR: 1.7 (1.2–2.5)) and CHD (HR: 1.8 (1.3–2.7)); although these associations did not change after risk factor adjustment in the presence of MetS components, IFG/IGT lost its association with incident CVD/CHD.
Conclusion
In Iranian population, MetS in the absence of diabetes does not predict CVD/CHD, and intervention strategies should be focused on the prevention of diabetes.

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NovoMix30® reduces hypoglycemic events with favorable weight change in poorly controlled type 2 diabetes patients: results from Indian cohort of IMPROVE Study

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Objective
IMPROVE study was designed to assess the safety and effectiveness of NovoMix30® in type 2 diabetes subjects under normal clinical practice conditions.
Methods
This large multi-national, open label, prospective, 6 month long observational study captured data for above 50 000 patients started on NovoMix30® with/without oral anti-diabetic drugs (OADs)/other insulin. This paper analyses safety data of 17 995 patients constituting Indian cohort of IMPROVE™ study.
Results
16 391 & 16 398 patients have been evaluated for incidence of major and minor hypoglycemic events respectively. There was reduction of 0.124 major hypoglycemic events/patient year (EPY) at the end of 6 months of NovoMix30 therapy (baseline 0.129 EPY, final 0.005 EPY, $P < 0.001$). Patients taking OAD alone at baseline and those shifted from other insulins ± OAD showed reduction of 0.050 EPY ($P < 0.001$) and 0.327 EPY ($P < 0.001$) respectively, while treatment naïve patients experienced no such events.
Minor hypoglycemic events were reduced by 1.92 EPY ($P < 0.001$) as compared to baseline (3.08 EPY). NovoMix30® therapy was associated with reduction of 61.9% ($P < 0.001$) and 64.1% ($P < 0.001$) in day time and nocturnal minor hypoglycemic events respectively.
A mean reduction of 0.3 kg ($P < 0.001$) body weight in all patients with 0.9 kg ($P < 0.001$) decrement in treatment naïve patients was noted at the end of 6 months.
Eight serious adverse drug reactions (SADR) including major hypoglycemia and 13 adverse drug reactions (ADR) were reported in 17 995 patients during the study period.
Conclusion
IMPROVE™ study confirmed the safety of NovoMix30® in real life clinical practice, with lesser incidence of hypoglycemia and weight loss.

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Plasma thrombin-activatable fibrinolysis inhibitor (TAFI) antigen levels in diabetic foot ulcers

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Objective
Diabetic foot ulcer is associated with increased morbidity and mortality. The most important factor related to the development of foot ulcer is peripheral neuropathy. Thrombin-activatable fibrinolysis inhibitor (TAFI) is associated with coagulation, fibrinolysis and inflammation. Plasma TAFI may participate in arterial thrombosis in cardiovascular diseases (CVD). TAFI may be involved in the mechanism of vascular endothelial damage in diabetic patients. The aim of this study was to investigate the association of plasma TAFI antigen level in the development of diabetic foot ulcer in type 2 diabetes.
Research design and methods
The TAFI antigen levels were determined retrospectively in 50 patients with diabetic foot ulcers and 34 patients without diabetic foot ulcers and 25 healthy individuals. We measured TAFIa/ai antigen in plasma samples with a commercially available ELISA Kit.
Results
Diabetic foot ulcer group and diabetic group were similar in terms of mean age and sex distribution. Diabetes duration, retinopathy, neuropathy, macrovascular disease and infection were related to diabetic foot ulcers. HbA1c, HDL-Cholesterol and Folic Acid levels were decreased in the diabetic foot ulcer group. Vitamin B_{12}, CRP and ESR were significantly increased in the diabetic foot ulcer group. TAFI levels were $99.44 \pm 55.94\%$ in control group, $135.21 \pm 61.05\%$ in diabetic foot ulcer group, $136.75 \pm 59.38\%$ in diabetic group and was statistically different ($P < 0.05$). But no difference was seen in TAFI levels between diabetic foot ulcer group and diabetic group ($P > 0.05$). No significant difference in plasma TAFI levels were seen between diabetic foot ulcer stages.
Conclusions
TAFI antigen levels are increased in type 2 diabetic patients but are not related to diabetic foot ulcer development.

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Relationship between adipocytokines and cardiovascular risk factors in patients with type 2 diabetes mellitus

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Aims
In this study, we examined the relationships between levels of adipocytokines and traditional and non-traditional cardiovascular risk markers in patients with type 2 diabetes mellitus (T2DM).
Methods
Serum leptin, resistin, adiponectin, visfatin, high sensitive C-reactive protein (hsCRP), homocystein, asymetric dimethylarginine (ADMA), fasting glucose, insulin, glycated haemoglobin (HbA_1C) and full lipid and lipoprotein profile, systolic blood pressure (SBP), diastolic blood pressure (DBP) were determined in type 2 diabetic patients. Results were compared with those control subjects.
Results
The levels of serum adiponectin were decreased and resistin, leptin and visfatin levels were increased in diabetic patients compared to that in controls ($P<0.001$, in each). Adiponectin showed significant negative correlations with body mass index (BMI), insulin, HbA_1C, triglyceride, homeostasis model assessment of insulin resistance (HOMA-IR), hsCRP, ADMA, visfatin, resistin, leptin and possitive correlations with high-density lipoprotein-cholesterol (HDL) and apolipoprotein A1 (Apo A1). Resistin showed significant possitive correlations with insulin, HOMA-IR, hsCRP, homocystein, visfatin, leptin and negative correlations with adiponectin, HDL, and Apo A1. Leptin showed significant positive correlations with age, BMI, HOMA-IR, homocystein, triglyceride, insulin, visfatin, resistin, SBP, DBP.and negative correlations with HDL and adiponectin. Visfatin showed significant possitive correlations with age, insulin, HOMA-IR, hsCRP, resistin, leptin, and negative correlations with adiponectin, HDL and Apo A1.
Conclusion
These findings may suggest that levels of serum leptin, resistin, adiponectin, visfatin are associated with obesity, insulin resistance and cardiovascular risk markers in T2DM patients. Therefore these adipocytokines may be useful indicators for assessing the cardiovascular disease risk in patients with type 2 DM.
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Prevalence of atopic profile in patients with type 1, type 2 and LADA diabetes mellitus

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Background
The existing information about an association between allergic diseases and diabetes mellitus is rather conflicting. However, a few studies demonstrated an inverse relationship between atopic diseases and the risk to develop type1 diabetes mellitus.
Objective
The aim of the present study was to analyze the frequency of allergic diseases in patients with type 1, type 2 and LADA (latent autoimmune diabetes in adults) diabetes mellitus and to evaluate the role of diabetes mellitus in atopy and allergic disease.
Methods
We studied 205 diabetic patients (type 1 DM, $n=30$; type 2 DM, $n=165$ and LADA, $n=10$). All patients responded to a detailed questionnaire of atopic diseases (allergic rhinitis and/or asthma, atopic dermatitis). Atopy was defined by one or more positive skin prick test (SPTs) responses (wheal of >3 mm) to 13 common aeroallergens (olive, birch, mix grasses, plantain, parietaria, mugwort, alternaria, cladosporium, aspergilus, Derm. farinae, Derm. pteronyssinus, cat fur and dog hair- (Stallergenes, France).
Results
No relationship was seen between history of atopy and diabetes mellitus (Pearson Chi Square P value $=0.129$). However, higher prevalence of atopy was observed in the group of type 1 as well as in LADA diabetic patients (odds ratio for type 1, 2 and LADA 1.31/0.66/0.59 consequently). Furthermore, no significant correlation was observed between SPTs positivity and diabetes mellitus, although higher SPTs positivity was seen in the group of autoimmune diabetes (odds ratio for DM 1, 2 and LADA 0.36/0.3/1 consequently).
Most common allergen sensitizations were: olive (12.2%), grasses (10.2%) and Derm. Pteronyssinus (5.9%).
Conclusion
According to our results there is no association between diabetes mellitus and allergic diseases. However, additional studies are needed to evaluate the effect of atopy and allergic diseases in diabetic patients.

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Prevalence of cardiovascular risk polymorphisms and its association with microvascular complications in an adolescent type 1 diabetes population

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Objectives
To determine the prevalence of several polymorphisms associated with increased cardiovascular risk in a group of adolescents with T1DM. To study the possible association of some polymorphisms with the occurrence of microvascular complications.
Methods
Patients were randomly selected from our outpatient clinic. The following polymorphisms were studied:: ACE Ins/Del, Apo B R3500Q, Apo E2, 3, 4, MTHFR C677T and A1298C, PAI 4G/5G, ITGB3 PL(A1)/(A2) and FGB G/A-45. We compared the prevalence of each genotype between the groups with and without microvascular complications (nephropathy and retinopathy). We took into consideration patient's age, diabetes duration, mean A1C values in the last year and lipid profile to characterize the population and adjust between groups.
Results
A total of 33 patients were studied (54.3% female), with a mean age of 19.8 ± 3.1 years and mean diabetes duration of 9.1 ± 5.3 years. Mean A1C was 8.2 ± 1.6%. Four patients (12.1%) had hypertension, five patients (15.2%) had incipient nephropathy and three patients had background retinopathy. The frequency of heterozygotes for each polymorphism was: ACE Ins/Del $=19$ (57.6%); Apo B R3500Q $=0$; Apo E $2=9$ (27.3%); MTHFR C677T $=14$ (42.4%); MTHFR A1298C $=13$ (39.4%); PAI 4G/5G $=17$ (51.5%), ITGB3 PL (A1)/A2 $=11$ (33.3%), FGB G/A-455 $=15$ (45.5%). None of the patients was homozygote. The ACE Ins/Del polymorphism was more frequent in the group of patients with hypertension ($P<0.001$), nephropathy ($P=0.001$) and retinopathy ($P<0.001$). The frequency of other polymorphisms was similar in the groups with and without complications.
Conclusions
The frequency of the polymorphisms studied was overall similar to the expected in a Caucasian population (PAI and ITGB3 slightly above the estimated). The ACE Ins/Del polymorphism was more frequent in the group of patients with hypertension, nephropathy and retinopathy. These data suggest that this polymorphism may have a role in determining blood pressure values and increased susceptibility to microvascular complications. Knowing this genotype may have implications regarding the therapeutic strategy designed to prevent both macrovascular and microvascular complications.

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Visfatin seems not to be related in insulin resistance

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Visfatin is a newly discovered adipocyte hormone. Recent studies showed a possible relationship between diabetes mellitus and visfatin level. But the effect of visfatin on the insulin resistance was not well understood. The aim of the present study was to explore the relation of visfatin with insulin resistance in patients with prediabetes and diabetes.
We included a total of 128 persons in the study and of these 128 persons, 36 were diabetic, 69 were prediabetic (impaired fasting glucose and impaired glucose tolerance patients) and the remaining 23 were healthy (control). In all cases,

HbA1c, microalbuminuria, fasting blood visfatin, insulin, glucose, lipid profiles, fibrinojen, BUN, creatinine and CRP levels were calculated and recorded. HOMA-IR scores were calculated.The results were evaluated statistically.
According to our results visfatin levels were not different between groups (11.5 ± 2.5 for control group; 12.1 ± 2.7 for pre-diabetes group and 11.2 ± 2.8 for diabetes group). Furthermore neither HOMA-IR nor other laboratory parameters (CRP, HbA1c, BMI etc.) were correlated with visfatin levels in any group. Also obesity, hiperlipidemi and gender differences did not effect on the plasma visfatin levels. Our investigation demonstrates that visfatin has no effect on insulin resistance in type 2 diabetes. Further research is required to investigate its role in insulin resistance and the investigations of the metabolic role of visfatin may be shifted to other areas.

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Anthropometric determinants of adiponectin levels in obese and non obese premenopausal women

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Background
Adiponectin is an important adipokine to which have been attributed anti-diabetic and anti-atherogenic properties. Excess of abdominal fat deposition is undoubtedly assumed as a cardiometabolic risk factor. It has been hypothesized that peripheral deposition of fat could exert a protective effect in cardiometabolic profile.
Aims
To evaluate the influence of anthropometric parameters on adiponectin levels in both obese and normal-weight premenopausal women.
Methods
We studied 80 obese (age = 34.3 ± 8.2 years, BMI = 43.1 ± 8.5 kg/m^2, waist = 117.8 ± 15.7 cm, hip = 133.4 ± 14.3 cm, waist:hip ratio (WHR) = 0.88 ± 0.07 cm, percentual total body fat (%TBF) = 47.4 ± 5.3%) and 57 normal-weight pre-menopausal women (age = 36 ± 7.5 years, BMI = 21.5 ± 1.8 kg/m^2, waist = 71.4 ± 5.9 cm, hip = 96.9 ± 4.7 cm, waist:hip ratio (WHR) = 0.74 ± 0.05 cm, percentual total body fat (%TBF) = 25.2 ± 4.6%). In each group, we looked for the correlation between adiponectin and each anthropometric parameter; we also tested the influence of the several possible parameters' combinations on adiponectin levels.
Results
Adiponectin levels were significantly lower in the obese group ($P < 0.001$). Adiponectin were inversely associated with waist ($P = 0.008$; $r = -0.293$), WHR ($P < 0.001$; $r = -0.483$) and %TBF ($P = 0.034$; $r = -0.237$) in the obese women and with waist ($P = 0.007$; $r = -0.355$) and WHR ($P = 0.001$; $r = -0.441$) in the normal-weight group. Despite the absence of significance, hip circumference and adiponectin values showed concordance in their variation. The stronger combination of 2 anthropometric parameters for the association with adiponectin levels was WHR + %TBF in obese ($r = 0.511$) as in non-obese ($r = 0.468$). The stronger combination of 3 parameters was WHR + hip + %TBF ($r = 0.532$) in obese and waist + hip + %TBF ($r = 0.523$) in normal-weight. In the normal-weight, but not in the obese group, a greater power of association with adiponectin levels was obtained with the combination waist + hip + WHR + %TBF ($r = 0.558$) and BMI + waist + hip + WHR + %TBF ($r = 0.57$).
Conclusions
There is an inverse association between abdominal fat and adiponectin levels in both obese and normal-weight premenopausal women. In both groups, when we use parameters that take into account the amount of peripheral fat mass we increase the level of prediction for adiponectin levels.

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Is brachial artery diameter most significant determinator of the flow-mediated dilatation?

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Endothelial dysfunction is assessed noninvasively by measuring flow-mediated dilation (FMD). However, FMD is strongly dependent on arterial size, which contributes to a wide range of FMD values. It was previously shown that FMD is greater in small arteries, because the shear stimulus during postocclusion hyperemia is greater in small arteries. We aimed to investigate the predictive value of brachial arterial size on FMD in 44–61 years old Turkish women while adjusting for a number of covariates.
Method
We examined FMD of 507 voluntary women participating in a study on predictors of natural menopause. FMD was evaluated in women who used no medications in the previous 15 days. Predictors of FMD were evaluated in univariate and multivariate regression models (UVA & MVA). Variables including age, body mass index, menopause status, current smoking, alcohol use, socioeconomic factors and chronic diseases (diabetes mellitus and hypertension) are included as predictors. A stepwise model selection algorythm was used in MVA with a cut off P value of 0.15.
Results and conclusion
Mean age was 50, mean brachial artery diameter was 3.69 ± 0.50 mm. The mean absolute FMD value was 0.30 ± 0.22 mm and mean percent dilatation of FMD was 8.4 ± 6.4%. Postmenopausal women compromised 46%.
In both UVA & MVA, the most significant determinant of FMD was basal brachial artery diameter. Greater basal diameter is associated with worse FMD. Other significant predictors included menopausal status, perceived economic status and presence of health insurance. Our findings support a strong association between arterial size and FMD. Baseline arterial diameter should be taken into account when investigating predictors of FMD.

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General and central obesity and risk of cardiovascular disease: results of the 7.6-year follow-up study in Iranian men

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Objective
Although body mass index (BMI) is commonly used to assess risk for cardiovascular disease (CVD), there is an obvious need for prospective studies of different ethnics to evaluate the predicting power of various obesity variables for CVD outcomes.
Methods
The study population consisted of 1931 men aged ≥40 years free of CVD at baseline. After a median follow up of 7.6 years, 254 CVD events occurred. Demographic data were collected at baseline; blood pressure and anthropometric variables such as BMI, waist circumference (WC), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were measured according to a standard protocol. CVD outcome was defined as any coronary heart disease events, stroke, or CVD death. Cox proportional hazards regression was used to calculate hazard ratio (HR) of CVD for each anthropometric variables in two model; age adjusted model and full model adjusted for age, hypertension, smoking, family history of premature CAD. Receiver operator characteristic (ROC) curves were constructed to assess sensitivity and specificity of the variables in prediction of risk.
Results
In the age adjusted model all measures of obesity predict CVD in a high level of significance. According to Cox proportional hazard modeling, after controlling confounding factors, HRs and 95% CI (for 1 S.D. increase) for CVD were 1.17 (1.01–1.03) with BMI, 1.24 (1.06–1.37) with WC, 1.19 (1.05–1.35) with WHR and WHtR. Area under ROC curve (95% CI) were 0.56 (0.5–0.6) for BMI, 0.59 (0.55–0.62) for WC, 0.59 (0.56–0.64) for WHR and WHtR.
Conclusions
Waist-related variables are superior clinical measures of obesity for predicting CVD outcomes in Iranian adult men. WHR or WHtR are not more useful than WC alone.
Keywords: Obesity, Cardiovascular disease, follow-up.

P395

Prevalence of undiagnosed glucose intolerance changes according to age and gender in Japanese middle aged working people

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Undiagnosed diabetes and impaired fasting glucose, a condition that increases the risk for diabetes, have important health consequence. About one-third diabetes was undiagnosed. Thus, prevalence estimates based on self-report or doctor diagnosed disease are underestimates of total prevalence of diabetes, which

includes individuals with both known and undiagnosed type 2 diabetes. To examine the prevalence of undiagnosed glucose intolerance, we did 75 g oral glucose tolerance test in consecutive 1142 health check middle aged subjects (age range 40–55 y.o.) in 2006 who were working in a company (914 men, mean 50.7 y.o., 228 women, mean 49.4 y.o.). Nobody had had the history of atherosclerotic diseases or been diagnosed as impaired fasting glucose, impaired glucose tolerance, or diabetes. Fasting glucose levels increased as old age in both men and women, and the levels were higher in men than women in each age. Glucose intolerance is more common in men as compared with women (Fasting 100.1±19.7 vs 92.9±9.6, $P<0.01$, 1-hour 170.7±52.1 vs 139.7±11.6, $P<0.01$, 2-hour 136.0±50.1 vs 119.8±31.5 mg/dl, $P<0.01$). The prevalence of IGT and DM was higher in men than women (IGT: 24.1 vs 16.7, $P<0.01$, DM 10.7 vs 1.4%, $P<0.01$). Blood pressure and triglyceride levels also higher in men than women (124.0±18.5/76.9±11.6 vs 114.8±19.4/70.6±12.5 mmHg, $P<0.01$, 148.1±109.4 vs 88.2±44.0 mg/dl, $P<0.01$). HDL cholesterol levels were lower in men than in women (58.8±16.0 vs 72.6±17.4 mg/dl, $P<0.01$). Coronary heart disease is more common in men as compared with women, and the incidence in women is about 10 years older than men. Glucose intolerance is often associated with other risk factors such as hypertension and dyslipidemia. These features of undiagnosed glucose intolerance during the working age people may contribute to the gender difference in the incidence of coronary artery disease in Japan.

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Diabetes and obesity associated difference in aggregation platelet capacity

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Diabetes mellitus and abdominal obesity are considered to be risk factors for venous and arterial thrombosis, but till now it still remains unclear, what factor is a primary in the genesis of these abnormalities: fibrinolysis dysfunction, vascular endothelium impairment or pathology of the platelet homeostasis component.
Forty-two persons included in the study were primary divided into 3 groups: 1 group – nearly healthy (10 males, 12 females), 2 group – overweight with body mass index (BMI) more then 25 kg/m^2 (9 males, 11 females), 3 group – patients with diabetes mellitus (10 males, 6 females).
Platelet adhesive and aggregative activity had been evaluated in a quantity method with the help of aggregometer device SOLAR AP 2110, ADP «Renam», Russia had been used as an aggregation activator. Disease compensation stipend in patients with Diabetes Mellitus had been determined according to the level of the glycated hemoglobin that at the average was 7.0±1.5 (12–5.2%).
Through the evaluation of the platelet adhesive and aggregative activity (Table 1) significant evaluation of the aggregation level in overweight and diabetes groups had been detected ($P<0.05$) in comparison with a control group, the longest time to reach the maximum aggregation level had been seen in diabetes group ($P<0.01$). Aggregation level in overweight and diabetic patients does not differ significantly.

Table 1

Homeostasis parameters	Nearly healthy	Overweight	$P_{I–II}$	Diabetes	$P_{I–III}$	$P_{II–III}$
Aggregation level (%)	53.02±10.89	62.62±11.96	<0.05	64.1±15.6	<0.05	>0.05
Aggregation speed (30), %/min	49.5±13.1	61.2±8.1	<0.05	46.4±16.44	>0.05	<0.05
Aggregation time (min)	4.14±074	4.07±0.6	>0.05	5.12±0.88	<0.05	<0.01

These dates are proving the fact, that one of the reasons for cardiovascular disease developing in association with diabetes mellitus and abdominal obesity is abnormality of the pathology of the platelet homeostasis component.

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Functional balance in diabetic neuropathy

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Introduction
Proprioceptive loss in diabetic peripheral neuropathy patients (DPN) seems to cause postural imbalance which may affect quality of functions and activities of daily living of these patients.
Aim
The aim of this study was to compare functional balance in diabetic neuropathic patients and normal subjects.
Methodology
In this case–control study, fifteen patients with DPN (which their neuropathy was diagnosed by Diabetic Neuropathy Examination (DNE)) and 15 healthy (gender-, age- and BMI-matched) subjects were evaluated with Berg Balance Scale (BBS), containing 14 balance tests. As well as overall functional balance, five groups of these tests were taken into more consideration in this study, based on the probable effects of proprioceptive loss on various functions. These groups were labeled as: ability to control weight shifting (CWS), ability to transfer (T), and ability to control balance under different base of support (BOS) and visual (V) conditions.
Results
Comparison of two groups showed a significant decrease in BBS, CWS, T, BOS, and V scores in DPN patients relative to healthy control group ($P<0.05$). There were negative significant (sig. level: 0.001) good to strong correlations between DNE score and BBS, CWS, T, BOS, and V, Scores (Pearson's correlation coefficient: −0.88, −0.91, −0.87, −0.76, and −0.70, respectively) in patients.
Conclusion
DPN results in a remarkable functional imbalance which may expose these patients to danger of falling during activities of daily living and becomes more severe as the severity of neuropathy aggravates. In order to control their balance, DPN patients rely on visual information.

P398

***Terminalia bellerica* (Belliric Myrobalan) stimulates the secretion and action of insulin and inhibits starch digestion and protein glycation**

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Traditional plant treatments have been used throughout the world for the therapy of diabetes mellitus. The aim of this study was to investigate the efficacy and mode of action of *Terminalia bellerica* Roxb. (Combretacea) used traditionally for treatment of diabetes in India. *Terminilia bellerica* aqueous extract stimulated basal insulin output and potentiated glucose-stimulated insulin secretion concentration-dependently in the clonal pancreatic beta cell line, BRIN-BD11 ($P<0.001$). The insulin secretory activity of plant extract was abolished in the absence of extracellular Ca^{2+} and by inhibitors of cellular Ca^{2+} uptake, diazoxide and verapamil, ($P<0.001$, $n=8$). Furthermore, the extract did not increase insulin secretion in depolarised cells and did not further augment insulin secretion triggered by tolbutamide or glibenclamide. *Terminilia bellerica* extract also displayed insulin mimetic activity and enhanced insulin-stimulated glucose uptake in 3T3 L1 adipocytes by 300%. At higher concentrations, the extract also produced 10–50% ($P<0.001$) decrease in starch digestion *in vitro* and inhibited protein glycation ($P<0.001$).
In Streptozotocin (125 mg/kg body weight) diabetes-mice, long term administration of *T.bellerica* decoctions (5 mg/ml) reduced ($P<0.01$) diabetic polydepsia, with no parallel recorded improvements of glucose homeostasis parameters.
This study has revealed that components in *T. bellerica* extract stimulate insulin secretion, enhance insulin action and inhibit both protein glycation and starch digestion. The former actions are dependent on the active principle(s) in the plant being absorbed intact. Future work assessing the use of *Terminalia bellerica* as dietary adjunct or as a source of active antidiabetic agents may provide new opportunities for the treatment of diabetes.

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The frequency of diabetic retinopathy and relevant factors for prediabetic subjects

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Objective
To determine the frequency of diabetic retinopathy in prediabetik individuals and to evaluate the relevant factors.

Materials and methods
A total of 118 persons between 20 and 60 years of age attending our internal medicine clinic between January 2006 and March 2008 were recruited for the study. The exclusion criterions were the presence of diabetes mellitus and use of antidiabetic agents. Fasting blood glucose between 100 and 125 mg/dl was defined as impaired fasting glucose (IFG); blood glucose after 2 h oral glucose tolerance test between 140 and 199 mg/dl was defined as impaired glucose tolerance (IGT). The individuals provided these criterions were included the study. In addition, diabetic history, physical examination (tension arteriale, pulse, fever) and laboratory investigations (creatinine, ALT, AST, Total cholesterole, HDL-C, triglyceride versus LDL-C) were carried out.
Results
Hypertension was found in 27.1% of participants and retinopathy was determined in 11% of them. The frequency of retinopathy in patients with isolated IFG, isolated IGT and both IFG and IGT were 2, 9.1 and 22.2%, respectively. The frequency of hypertension were found to be 15.7, 36.4 and 35.6%, respectively. There are significant correlations between retinopathy and postglucose 2 h blood glucose (PG2hr BG) (*P*: 0,001, *r*: 296), the presence of isolated IGT (*P*: 0.006, *r*: 252), coexistence of IFG and IGT (*P*: 0.002, *r*: 281). In logistic regression analyse, statistically significant parameters for retinopathy according to impotance are as follows: (1) coexistence of IFG and IGT (*P*: 0.002), (2) PG2hr BG (*P*: 0.003), (3) presence of isolated IGT (*P*: 0.006). Although HbA1C was no statistically significant for retinopathy, it was in fourth line as predictive (*P*: 0.073).
Conclusion
In our study, frequency of retinopathy among prediabetic subjects were significantly higher in group of coexisting IFG and IGT than other groups. Additionally, PG2hr BG was the most important predictor of retinopathy.

P400

Prognostic values of diabetic retinopathy progression for the assessment of lower limbs and heart muscle perfusion disturbances in patients with diabetes 2

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Introduction
Diabetic retinopathy and lower limbs and heart muscle perfusion disturbances are important complications in diabetes. Early recognition of diabetic retinopathy and acroangiopathic disturbances reduce the complications frequency.
Aim
Correlation of lower limbs and heart muscle perfusion disturbances and diabetic retinopathy progression level in patients with diabetes 2. The assessment to what extend diabetic retinopathy progression level may be a direction for diagnostics of lower limbs and heart muscle perfusion disturbances.
Material
One hundred patients with diabetes 2 and retinopathy divided into three groups: PHL – diabetic angiopathy (34 patients); SIM – simple retinopathy (33 patients); PRO – prolixpherating retinopathy (33 patients).
Methods
Full range of ophthalmological examinations: indirect ophthalmoscopy, color photography, fluorescent angiography. The assessment, at rest and after exercise, of heart muscle perfusion with SPECT technique (including SDS – summary defect score) and lower limbs muscles with perfusion scintigraphy. The examinations were performed with gamma-camera with the own programs after application of radiopharmaceutic Tc^{99m}MIBI.
Results
Deterioration of lower limbs muscle perfusion after exercise: PHL – 42%; SIM – 60%; PRO – 93%. Deterioration of heart muscle perfusion and SDS after exercise: PHL – 3%; SIM – 14%; PRO – 25%.
Conclusions
A dependency between lower limbs and heart muscle perfusion disturbances and diabetic retinopathy progression was stated. The level of diabetic retinopathy is the exponent for macroangiopathic disturbances and correlates with the changes of heart and lower limbs muscles. Statistic analysis of heart muscle perfusion showed an important dependency on the level of progression of eyegrounds changes. The patients with advanced diabetic retinopathy should undergo the assessment of lower limbs and heart muscle perfusion disturbances despite the lack of subjective complaints.

P401

Association between inflamation markers and metabolic parameters in type 2 diabetic patients, metabolic syndrome, impaired glucose tolerance

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Impaired glucose tolerance (IGT) and metabolic syndrome (MS) is considered as the pre-diabetic stages. Recently, it has been suggested that inflammation is associated with the components of MS and it may also have a role in the development of type 2 Diabetes Mellitus (DM). The aim of this study was to investigate the effects of inflammation in the pathogenesis of DM.
The study groups consisted of 51 patients with type 2 DM, 28 patients with MS, and 26 patients with IGT. DM patients had either initial diagnosis or recent history with the ultimate period of 5 years. A total of 21 healthy person was included as the control group. Highly sensitive CRP (hsCRP), fibrinogen, ferritin and white cells were utilized as the markers for inflammation. The relationships between the inflammation markers and parameters of MS were investigated in all groups.
The levels of hsCRP and fibrinogen were significantly higher in DM, IGT, and MS than control group. The level of hsCRP was highest in the DM group. On the other hand, ferritin was found to be higher in the DM group than the controls. When the whole study group was considered, hsCRP had positive correlations with WC, insulin, BMI, TG, HOMA, postprandial glucose (PPG), fasting blood glucose (FBG), cholesterol, LDL, and a negative correlation with HDL.
In conclusion result of the current study suggests that inflammation parameters particularly elevated levels of hsCRP were strongly associated with DM and features of MS.

P402

C-reactive protein level early after starting standard treatment of diabetic foot infection is associated with amputation risk

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Prediction of amputation would aid clinicians in the management of the diabetic foot infections. We aimed to assess the predictability of baseline and early post-treatment levels of acute phase reactants on the outcome of patients with diabetic foot infections.
In this study, we included patients with infected diabetic foot ulcers who were hospitalized in Dokuz Eylul University Hospital between January 2003 and January 2008, of which data were collected prospectively during a minimum follow-up of 6 months. After exclusion of patients who underwent an urgent amputation (within 1 week of admission) and patients who did not attend the hospital for follow-up visits regularly, finally, data from 165 foot ulcer episodes were analyzed.
Univariate analysis showed that one standard deviation increase in baseline and post-treatment C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white blood cell count (WBC), and one standard deviation decrease in post-treatment albumin levels were significantly associated with increased risk for amputations. Post-treatment CRP levels were more strongly related to amputations (AUC: 0.809, 0.744–0.874, 95% CI).
We suggest that CRP level obtained early after standard treatment is a strong predictor of amputation in patients with diabetic foot infections.

P403

Use of metformin in pregnancy: a survey of Turkish physicians' attitudes

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Although metformin crosses placenta, there is no current evidence whether the use of metformin in pregnancy is associated with increased risk of fetal and

maternal complications. The aim of this present study is to investigate the attitudes of Turkish physicians in different specialties in terms of metformin use in pregnancy.
Current opinions were assessed by interviewing physicians using a questionnaire. Nine hundred forty physicians were invited to join the study; however completely fulfilled questionnaires could be obtained from a total of 407 physicians (170 family physicians, 110 internists, 98 obstetricians, 29 endocrinologists).
One hundred fifty-one physicians (37.1%) stated that they recommended metformin use in pregnancy for any of the indications (pregnant women with PCOS, type-2 diabetes or gestational diabetes). Among physicians, obstetricians were more likely to suggest metformin use in pregnancy. Rationales of physicians for the metformin use were lower risk for abortus, decreased prevalence of maternal and neonatal complications, improvement of insulin resistance, prevention of excess weight gain, better glycemic control in diabetics and decreased insulin need in diabetics taking insulin.
Despite limited data on metformin use in pregnancy, significant number of physicians in Turkey supported metformin use. Obstetricians were more likely to recommend metformin treatment in pregnancy.

P404

Predictors of amputation in diabetic foot ulcer: single centre experience in a large Turkish cohort

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Background
Prediction of diabetic foot ulcer outcome might be helpful for clinicians in optimizing individual treatment strategy. The aim of the present study was to determine potential benefits of easily assessed clinical and laboratory factors at baseline in the prediction of the outcome in patients with diabetic foot ulcers.
Methods
In this observational study, data was collected prospectively in 670 consecutive diabetic foot ulcer episodes in 510 patients between January 1999 and June 2008, and were used retrospectively to evaluate potential predictors of amputation. After exclusion of patients who did not attend to the hospital for follow-up visits for minimum 6 months, data of 574 foot ulcer episodes were evaluated.
Results
Limb ischemia, osteomyelitis, and presence of gangrene and ulcer depth, which were determined by Wagner classification system, were major independent predictors of overall and major amputations. Older age, presence of coronary artery disease, smoking and ulcer size were found to be associated with either overall or major amputations. Baseline levels of acute phase reactants (white blood cell count, polymorphonuclear leukocyte count, platelet count, erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP) and albumin) and decreased hemoglobin levels were associated with amputation risk. Multivariate analysis showed that one standard deviation increase in baseline CRP and ESR levels were independent predictors of overall and major amputations, respectively.
Conclusions
Presence of limb ischemia, osteomyelitis, local and diffuse gangrene and ulcer depth were determined as independent predictors of amputation. Baseline levels of ESR and CRP seemed helpful for clinicians in prediction of amputation.

P405

The prevalence of anemia in Iranian type 2 diabetic patients and the role of nephropathy

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Background
Anemia is a common problem in diabetic patients. Diabetic patients have a greater degree and severity of anemia for their level of renal impairment compared to non-diabetic patients. This study examines the prevalence of anemia in type 2 diabetic patients and to determine the contribution roles of different stages of nephropathy in development of anemia in this patient.
Methods and materials
Of 1962 outpatients type 2 diabetes were selected. A full blood count, iron indices were obtained from all patients. The prevalence and correlation of anemia with other variables identified with multivariate logistic regression.
Results
Of 9.2% of male and 10.4% of female patients had anemia. Prevalence of elevated albuminuria (micro or macroalbuminuria) was 38.1%. Of 8.1% of our patients had moderate (creatinine clearance <60 ml/min per 1.73 m^2) and 31.4% had mild (CCr = 60–90) renal impairment. Patients with moderate renal impairment had significant more anemia than patients with mild renal failure (30 vs 9%, $P=0.000$). Patients with diabetes and macroalbuminuria were also likely to have more anemia than patients with microalbuminuria (32.4 vs 8.4%, $P=0.000$). Also patients with microalbuminuria were more likely to have anemia than patients without elevated albuminuria (8.4 vs 5.7%, $P=0.000$). Cardiovascular disease and retinopathy were more in diabetic patients with anemia than patients without anemia ($P=0.01$, 0.001 respectively).
Conclusion
Anemia is a high prevalent finding in type 2 diabetic patients. Any degree of renal impairment and albuminuria are greatest risk factors for anemia in this patients.

P406

Frequency of hypogonadism in men with type 2 diabetes

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Introduction
Type 2 diabetes is associated with lower total testosterone (TT) levels in cross – sectional studies. However, it is not known whether the defect is primary or secondary.
Method
We investigated the prevalence of hypogonadism in type 2 diabetes in men by measuring serum total testosterone (TT), SHBG, LH, FSH, prolactin (PRL) in 85 men with type 2 diabetes. Free testosterone (FT) was calculated by using TT and SHBG (CFT). Hypogonadism was defined as low CFT.
Results
The mean age was 51.4 ± 5.87 years, Mean BMI was 26.6 ± 3.6 kg/m^2, mean HbA1C was $8.815 \pm 2.1\%$, mean FBS was 197.7 ± 74.5, mean TT was 460 ± 20.5 ng/dl, mean CFT was 7.5 ± 2.34 ng/dl, mean BT was 172.8 ± 62.2 ng/dl, mean SHBG was 51.7 ± 29.5. Of 36.6% of patients had hypogonadism. LH and FSH levels were not increased. There was a significant inverse correlation between BMI and TT ($r=-0.367$; $P=0.001$) but there wasn't correlation between BMI with CFT. There was inverse correlation between SHBG and BMI ($r=-0.25$; $P=0.02$) and direct correlation between SHBG and age ($r=0.4$; $P<0.001$). There was inverse correlation between CFT and age ($r=-0.2$; $P<0.05$).
Conclusion
Hypogonadotropic hypogonadism is a common defect in type 2 diabetes that requires further assessment in terms of etiology of the defect and the possible consequences, complications, and treatment.

P407

Is testosterone therapy safe for cardiovascular system? The impact of cardiac ultrasound monitoring

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Background
The benefit of testosterone replacement therapy in men with hypogonadism nd metabolic syndrome is known. Safety of testosterone therapy is still a question of a high interest, especially regarding influence on cardiovascular system.

Objective
To study the safety of testosterone therapy in hypogonadal men with metabolic syndrome based on cardiac ultrasound data.
Methods
We studied 39 men aged 35–69 with hypogonadism (total testosterone <12 nmol/l) and metabolic syndrome (IDF criteria). All men were hypertensive. Of 23 men received testosterone undecanoate (Nebido, Schering) (group 1) and 16 men were the control (group 2). Individual daily monitoring, electrocardiogram (ECG) and echocardiography were performed before and after 30 weeks. Left ventricular *end*-diastolic volume *(LVEDV),* left ventricular *end*-systolic volume (LVESV), LV ejection fraction (LVEF), left ventricular posterior septum width (LVPSW), interventricular septum width (ISW) were investigated. Statistical analysis was performed using Wilcoxon test.
Results
In group 1, LVEDV was 117 (110;123) and 116 (110;121) mm (normal range, NR, 96–157), LVESV was 45 (36;46) and 43 (36;45) mm (NR 33–68), LVEF was 63 (61;65) and 63 (61;65) % (NR 58–65), LVPSW was 13 (11;12) and 12 (11;12) mm (NR <12), ISW was 13 (11;12) and 13 (11;11.8) mm (NR <12), respectively. In group 2, LVEDV was 116 (110;120) and 114 (107;121.5) mm, LVESV was 45 (38;47) and 44.5 (38;47) mm, LVEF was 62 (61;66) and 62 (61;65.5) %, LVPSW was 13 (11;12) and 13 (10.05;11.5) mm, ISW was 12 (9.5;12) and 11 (10;11) mm, respectively. All changes were not significant ($P>0.05$), except LVESV in the group 1 ($P=0.02$) and LVPSW in group 2 ($P=0.02$). We did not notice any worsening of hypertension or any significant ECG changes.
Conclusion
Androgen therapy has no adverse influence on cardiac function.

P408

Effects of alteration in serum testosterone levels on beta cell functions in male patients with hypogonadism and in female patients with polycystic ovary syndrome

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While low testosterone levels are associated with higher incidence of type 2 diabetes mellitus in males. In contrast, hyperandrogenism is associated with higher risk of type 2 diabetes mellitus in females.
We, therefore, have assessed alterations in beta-cell functions before and after testosterone replacement therapy in male patients with idiopathic hypogonadotropic hypogonadism (IHH) and before and after anti-androgen therapy in patients with polycystic ovary syndrome (PCOS). The study population consisted of 17 female patients with PCOS, 15 appropriate controls and 33 patients with IHH, 30 appropriate controls. Patients with IHH were treated with intramuscular injections of chorionic gonadotropin 1500 U twice in a week for 6 months. Patients with PCOS were followed during treatment of 0.035 mg ethinylestradiol/2 mg cyproterone acetate combined pills (DIANE-35 tablet®) for six menstrual cycles. After 6 months treatment with antiandrogen in patients with PCOS, levels of total testosterone (TT), free testosterone (fT), fasting plasma glucose (FPG), 2-hour post-challenge glucose, 2-hour post-challenge insulin, c-peptide, and amylin concentrations decreased significantly. Antiandrogen treatment did not cause alteration in body mass index, Waist/Hip ratio, HOMA-IR, fasting insulin, proinsulin and amylin levels. Fasting plasma levels of TT, fT, proinsulin, c-peptide, and amylin increased, but fat mass, HOMA-IR and FPG decreased significantly in patients with IHH after chorionic gonadotropin replacement therapy.
Androgen replacement may increase beta cell dysfunction despite increase in insulin sensitivity and antiandrogen treatment may restore beta cell dysfunction in patients with PCOS.

P409

Endothelium dependent hemostatic factors and subclinical inflammation in women with previous gestational diabetes mellitus

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Women with a history of GDM may be at increased risk for future diabetes and cardiovascular disease. The aim of this study was to investigate the endothelium dependent hemostatic factors as well as the parameters of endothelial function and inflammation in women with pGDM.
Eighty women with pGDM and 40 women (control) healthy women with normal glucose tolerance during pregnancy were studied. In all women, plasma glucose, insulin, C-peptid, lipid profile, CRP and endothelium dependent hemostatic factors PAI–1, vWF, fibrinogen, tissue factor (TF), t-PA, total TFPI were measured.
Subjects with pGDM and healthy control subjects did not differ in mean age or number of years postpartum. The women with a pGDM had higher fasting glucose, postprandial glucose, total cholesterol, LDL-cholesterol, and triglyceride levels than women with a history of normal pregnancy; homeostasis model assessment, insulin and C-peptid were also higher in the pGDM group. Compared with the control group, women with a pGDM had higher levels of CRP, fibrinogen, PAI-1, TF, and total TFPI. After adjusting for BMI, CRP and PAI–1 remained higher in the women with pGDM. PAI–1 levels correlated with BMI, WHR, LDL-cholesterol, triglyceride, HDL-cholesterol, fasting glucose, 2 h plasma glucose, insulin, HOMA-IR, CRP and TF in the whole group.
The increase of endothelium dependent hemostatic factors and inflammatory markers may be one of the first detectable markers in high risk of diabetes, like those with a pGDM. Higher levels of fibrinogen, PAI–1, TF and TFPI in particular may help to explain this higher prevalence of diabetes in pGDM subjects.

P410

Metabolic syndrome and meal stimulated C-peptide levels as useful clinical markers in choosing metformin for combination therapy with sulphonylurea in type 2 diabetic subjects

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Introduction
Monotherapy failure in diabetes mellitus type 2 occurs early in the course of disease. Evaluation of the primary pathogenetic mechanism is of primary importance in choosing the optimal combination therapy.
Objectives
To investigate influence of meal stimulated C-peptide levels and HOMA B, as markers of residual insulin secretion on the efficasy of adding metformin to sulphonylurea therapy in subjects with metabolic syndrome, type 2 diabetes and sulphonylurea failure.
Method
In the group of thirty subjects with diabetes type 2, metabolic syndrome and SU monotherapy failure, metformin was added on (SU+MET) for the six months. During the six months following-up, fasting (FPG), postprandial (PPG), mean daily plasma glucosae (MPG) and glycosylated hemoglobin (A1C) were evaluated. Fasting and stimulated C- peptide levels after standard meal test (300 kcal) were measured at start; absolute and relative increase of C-peptide (delta CP, delta CP%), and HOMA B were calculated. Correlation between markers of residual insulin secretion at start and glycaemic control after six months of SU+MET therapy were evaluated by using Pearson correlation coefficient.
Results
Glycaemic control after six months of SU+MET therapy was significantly improved (FPG 7.89 vs 11.61 mmol/l, $P<0.01$; PPG 12.61 vs 11.12 mmol/l, $P<0.01$; MPG 9.32 vs 11.78 mmol/l; A1C 7.81 vs 9.73%, $P<0.01$), but target values were not reached. Stimulated C-peptide levels inversely correlated high singnificantly, with all parameters of glycaemic control (r -0.479 to -0.791; $P<$ 0.01). HOMA B significantly correlated only with A1C ($r=-0.382$; $P<0.05$). However, fasting C- peptide levels were found not to correlate with parametars of glycaemic control.
Conculsion
Not only presence of metabolic syndrome, but also evaluation of residual insulin secretion are necessary in the choosing the best combination therapy in type 2 diabetic subjects. Standard meal stimulated C- peptide level is a simply and useful marker of residual insulin secretion.

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Abstract unavailable.

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Clinical characteristics and mother-fetal results in patients with gestational diabetes

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Objective
To evaluate the main clinical characteristics and the obstetrical results of perinatal morbi-mortality of women with gestacional diabetes in our Service.
Material and method
We made a descriptive study of 83 gestational diabetes patients which became a pursuit coordinated between the services of Endocrinology and Gynecology. The following main aspects were analyzed: age, toxic habits, BMI pre-gestational, first pregnancy or not, previous antecedents of gestational diabetes, hypertensive pathology, weeks of gestation, restored treatment, levels of HbA1c to the diagnose and to end of the gestation, childbirth, obstetrical complications and weight of newborn.
Results
The average age of the pregnants were 32.7±5 years and had been diagnosed of gestational diabetes in the week 29±7.8 with BMI pre-gestational of 27.6±5.1 kg/m^2 (37.3% normal weight, 34.9% overweight, 27.7% obesity). The 32.5% were first pregnants showing more than 30 years in the 46.1. Of 30.3% had antecedents of gestational diabetes. In the 15.7% hypertensive pathology were associated (4.8% antecedents of hypertension, 7.2% pregnancy hypertension and 3.6% preeclampsia), and the 31.3% were smokers. The average HbA1c to the diagnosis was 5.29±0.5, and at the end of the gestation 5.3±0.46, need of insulinization 64% (15.55±10 U/día). Of 66 patients ended the gestation with an average duration of 38.6±1.5 week (15.1% childbirths preterminal, 78.8% upon maturity and 6% posterminal). The global percentage of Caesarean was 34.8%, spontaneous childbirth 46.9% and instrumental childbirth 18.1%. The average weight of new born was of 3288±489 g with macrosomia in 7.6%. The total percentage of obstetrical trauma was of 10.6%. The Apgar average to the 5 min was of 8.7±1.5 and to the 5 min 9.8±1.2. Entrance in pediatry were needed in the 4.5% and a neonatal mortality of 1.5% was determined.
Conclusions
Patients with gestational diabetes in our service showed a suitable metabolic control with a discreetly superior macrosomia percentage in comparison with nondiabetic general population
We found a higher percentage of Caesarean in our patients in comparison with other published series.

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Quality of life in diabetes mellitus: conditional issues of treatment and coping strategies

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Introduction
Quality of life (QoL) is a subject of increasing interest in the health context. Applied to a chronic condition like diabetes, this issue could give an overall perspective of the health outcome.
Objective
To evaluate the coping mechanisms, the treatment issues and its contribution to QoL of diabetes patients.
Patients and methods
We gathered a sample of 94 diabetic subjects, 50% males, 55.3% type 1, with a mean age of 42.02±16.68 (17–77) years. To accomplish our work we applied several instruments: a general biographical questionnaire, audit of diabetes-dependent quality of life (ADDQoL), problem areas in diabetes survey (Paids), experience of treatment benefits and barriers (ETBB) and problem solving inventory (PSI). We used a mean comparison *t*-test, the Pearson and Spearman's correlations tests.
Results
Type 1 diabetics showed higher values in ADDQoL questionnaire than Type 2 diabetics (−1.04±1.19 vs −1.74±1.34; $P=0.009$), as well as patients with none diabetes related complications (−2.03±1.49 vs −0.95±0.98; $P<0.001$). Relatively to the problem areas, we found that patients with insulin treatment and patients with later complications reported higher levels of psychological suffering than patients on tablets (33.9±16.1 vs 22.3±13.8; $P=0.01$) and with none diabetic complications (37.5±17.9 vs 28.8±14.5; $P=0.01$), respectively. In terms of coping, better coping strategies are associated with better QoL ($r=0.29$; $P=0.005$). Last, we didn't find any correlation between A1c levels and QoL.
Results
In this study we acknowledged that QoL is directly linked and influenced by subjects like personal problem solving mechanisms, treatment methods and perceptions of problem areas, namely emotional materials. These results are somewhat consistent with findings in previews studies.

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Thyroid dysfunctions and serum creatine kinase levels in statins-using patients with hyperlipidemia

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Introduction
Hypothyroidism is a well-known cause of secondary dyslipidemia, and its link to atherosclerosis has been known for long time. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) offer important benefits for the large population of individuals at high risk for coronary heart disease. It was aimed to invastigation the relations of the secondary hyperlipidemia included overt and subclinical hypothyroidism and increased serum creatine kinase (CK) levels in patients who were taken statins to treatment for hyperlipidemia, retrospectively.
Materials and method
There were 1765 patients who were taken statins for hyperlipidemia in last 5 years. The rates of hypothyroidism and subclinical hypothyroidism were determined, then increased serum CK levels as a statin induced myopathy were analysed.
Results
There were 51 (2.9%) patients with subclinical hypothyroidism and 26 (1.5%) hypothyroidism in study population. However, increased CK levels were not determined in subjects with thyroid dysfunctions.
Conclusion
The rate of hypothyroid-induced myopathy is unknown. Patients' thyroid status should always be considered before initiating lipid-lowering medications. Statins offer important benefits with decreased statin induced myopathy for the large population of individuals at high risk for coronary heart disease.

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Histopatological effects of stress on the heart muscle in rats

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Objective
Stress causes negative effects at all the body especially the heart. As a result of the damage of secreting various hormones which stress cause to secrete in the body. During the stress, respiration, tension, heart speed and catecholamine level increase. All these can be considered as a risk factor for the heart disease. In our study, we aimed to examine stress' possible histopathological changes on heart muscle.
Methods
Eight adult female Sprague Dawley rats were used in this study. Rats were randomly divided the control ($n=4$) and stress ($n=4$) groups. Chronic mild stress (CMS) model of depression was performed to the stress group during two weeks.

At the end of the test, rats were slept with ketamin HCI and hearts were removed after opening their chest cage and their volumes were measured by the water immersion method. After this process tissue samples were blocked in paraffin blocks following routine histological protocol. Sections were taken with *Leica RM2125RT* microtome at the thickness of 4–5 mm and stained with hematoxylin - eosin. Preparations were light microscopically examined.
Results
It was observed that volumes of the hearts which belongs to the stress performed rats were significantly increased when compared with the control group ($P < 0.05$; independent samples *t*-test). In the sections of test group, there was an extension at the length of muscle fibers because of cytoplasmic swelling. Expansion of blood vessels in interstitium and presence of fat cells in some vessels were remarkable. Extensive vacuolar degeneration was also determined in the muscle fibers.
Conclusions
According to our findings, it is concluded that CMS can cause histopathological changes in the heart, such as coronary heart disease and high blood pressure.
Aknowledgement
This study was supported by the 2008/20-numbered Scientific Research Fund of our University.

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Insulin casts in type 2 patients different shadows with proliferate retinopathy and those with wtent or bypass on coronary rrteries

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Background and aims
The aim of this study was to examine the prevalence of neuropathy and nephropathy in patients with type 2 (T2DM) complicated by proliferate retinopathy (PDR) and coronary artery occlusive disease (CAOD) treated with stent or bypass surgery.
Materials and methods
Comparisons were made between 22 patients with T2DM and bypass or stenting of coronary arteries with normal fundoscopic finding (G1) and 21 age matched controls without diabetic retinopathy and CAOD, and also between 23 patients with T2DM and PDR (G2) and 21 age and diabetes duration matched controls. Clinical examination of the eyes was performed through dilated pupils using a slit lamp and a magnifying lens. Vibration perception threshold (VPT) was measured by semi quantitative tuning fork C128 (grade 0–8) and ankle reflexes were recorded. Body weight (kg), serum creatinine, fibrinogen and urine protein concentration were measured, and the presence of macro vascular (coronary, cerebrovascular and peripheral arterial) complications was also documented.
Results
In T2DM patients with CAOD were ones with lower HDL than it was the case with the control group of patients (1.14 ± 0.22 vs 1.38 ± 0.44 mmol/l, P 0.03) that had higher triglycerides (Tg)/ HDL (2.9 ± 2.36 vs 1.64 ± 1.43), shorter duration of diabetes (15 ± 6.8 vs 22.5 ± 6.4 years), higher creatinine (121.2 ± 28.7 vs 95.9 ± 28.7 mmol/l; P 0.002). However, VPT was not significantly different between the two groups (6.8 ± 1.8 vs 6.9 ± 1.3). A positive history of hyperlipidemia was more common in T2DM with CAOD than among Controls (90.9 vs 61.9%; $P = 0.004$). In addition to that, the tendency was present for hypertension (50 vs 33.3%; P 0.08) and HDL cholesterol was negatively correlated with HbA1c ($r - 0.42$, P 0.05). VPT was significantly worse in patients T2DM and PDR compared with controls (2.98 ± 2.9 vs 6.88 ± 1.33, $P < 0.001$), as it was the case with ankle reflexes (3.5 ± 0.9 vs 2.8 ± 1.74, $P < 0.05$), fibrinogen (4.1 ± 0.89 vs 0.89 g/l), proteinuria (1488.9 ± 2676 vs 225 ± 209 mg/dU), creatinin (135.8 ± 75 vs 95.9 ± 28.7 mmol/l; P 0.02). A positive history of hyperlipidemia was more common in G2 than among Controls (82.6 vs 61.9%, P 0.03), secondary insulin dependence (73.9 vs 38.1%; P 0.0001), low smoking habit (34.8 vs 76.2%, $P < 00001$). Total cholesterol showed positive correlation with HbA1c ($r = +0.51$, P 0.01) and pulse rate ($r = +0.38$, P 0.07). Vibration perception for all three groups (G1, G2, Controls) was negatively correlated with pulse rate ($r = -0.23$, P 0.07), HDL cholesterol ($r = -0.22$, P 0.07), fibrinogen ($r = -0.30$, P 0.018), duration of diabetes ($r = -0.28$, P 0.02), proteinuria ($r = -0.31$, P 0.01), creatinin $r = -0.23$, P 0.09), ankle reflexes ($r = -0.34$, P 0.006). After multiple regression analysis the correlations with duration of diabetes ($P = 0.0001$) remained significant.
Conclusion
Worsening VPT in T2DM is strongly associated with duration of diabetes where the worst outcome is in patients who developed diabetic proliferate retinopathy and nephropathy. In T2DM with coronary artery occlusive disease endogenous insulin exerts protective effect on microvascular complications. On the other hand, the same endogenous insulin, in the presence of classical macro vascular risk factors and compromised reverse cholesterol transport caused by hyperglycemia, exerts atherogenesis on coronary arteries.

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Evaluation of the severity of hepatic steatosis in type 2 diabetes

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Objective
The objective of this study was to examine the factors influencing the severity of hepatic steatosis in type 2 diabetes.
Methods
Computed tomography imaging was used to assess hepatic fat content in 80 men and women with type 2 diabetes. Inclusion criteria included a confirmed diagnosis of type 2 diabetes (≥ 1 year of duration), without history of hepatic disease or daily consumption of alcohol drink. The ratio of liver to spleen attenuation (L/S ratio) was calculated. Patients with an L/S ratio ≤ 0.8 were considered to have severe fatty liver. We compared the 2 groups with moderate and severe steatosis.
Results
The prevalence of hepatic steatosis was 30% (24/80). Steatosis was severe in 29% (7/24) and moderate in 71% of patients. Mean alanine aminotransferase level and C reactive protein level are significantly higher in diabetes with severe steatosis (respectively 25.8 ± 5.3 vs 20.4 ± 6 $P = 0.04$–12.6 ± 15.1 vs 4.7 ± 2.7 $P = 0.04$). No significant difference was found concerning age, duration of diabetes, anthropometric parameters, HbA1C, triglycerides level, HDL cholesterol level and total cholesterol level between the 2 groups.
Conclusions
Type 2 diabetic patient with higher liver enzyme especially alanine amino-transferase and positive inflammatory parameters had a higher risk of severe steatosis.

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Microalbuminuria and cardiovascular risk in diabetic patients

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Background
Microalbuminuria was originally established as a predictor of renal failure and an independent risk factor for cardiovascular disease in patients with diabetes mellitus as well as in general population. The aim of our study is to assess the relationship between microalbuminuria and the other risk factors in diabetics and their prevalence.
Methods
Sixty five patients, 22 men and 43 women with mean age 58.6 ± 10.09, with type 2 diabetes, were hospitalized in the Department of Internal Medicine in the University Hospital Center 'Mother Teresa' in Tirana, Albania, between March 2007 and February 2008. These patients with a mean duration of diabetes 6.09 ± 5.41 were divided in two groups: with (Group A: 24 patients) and without (Group B: 41 patients) microalbuminuria and each group was evaluated for left ventricular mass index (LVMI), body mass index (BMI), glycosylated hemoglobin (HbA1C), lipid profile and intima media thickness (IMT).
Results
The prevalence of microalbuminuria in our study was 32.3%. The prevalence of microalbuminuria in males was 37.5 and in females 62.5%. The microalbuminuric patients were older (59.71 ± 9.87 vs 57.07 ± 10.32) and had a longer duration of diabetes (7.74 ± 5.74 vs 4.45 ± 5.08) compared with normoalbuminuric patients ($P = 0.01$). The Group A had significantly higher LVMI compared with Group B ($P = 0.02$). The prevalence of obesity (BMI > 30 kg/m^2) in our sample was 44.6%. In Group A the mean BMI (30.13 ± 4.98) was significantly higher compared with Group B (28.00 ± 3.72, $P = 0.04$). Diabetic retinopathy was more frequent in Group A compared with Group B (33.3 vs 14.6%, $P = 0.05$). The mean value of IMT was higher in Group A compared with Group B (1.28 ± 0.35 vs 1.09 ± 0.28, $P = 0.03$)
Conclusion
In patients with type 2 diabetes and microalbuminuria LVMI, IMT, BMI, duration of diabetes was significantly higher compared with patients with type 2 diabetes and normoalbuminuria.

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Type 2 diabetes mellitus: failure to recognize cardiovascular risk jeopardizes prevention of diabetes complications in type 2 diabetic patients in daily practice

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There are still considerable deficits in the prevention of cardiovascular diabetes complications as in effective antihypertensive treatment in type 2 diabetic patients in daily practice. There is controversy, however, to which extent lack of information regarding potential risk factors or deficits in disease awareness of the patients themselves contributes to this unfavourable situation.
In 45 female and 51 male type 2 diabetic patients, age, 56 + 16 years (mean + S.E.M.), known duration of diabetes, 10 + 11 years, who were referred to our institution by their general practitioners for start or optimization of insulin therapy, we assessed the knowledge concerning risk markers and chances of prevention of cardiovascular diabetes complications.
Patients who were aware of risk markers had lower levels of cholesterol and triglyceride ($P<0.05$) as well as urinary albumin excretion than patients without this awareness, but blood pressure levels were not different. Awareness of the significance of hypertension was most prevalent (55%, $P<0.05$), but self-reported blood pressure levels were considerably lower than measured blood pressure ($P<0.05$). Although 70% of the patients knew about the significance of treatment with thrombocyte aggregation inhibitors, only 29% of the patients were treated by aspirin or clopidogrel ($P<0.05$).
Lack of information, wrong beliefs and the 'behaviour gap', the difference between the informations the patients have about their disease and what they actually do, contribute to insufficient prevention of cardiovascular risk and disease complications in patients with type 2 diabetes in daily practice. More concerted efforts are required to improve the quality of teaching of all type 2 diabetic patients by competent diabetes teachers and to increase disease awareness and, hopefully, cardiovascular outcome of the patients.

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Prevalence of diabetic retinopathy and its relation with other risk factors

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Background
Micro and macroangiopathy are two major complications of diabetes. Diabetic retinopathy (DR) and nephropathy (ND) which represented microangiopathy, are present early in type 2 diabetes. Macroangiopathy represented by atherosclerotic process assessed through intima-media thickness (IMT) of carotid artery. Microalbuminuria (MI) predictor of ND is associated with DR in type II diabetic patients and is a reliable marker of DR. The aim was to search for association between DR and IMT and to identify risk factors for the development of DR and MI and their correlation.
Materials and methods
Of 65 patients, 22 men and 43 women with mean age 58.6 ± 10.09, with type 2 diabetes, for at least 5 years, were examined at the Department of Internal Medicine. The ocular fundi were examined by a specialist, by direct ophthalmoscope and common carotid artery IMT was measured by a B-mode ultrasound, 10 MHz transducer. These patients were evaluated for presence of MI (a urinary albumin excretion between 30 and 300 mg/l per day), body mass index (BMI) and glycosylated hemoglobin (HbA1C).
Results
DR was found in 25(38.5%) diabetic patients. IMT was higher in patients with DR than in patients without DR (0.91 mm vs 0.82 mm, $P=0.001$, respectively).
The relationship between DR and risk factors such as HbA1C, BMI, duration of illness and age revealed to be significant. HbA1C was higher in patients with DR (mean = 10.5%) than in patients with no signs of retinopathy (mean = 9.5%) and this difference was statistically significant ($P=0.001$). As expected, DR and renal involvement were highly positively correlated. ($P=0.001$).
Conclusion
DR is associated with increased IMT. Elevated HbA1C predicts DR.
Retinopathy is associated cross sectionaly with the presence of MI in persons with diabetes type 2. So, diabetic patients with DR need particularly intensive cardiovascular screening.

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Screening of the Romani population in Serbia for diabetes

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It is well known that Romani people suffer from respiratory and cardiovascular diseases. The aim of our study was to investigate the prevalence of diabetes in the Romani population in Serbia.
Methods
During the period October 2006–May 2008, diabetes association of Serbia performed blood glucose measurements in 11 urban and 8 rural Romani communities in Serbia. Blood glucose values, name, age, presence of diabetes, family history for diabetes, time of last meal and presence of obesity were noted.
Results
Statistical analysis was performed on 1465 Romani people (825 in urban and 641 in rural communities) with complete findings. Mean age of the Romani people investigated was 42.42 ± 15.69 years. Obesity was present in 577 (39.4%) people. Some 87 of the 1465 Romani people (5.9%) already had diabetes. Blood glucose measurements discovered 76 (5.2%) new cases of diabetes type 2. Romani people with diabetes were significantly older ($F=28.33$; $P<0.01$). Family history for diabetes was positive in 1/3 of the Romani people. Risk for diabetes is 3.48 times higher if a Romani person has positive family history (OR 3.47; 95% confidence interval 2.37–5.1; $P<0.01$). Obesity was significantly more prevalent in Romani people with diabetes ($X^2=32.555$; df = 3; $P<0.01$). The risk of diabetes in an obese Romani person is 2 times higher than in the non-obese (OR 2.107, 95% confidence interval 1.249–3.554; $P<0.01$). Diabetes was significantly more present in urban communities ($X^2=25.205$; df = 2; $P<0.01$). The risk of developing diabetes is 3.649 times higher in Romani people that live in urban settlements (OR 3.649, 95% confidence interval 1.998–6.662; $P<0.01$).
Conclusion
Prevalense of diabetes in Romani people living in Serbia is possibly higher from the prevalence in the general population in Serbia. The risk factors for diabetes are middle age, family history, obesity and life in urban communities.

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Thyrotropin and thyroxine are associated with fasting insulin and insulin resistance in euthyroid impaired glucose tolerant subjects

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Objective
To investigate the relationship of thyroid hormones and insulin secretions in glucose homeostasis in impaired glucose tolerant and type 2 diabetic subjects, with normal thyroid functions.
Methods
Retrospective cross sectional analysis was carried out on ($n=266$) impaired glucose tolerant, type 2 diabetics and normal glucose tolerant subjects. Thyrotropin (TSH), total triiodothyronine (TT_3), total thyroxin (TT_4) and insulin were assessed by enzyme linked immunoassays (ELISA). Insulin and TSH were assessed by Immunoenzymometric Assay (Type 3), while TT_3 and TT_4 were assessed by competitive enzyme immunoassay (type 5). Fasting plasma glucose and HbA1c were measured by glucose oxidase and low pressure cation exchange chromatography. Homeostasis model of assessment (HOMA-IR) was employed to assess the level of insulin resistance. Anthropometric measurement and habits were recorded.
Results
Serum TT_3 levels were significantly lower in the IGT and diabetics as compared to normal glucose tolerants (controls). TT_4 and TSH were higher in IGT subjects as compared to control and diabetics. IGT subjects were more hyperinsulinemic and insulin resistant as compared to diabetics. There was a significant positive correlation of TSH with BMI only in control group ($r=0.351$; $P<0.05$). TT_3 had significant and positive correlation with TT_4 ($r=0.700$, $r=0.577$) in control and diabetic respectively ($P<0.01$). Correlation of insulin with TSH was significant ($r=-0.457$) in IGT subjects. In multiple regression analysis TSH, TT_4 contributed significantly to the variance of fasting insulin in IGT subjects.

Pathophysiology of chronic hyperglycemia and persistent insulin resistance suppressed the true picture of thyroid hormone status in diabetic subjects.
Conclusion
T_4 and TSH are associated with insulin secretions in IGT subjects. Impaired glucose tolerant subjects can be targeted for better therapeutic options.
The study was approved by ethical committee of the hospital.

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The effects of *Urtica diocia* on rat pancreatic β cell

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Introduction
Urtica diocia extract has been used for treatment of diabetes mellitus for many centuries. Hypoglycemic effects of *U. diocia* have been shown in multiple studies. The present study was designed to determinutese the possible mechanisms of hypoglycemic effects of *U. diocia* on RIN5F Rat Pancreatic β cells.
Methods and materials
In cell culture laboratory of Drug Applied Research Center of Tabriz University (Medical Sciences) pancreatic β cell prepared in multiple flasks containing culture media. Alcoholic extract of *U. diocia* with doses of 50, 100 and 200 μg were added to flasks containing RIN5F Rat Pancreatic βcell. Insulin and C-peptide levels were measured in 0, 60, 120 and 180 min.
Results
Insulin level in pancreatic cell medias before and after adding of *U. diocia* with varying doses and in different times were $\leq$0.2 μg/ml. C-peptide (μg/ml) level in these medias with dose of 50 μg of *U. diocia* and in above mentioned times were 0.31, 0.33, 0.86 and 0.8; with dose of 100 μg were 0.7, 0.2, 0.4 and 0.39; and with dose of 200 μg were 0.32, 0.33, 0.93, 0.77 respectively.
Conclusion
The results of the present study show that alcoholic extract of *U. diocia* was not able to increase Insulin and C-peptide secretion from RIN5F pancreatic βcells

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***Urtica diocia* effects on glucose utilization by human muscle cells**

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Introduction
Urtica diocia extract has been used for treatment of diabetes mellitus for many centuries. Hypoglycemic effects of *U. diocia* have been shown in multiple studies. The present study was designed to determinate the possible mechanisms of hypoglycemic effects of *U. diocia* on human muscle cells.
Methods and materials
In cell culture laboratory of Drug Applied Research Center of Tabriz University (Medical Sciences) in IRAN human muscle cells prepared in multiple flasks containing culture media. Alcoholic extract of *U. diocia* with doses of 50, 100 and 200 μg were added to muscle cells flasks. The same doses of extract plus insulin added to other muscle cells flasks. In all flasks glucose levels were measured before and 60, 120 and 180 min after adding of extract.
Results
Mean ± standard error (S.E.M.) of glucose (mg/dl) level in muscle cells medias containing of *U. diocia* with dose of 50 μg were 212 ± 2.35 in 0 min, 210 ± 2.11 in 60 min, 216 ± 2.35 in 120 min and 218 ± 2.94 in 180 min; with dose of 100 μg were 212 ± 2.11 in 0 min, 212 ± 2.35 in 60 min, 212 ± 2.35 in 120 min and 211 ± 2.11 in 180 min; with dose of 200 μg were 204 ± 2.35 in 0 min, 203 ± 2.11 in 60 min, 206 ± 2.11 in 120 min and 208 ± 2.94 in 180 min.
The glucose level in muscle cell medias containing of *U. diocia* plus insulin with dose of 50 μg were 204 ± 2.11 in 0 min, 214 ± 2.94 in 60 min, 213 ± 2.94 in 120 min and 213 ± 2.35 in 180 min; with dose of 100 were 206 ± 2.11 in 0 min, 208 ± 2.35 in 60 min, 210 ± 2.35 in 120 min and 211 ± 2.94 in 180 min; and with dose of 200 μg were 202 ± 2.11 in 0 min, 209 ± 2.35 in 60 min, 204 ± 2.11 in 120 min and 207 ± 2.94 in 180 min. The results showed that glucose level were not changed significantly in muscle cell medias with addition *U. diocia* alone or *U. diocia* plus insulin.
Conclusion
The results of the present study show that alcoholic extract of *U. diocia* was not able to increase insulin sensitivity in muscle cells.

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Effects of oral contraceptives versus no treatment on glucose tolerance and patients' satisfaction during long time follow up in 69 hirsute patients

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Objective
To evaluate the long term risk for diabetes and insulin resistance in untreated and oral contraceptive (OC) treated hirsute patients.
Design
Cross sectional study.
Setting
Academic tertiary-care medical centre.
Patients
Of 233 Caucasian hirsute women were evaluated during 1997–2002 (baseline) and re-contacted in 2003–2004. Of 159 patients returned questionnaires and 69 attended clinical examinations.
Interventions
Two-hour oral glucose tolerance test (OGTT).
Main outcome measures
Diabetes and impaired glucose tolerance (IGT), hirsutism.
Results
The median follow-up period was (median (range)) 4 (2–7) years. Evaluated by questionnaires, 91/130 (70%) patients had terminated OC treatment at follow-up. OC treatment significantly improved hirsutism. Cosmetic treatment compared to no cosmetic treatment had no significant long-term effects on hirsutism.
During clinical examinations ($n = 69$), BMI was 24.9 (22.4–29.0) kg/m^2 and total Ferriman–Gallwey score was 10 (7–15) (median (25–75 quartile)). Medically untreated patients (47/69) had increased fasting and 2 h glucose levels compared to baseline, whereas BMI was unchanged. Of 4/47 (8.5%) untreated patients developed diabetes and 5/47 (10.6%) developed IGT. OC treated patients had significantly decreased AUC insulin during follow up, whereas HDL and AUC glucose increased.
Conclusion
Of 8.5% untreated patients developed diabetes during follow-up, suggesting a high diabetes risk in hirsutism. OC treatment improved hirsutism.

P426

The effect of creatinine clearance on the short-term outcome of neuropathic diabetic foot ulcers

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Introduction
Reduced creatinine clearance is related to an increased risk of diabetic foot ulcer development. Wound healing has been reported to be worse in diabetic patients with impaired kidney functions than general diabetic population. This study aimed to investigate the effect of creatinine clearance on the short term outcome of neuropathic diabetic foot ulcers.
Materials and methods
Data from 147 neuropathic diabetic foot ulcer episodes were included in this observational study. Patients applied to Dokuz Eylul University Hospital between January 2003 and June 2008. Patients were excluded if they had limb ischemia. Diabetic nephropathy was investigated by 24 h urinary albumin excretion and serum creatinine levels. Creatinine clearance was calculated according to Cockcroft–Gault formula. Foot ulcers were followed up for 6 months to determine the outcome.
Results
Our short term follow-up revealed that neuropathic diabetic ulcers healed worse in patients with decreased creatinine clearance than in those who had normal creatinine clearance. Amputation rates were also found to be higher.
Conclusions
Our results suggest that creatinine clearance is an important factor affecting wound healing in patients with neuropathic diabetic foot ulcers.

P427

Mean platelet volume in women with previous gestational diabetes and its alteration during pregnancy

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Previous gestational diabetes mellitus (pGDM) is associated with insulin resistance which is a well-known risk factor for atherosclerotic diseases. Mean platelet volume (MPV) is an indicator of platelet activation which plays a crucial role in pathogenesis of atherosclerosis.
One hundred and five consecutive women with pGDM and 40 healthy women with normal previous pregnancies were included in the study. At the time of enrollment, anthropometric measurements and laboratory tests including fasting and post-load glucose levels, lipid profiles, insulin, fibrinogen and MPV were performed. Women with pGDM were classified as type-2 diabetes, IFG-IGT and normal glucose tolerance (NGT) according to 75-g oral glucose tolerance test (OGTT). Retrospectively, perinatal data of the subjects regarding anthropometric measurements, 100-g OGTT and MPV values during pregnancy were recorded.
Women with pGDM were found to have higher insulin resistance, more atherogenic lipid profile and increased levels of plasma fibrinogen than control subjects. MPV was not statistically different between women with pGDM and women with normal prior pregnancies. MPV was associated with fasting blood glucose levels. MPV values were tended to be increased in women with pGDM who had type-2 diabetes when compared to those who had NGT ($P=0.092$). In retrospective analysis, MPV at the time of screening of GDM and MPV late in third trimester were found to be significantly higher in pGDM group.
In conclusion, MPV values of women with pGDM were not statistically different from those of women with normal pregnancies. MPV tended to be increased in women with pGDM who had type-2 diabetes. During pregnancy, MPV was significantly higher in women with gestational diabetes.

P428

Reduction of major amputations after starting a multidisciplinary diabetic foot care team: single centre experience from Turkey

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Introduction
It is widely recognized that a multidisciplinary team is effective in the management diabetic foot ulcers. Contrary to developed countries, multidisciplinary diabetic foot care teams and/or clinics have not been constructed in most centres in developing countries. The aim of this study was to present our data regarding amputations rates and profiles before and after starting the Dokuz Eylul University multidisciplinary diabetic foot care team.
Methods
This study includes data from diabetic foot ulcer episodes which were managed in Dokuz Eylul University Hospital between January 1999 and January 2008. The data was collected prospectively during a minimum follow-up of 6 months in all ulcers. After January 2002, management of ulcers was coordinated by the diabetic foot care team ($n=437$). Amputation rates were compared to those who were admitted before January 2002 ($n=137$).
Results
Overall amputation and minor amputation rates were similar for both periods. However, major amputations were observed to be decreased after starting the Dokuz Eylul University multidisciplinary diabetic foot care team (20.4 vs 12.6%, $P=0.026$).
Conclusions
Our results demonstrated that major amputation rates can be reduced by team work. Formation of multidisciplinary diabetic foot care teams and clinics should be encouraged in Turkey.

P429

Prediction of developing metabolic syndrome after gestational diabetes mellitus

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It has been shown that women with previous GDM more likely developed metabolic syndrome (MS). This study aimed to determine predictors of the later development of MS in women with previous GDM.
One hundred sixty-four consecutive women with previous GDM were evaluated after a mean follow-up of 40.54 months from index pregnancy. Sixty-five lean women with negative screening for GDM were included as a control group. Data regarding antenatal and peripartum characteristic of participants were collected prospectively. Subjects were evaluated for the diagnosis of MS according to criteria of NCEPATP III and IDF. Tests were performed including 75 g. OGTT, fasting insulin, lipids, plasma fibrinogen, blood pressure, and body measurements. HOMA score was calculated.
MS prevalence was higher in women with previous GDM according to both definitions. Women with previous GDM were more overweight and insulin resistant. They had more atherogenic lipid profile and increased fibrinogen levels. Univariate analysis showed that prepregnancy obesity, weight gain during follow-up and fasting glucose level at the OGTT of the index pregnancy were predictors of developing MS. Multivariate analysis showed that fasting glucose level >100 mg/dl at the OGTT of the index pregnancy was an independent predictor of the MS development.
We suggest that early prediction of women with previous GDM who are at high risk for developing MS is possible, and it is vital to prevent MS related complications.

P430

Exercise induced oxidative stress in type 2 diabetes: relation to diastolic dysfunction and hypertension

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Background
Lipid peroxidation and antioxidant systems are important factors affecting the oxidation of lipoproteins. Left ventricular dysfunction and hypertension are much more common in subjects with type 2 diabetes mellitus and limit exercise performance.
Aims
The aim of our study was to evaluate the oxidative stress in patients with diabetes mellitus type 2 and to determine influence of acute exercise training on the investigated parameters.
Methods
To asses' oxidative stress of the patients, we determined the following parameters: total cholesterol, low density cholesterol, Ox LDL cholesterol, superoxide dismutase, glutathione peroxidase, plasminogen activator-type 1 which was measured at rest and immediately after the acute bout of cardiopulmonary exercise cycle ergometer test.
Results
In basal condition, diabetic patients have significant increase of Ox LDL cholesterol and SOD enzyme activity compared to controls. During acute exercise test, there were significantly greater levels of Ox LDL in study patients and in control group. SOD significantly increases in both groups during exercise, in diabetic patients and in controls. GSH-Px was significantly increased only in diabetic patients after acute exercise. Type 2 diabetic patients with cardiovascular complications have only significant increase of GSH-Px activity.
Conclusion
Elevated OxLDL, SOD and GSH-Px levels are associated with exercise in type 2 diabetic patients.

P431

Serum homocysteine levels in diabetes and its relationship to nephropathy

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Introduction
Diabetes is a common disease with many complications; therefore many researches are done on possible factors witch affect on diabetes control and its complications. Homocysteine is one of these possible factors. We studied correlation of homocysteine and diabetes. We also compared homocysteine levels in diabetic patients with nephropathy and patients without nephropathy.

Materials and methods
Of 105 diabetic patients and 32 controls were enrolled in study. Smokers, addicts, pregnant women, patients with macrocytic anemia, thyroid disease and renal failure were excluded. Only patients with type 2 diabetes were studied. Serum homocysteine, FBS, creatinin and urine microalbumin and creatinin were measured.
Results
The patients and controls were matched in age, sex, hypertension, BMI and family history of diabetes. There was no significant difference in homocysteine between patients and control. Homocysteine level in diabetic patients was 12.9 ± 9.5 μmol/l and 11.6 ± 8.6 μmol/l in control group. Also there wasn't any significant difference between diabetic patients with and without nephropathy in homocysteine level. Serum homocysteine was 13.2 ± 11.08 μmol/l in patients with nephropathy and 12.5 ± 9 μmol/l in patients without nephropathy ($P = 0.9$).
Conclusion
In contrast to most of other studies, our study showed no difference in homocysteine between diabetics and control. This may be due to number of cases or racial difference.

P432

Can testosterone therapy be included into diabetes treatment in men with metabolic syndrome and hypogonadism?

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Background
In 1998 the UKPDS study showed that over 10 years of observation there were no significant changes in haemoglobin A1c (HbA1c) in intensive insulin therapy group compared with conventional therapy group. Therefore, we still need new options to treat diabetes. Diabetes mellitus type 2 (DMT2), is one of the MS components. Obesity leads to insulin resistance (IR) and DMT2. Androgen deficiency (AD) is well-known factor to predict the development of diabetes.
Objective
To study the glycaemic status and changes in hypoglycemic therapy in men with androgen deficiency (AD), MS and DMT2, treated with testosterone undecanoate (TU).
Materials and methods
Of 18 men with MS (IDF criteria), DMT2 (6 patients were on insulin therapy) and AD (total testosterone (TT) < 12 nmol/l or free T < 250 pmol/l) were treated with TU (Nebido, Schering) for 102 (72;132) weeks. Fasting plasma glucose (FPG), HbA1c, TT and hypoglycemic therapy were estimated at baseline and at the endpoint. Statistical analysis was performed using Wilcoxon test and Spearman correlation test.
Results

	TT, nmol/l (NR 12–33)	**FG, mmol/l** (NR 3.3–6.4)	**HbA1c, %** ($n=11$)	**Insulin, U** (mean)	**Sulfonylurea + biguanides**	**Biguanides monotherapy**
Baseline	10.1 (9.3;12.4)	9 (7.3;10.8)	8.9 (8;10.8)	44.5	38.8% ($n=7$)	22.2% ($n=4$)
Endpoint	16.4 (13.2;20)	7.55 (6.7;8.7)	7.1 (7;7.9)	14	11.1% ($n=2$)	55.5% ($n=10$)
P	0.0003	0.046	0.05			

Of 3 patients were withdrawn from insulin. A negative correlation between duration of treatment and HbA1c at the endpoint was found ($r = -0.67$).
Conclusion
AD correction in men with MS and DMT2 improves glycaemic parameters and gives the opportunity to withdrawal from insulin treatment. Biguanides monotherapy is more preferable in DMT2 treatment in men with MS. Level of diabetes compensation depends on duration of testosterone treatment. Testosterone therapy can be included into diabetes treatment in men with metabolic syndrome (MS) and hypogonadism together as well as lifestyle modification and conventional therapy.

P433

The effect of telmisartan and irbesartan treatments on insulin resistance of patients with type 2 diabetes and hypertension

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Hyperinsulinemia that occurs due to insulin resistance play an important role on the development of type 2 diabetes and hypertension. It is shown that apart from their angiotensin II receptor blockage effect, telmisartan and irbesartan can activate intracellular hormone receptor PPARγ. Owing to this dual effect, telmisartan and irbesartan can improve cardiovascular risk factors related to insulin resistance. The aim of this study was to compare the effects of telmisartan and irbesartan on insulin resistance, inflammation, lipid and carbohydrate metabolism of patients with type 2 diabetes and hypertension. Of 53 patients with type 2 diabetes and hypertension were included in the study. Patients were separated into three groups. Only diet and exercise treatment were applied on the first group ($n=18$), in addition to this treatment, second group ($n=18$) was administered telmisartan and third group ($n=17$) was administered irbesartan and they were followed for 6 weeks. Significant decrease was detected in body mass index, average weight, waist/hip ratio, systolic/diastolic blood pressure and insulin resistance in each group at the end of treatment. Serum high sensitive C-reactive protein level significantly decreased in telmisartan and irbesartan group in comparison to pre-treatment period. When compared to the pre-treatment period, significant increase in high-density lipoprotein cholesterol (HDL-C) and significant decrease in fasting plasma glucose (FPG), postprandial plasma glucose (PPG) and glycosylated hemoglobin (A1c) were detected in telmisartan group. Insignificant decreases were detected in total cholesterol, triglyceride, low-density lipoprotein cholesterol, HDL-C, FPG, PPG and A1c in diet-exercise applied control group and in irbesartan group. In conclusion, it was found that there was no difference between the effects of telmisartan and irbesartan treatments on insulin resistance and that they were not statistically superior to the exercise and control group in terms of decreasing insulin resistance.

P434

Insulin treatment intensification in daily diabetes hospital

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Objectives
To verify the results of the insulin treatment intensification in the control of Diabetes Mellitus type 1 and 2 (DM1 and DM2).
Methodology
Of 100 diabetic patients (30% DM1 and 70%DM2) derived to Day Diabetes Hospital (DDH) by glycemic decompensation during year 2007 were studied, 45% women and 55% men (we excluded the debut). The average age of the patients was of 35.1 years in DM1 and 64.4 years in DM2. The Hba1c in the first visit and to the 3 months was analyzed, as well as weight, dose of insulin, glycemic average and glycemic variability. The treatment intensification consisted of modifying the insulin guidelines (from mixtures to bolus-basal in DM1; from basal to mixtures, from 2 to 3 mixtures and mixtures to bolus-basal in DM2) and re-education. In this period of time there were an average of 3.06 revisions by patient.
Results
In DM1: the HbA1c happened from 10.2 ± 2.3% ** to 8.3 ± 1.6% **; the initial average weight was of 66 kg and the end of 67.6 kg; the initial insulin metering was of 0.78 ± 0.3 UI/kg and the ending of 0.86 ± 0.3 UI/kg; glycemic average evolved from 213 to 167 mg/dl and the glycemic variability average happened from 86 to 80.3 mg/dl.
In DM2: the HbA1c happened from 9.9 ± 1.9% ** to 7.9 ± 1.3% **; the initial average weight was of 80.6 kg and the end of 81 kg; the initial insulin metering was of 0.78 ± 0.4 UI/kg and the ending of 1.37 ± 0.9 UI/kg; glycemic average evolved from 243.6 to 174.1 mg/dl and the glycemic variability average happened from 72.9 to 59.6 mg/dl.
Conclusions
- DDH has demonstrated to be a useful device to improve the metabolic control of the patients, improving the HbA1c, glycemic average and glycemic variability as much in DM1 as in DM2
- Reduction of HbA1c levels was greater in those patients whose initial HbA1c was higher.

P435

Welfare activity in day diabetes hospital in our area: our experience in 2007

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Objectives
The daily diabetes hospital (DDH) is a functional device that offers to diabetics a close and customized attention by specialized staff. The objective is to evaluate the welfare activity, the diagnose and educative program of the DDH in 2007.
Methodology
The following parameters have been analyzed: attended users, origin, main reason for consultation, previous Hba1c and to the 3 months, number revisions, income avoided, derivation place, telematic attendance, diabetologic education sessions.
Results
1. Patient attended: Of 698 new patients and 3646 revisions were seen (3.4 new/17.8 revisions per day) with a 48.2 ± 19.4 average age. The average HbA1c was: 8.8 ± 2.6%. In 2007 there was 93 diabetic debuts (36.5% DM1, 41.9% DM2 and 21.5 DM2 insulin dependents). By type of diabetes we classified in: DM2 49.7%, DM1 31.4%, and gestational DM (GD): 16.2%. Origin: Endocrinology (EEC): 34.5%, emergencies: 28.4%, gynecology: 12.3%, hospitalization: 8.2%, primary attention (PA): 7% and external consultations: 4.3%. Derivation place: Of 27.4% in revision, 47.3% derivatives to the EEC, 17.2% to PA, income 0.1%, exitus 0.5% (oncology). Avoided income: Of 66, 43 debuts, 9 hyperosmolar decompensations, 14 ketosis hyperglycemic. Precocious discharges of planta/observacion: 78. Attendance telematics: telephone attendance 24 h and emminens conecta plus program. Types of treatment: Basal 47%, three mixtures 22.4%, two mixtures: 4.6%, Basal + OAD: 5%, insulin pump: 4.2%, Exubera: 2.6%.
2. Diabetological education (1970sessions): Of 18% in group and 82% individual. Type of programs: basic education, DM1 debut, insulin pump, GD and diabetes and adolescence.
3. Tests you diagnose (1965): MAP: 88 accomplishment + interpretation (R + I); glucose sensors: 6 (R + I), EKG: 131, pulmonary function: 200; HbA1C, glycemia, ketonemia: 755; advanced test for neuropathy: 86; ECODOPPLER MMII: 6; RETINOGRAPHY 215 (R + I); Impedianciometria: 169.
Conclusions
1. DDH is a useful instrument for the integral attention of DM1 debut without criteria of hospitable entrance, diabetes and pregnancy, decompensation of diabetes that not require entrance, welfare continuity to the hospitable discharge and treatment with insulin pumps.
2. DDH is a more adapted tool to make the individual and group education.

Obesity and Metabolism

P436

Association between visceral fat and cardiovascular disease risk factors

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Objective
We designed this study to evaluate whether visceral fat area (VFA), and subcutaneous fat area (SFA) are associated with atherosclerotic parameters in obese and non-obese subjects.
Material and methods
Of 104 healthy volunteers were recruited for the study. Participitants were divided into two groups according to their body mass index (BMI). Group 1 has a BMI of <25 kg/m^2 ($n=31$) and the BMI of the group 2 was ≥ 25 kg/m^2 ($n=73$).
Results
The average age- and sex-specific distribution patterns of the groups were similar. While group 2 had impaired glucose tolerance (IGT) (21.9%), impaired fasting glucose (IFG) (30.1%), hypertension (HT) (13.7%) and metabolic syndrome (MS) (30.1%), group 1 didn't. There was a positive correlation between VFA and triglyceride (TG), waist circumference (WC) ($r=0.443$, $P=0.013$; $r=0.649$, $P<0.001$ respectively). In group 1, WC and TG had an statistically significant effect on the visceral fat alterations, respectively. In group 2, there was a correlation between VFA and age, fasting glucose, OGTT-1 h, OGTT-2 h, systolic BP, diastolic BP, TG, HOMA-IR, uric acid, WC and waist-to-hip ratio, ($r=0.363$, $P=0.002$; $r=0.44$, $P<0.001$; $r=0.529$, $P<0.001$; $r=0.315$, $P=0.007$; $r=0.374$, $P<0.001$; $r=0.324$ $P=0.005$; $r=0.316$ $P=0.006$; $r=0.55$ $P<0.001$; $r=0.431$, $P<0.001$; $r=0.791$, $P<0.001$; $r=0.439$, $P<0.001$ respectively). In group 2, WC, OGTT-1 h, uric acid and age had an statistically significant effect on visceral fat alterations. There was also a negative correlation with HDL-C in both group 1 and group 2. While there was no correlation between SFA and any of the parameters in group 1, in group 2 there was an statistically significant effect of WC on SFA alterations. VFA and SFA had an statistically significant concurrence with IFG, IGT, HT and MS.
Conclusion
We consider that the insulin resistance which is the result of increase in visceral fat tissue compatible with BMI is responsible for dysglisemia and hyperuricemia.

P437

Severe hypertriglyceridemia and triglyceride apheresis

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Introduction
Patients with extremely high triglyceride levels and associated lipemia are at high risk for acute pancreatitis. Hypertryiglycerideamia can be can be provoked when triglyceride levels exceed 1.000 mg/dl of acute pancreatitis.
Materials and method
In 7 patients with hyperlipidemic pancreatitis was evaluated. In addition to the standard therapy, they were treated with triglyceride apheresis. Acute pancreatitis was diagnosed based on the presence of clinical manifestations and consistent imaging finding on ultrasound and computed tomography in all patients. Plasma exchange was carried out using cascade filtration. Albumin, globulin, cholesterol, triglyceride, HDL, LDL, VLDL, lipase measured serially before and after each session of plasma exchange.
Results
The mean serum concentration of triglyceride after a single session of plasma exchange fell significantly 21 125 ± 318–318 ± 178 mg/dl.
Conclusion
In patients with triglyceride levels over 1000 mg/dl are at high risk for acute pancreatitis, plasma exchange can dramatically lower excessive triglyceride levels.

P438

Lower dietary acid load is related to a lower incidence of the metabolic syndrome

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Introduction
Bone metabolism and osteoporotic fractures have been related to inflammatory status and associated to metabolic disorders such as obesity, cardiovascular disease or metabolic syndrome (MS). Evidence suggests that a more acidic diet could be considered detrimental in terms of bone health. Thus, dietary acid load could be associated to the metabolic syndrome evolution.
Methods
A longitudinal study was conducted with 282 elderly subjects at high risk of cardiovascular disease randomly assigned to three interventional groups, a recommended low-fat diet (control diet group), a Mediterranean diet (Med-diet) supplemented with virgin olive oil or a Med-diet supplemented with mixed nuts. Main outcome was the evolution and reversion rate of MS defined by the updated National Cholesterol and Education Program Adult Treatment Panel III criteria after 1 year of nutritional intervention.
Results
From the 282 subjects, 168 were diagnosed of MS at the beginning of the study. The dietary potential renal acid load (PRAL) and the daily net endogenous acid production (NEAP) at baseline did not differ between the interventional groups. PRAL and NEAP were not related to MS at the beginning of the study. After nutritional intervention, subjects allocated to the Med-diet supplemented with mixed nuts but not with olive oil had a significant increase in PRAL and NEAP in relation to control group. The decrease of PRAL and NEAP during the intervention were good indicators of a lower incidence of the metabolic syndrome (OR (95%IC)) 0.96(0.93–0.99) and 0.93(0.87–0.99); $P<0.024$, respectively after adjusting by sex, age, intervention group and differences in total energy intake.

However reversion rate of MS did not differ in relation to dietary acid load changes during the intervention.
Conclusion
The present data suggest that a more acidic diet has a negative effect on metabolic syndrome evolution but not effect on the reversion of metabolic syndrome.

P439

The evaluation of metabolic effects following ghrelin and salbutamol administration

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Background
The activation of the GHS-R1a receptor by acylated ghrelin (AG) or by synthetic GH secretagogues (GHS), potently stimulates GH release and mediates other neuroendocrine/metabolic effects. Beta-adrenergic receptor agonists negatively influence GH secretion and other metabolic functions. The GH response to AG and GHS is refractory to the inhibitory effect of β2-adrenoceptors activation but no other report has evaluated the interactions between AG and salbutamol on metabolic parameters. Therefore, the present study intended to investigate insulin, glycemic and FFA values in response to a combined treatment with AG and Salb (AG/Salb).
Methods
Six healthy young male volunteers underwent the following testing sessions in random order: a) Salb (iv infusion of 0.06 μg/kg per min from 0 to 60 min), b) co-infusion of AG (1.0 μg/kg per min iv from −240 to 60 min) and Salb (0.06 μg/kg per min iv 0–60 min) and c) isotonic saline (iv infusion from 0 to 60 min). Insulin, glycemia and FFA levels were evaluated every 15 min.
Results
During the AG/Salb treatment, a significant elevation from 0 min insulin and FFA levels was observed at all time points ($P<0.05$). Incremental insulin AUC values were significantly higher in both AG/Salb and Salb treatments when compared to Saline ($P<0.001$). During AG/Salb, glycemic AUC values were increased when compared to those observed during treatments with Salb alone ($P=0.02$) or Saline ($P=0.053$). In addition, FFA AUC values were increased in AG/Salb when compared to Salb ($P=0.05$) and Saline treatments ($P=0.005$). Interestingly this effect was accentuated when incremental FFA AUC values were compared between the three treatments: AG/Salb when compared with Salb ($P=0.045$) and Saline ($P<0.001$).
Conclusion
Taken as a whole, the present study indicates that Salb and AG both exert effects on metabolism and furthermore, results strongly suggest that it could act in a synergetic manner to increase lipolysis.

P440

TNFR-1 knockout protects against diet induced obesity

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Abstract
Obesity results from an imbalance between caloric intake and energy expenditure. Specialized neurons of the hypothalamus coordinately control the integration between feeding and thermogenesis and a defective regulation of these parameters contribute to the progressive accumulation of body fat. Recent studies have revealed that at least part of hypothalamic dysfunction contributing to the development of obesity results from the activation of a local inflammatory response. TNF-α is one of the main players in this context. Inhibition of TNF-α signaling by both genetic and pharmacological approaches can, at least partially, rescue the obese phenotype. In the present study we evaluate the role played by hypothalamic TNFR-1 (TNF-α receptor type 1) in the transduction of the TNF-α signals that contribute to the development of obesity. For that, TNFR1 knockout mice (TNFR1KO) were fed a high-fat diet for eight weeks and a number of metabolic and molecular parameters were evaluated by respirometry, real-time PCR, immunoblot, immunohistochemistry and mitochondria respiration assay. The TNFR1KO were protected from diet-induced obesity, after 8-w high-fat diet consumption KO mice gained 15% less body mass than high-fat diet fed control mice. This was due to increased energy expenditure, as evaluated by *in vivo* respirometry and by isolated mitochondrial respiration assay, and by reduced cumulative food intake. At least part of the differences in food intake and energy expenditure were due to increased responsiveness to hypothalamic leptin and insulin. Thus, ip leptin injection led to an increase in 12 h suppression of feeding and increased STAT3 activation and SOCS-3 expression. Therefore, we conclude that TNFR1 is at least partially, implicated in the transduction of the TNF-α inflammatory signals that contributes to diet-induced hypothalamic dysfunction in obesity.

P441

Acute inflammatory biomarker modifications as a result of a single session of submaximal exercises in obese subjects

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Background
Classical risk factors are not capable to explain all the cardiovascular events and new markers are being evaluated to predict events.
Aims
We examined the effects of a single session of submaximal exercise (cardiopulmonary exercise cycle ergometer test) on atherogenic lipids in obese subjects focusing on inflammatory biomarker high-sensitivity C-reactive protein (hs-CRP), and oxidized low-density-lipoproteins (oxLDL) as a marker of oxidative stress.
Methods
The study group consisted 30 obese subjects (age: 48±3.8, f/m: 20/10, body mass index (kg/m^2) 31.55±2.3), participated in a bicycle ergometer 45 min submaximal exercise test. Blood samples were drawn immediately before, 30 minutes and 1 h after completion of the exercise. We determinate the follows lipids profiles: oxidized LDL (oxLDL), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). High-sensitivity C-reactive protein (hs-CRP) served as an inflammatory biomarker.
Results
Post-exercise levels of oxLDL (91.50±12.25 vs 96.83±11.20 UI/l $P=0.01$), and LDL-C (4.15±0.62 vs 4.19±0.63 mmol/l), compared to pre-exercise levels were increased, whereas 1 h after exercise, levels of oxLDL (91.50±12.25 vs 82.2069±11.29 UI/l, $P=0.001$), LDL-C (4.15±0.62 vs 3.86±0.62 mmol/l, $P=0.05$), and hs-CRP (2.42±0.98 vs 1.66±0.83 mg/ml, $P=0.001$) significantly decreased. There were no significant TG and HDL-C changes. Post-exercise levels of hs-CRP were negative correlated with body mass index ($r=-0.388$, $P=0.05$).
Conclusions
We found that a single session of submaximal exercise in obese subjects favorably modulates inflammatory mediators known to contribute in atherogenesis mechanisms.
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Adipokines' variations due to a lower caloric intake in endocrine obese patients associating the metabolic syndrome

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Adipokines and cytokines have modified values in the metabolic syndrome (MetSyn), without knowing if they are the cause or the effect of insulin resistance. Multiple studies evaluating adipokines and cytokines in MetSyn presented controversial results.
The aim of this study was to evaluate adiponectin, leptin, resistin, CRP and TNFα in obese subjects with endocrine diseases that associate insulin resistance before and after a low calorie diet.
Subjects and methods
This study enrolled 59 subjects that were clinically evaluated. Blood samples were taken in fasting conditions in order to determine the biochemical profile,

adipokine and cytokine levels, before and after a 3 months diet. Insulin resistance was appreciated using HOMA index. The MetSyn was diagnosed according to Diabetes International Federation definition.
Results were statistically analyzed using SPSS 15 program.
Results
Subjects were divided into 2 groups-37 subjects with MetSyn and 22 subjects-control group.
Compared mean values from both groups during the first visit, revealed statistically significant differences for both groups regarding triglycerides ($P<0.001$), HDL-cholesterol ($P<0.001$), diastolic blood pressure ($P=0.012$), leptin ($P=0.005$), insulin ($P=0.049$), glycaemia ($P=0.013$), HOMA ($P=0.033$) and TNF-α ($P=0.026$), differences kept for triglycerides ($P=0.03$) and glycaemia ($P=0.033$) after 3 month diet.
Adiponectin values were low, leptin, CRP and TNFα levels were high and resistin concentration was normal in both groups. Adipokines and cytokines values were lower after 3 months diet, but with higher adiponectin and resistin values in the MetSyn group. Weight loss determined a slightly improvement in insulin sensibility.
Conclusions
Obese subjects with different endocrine diseases that associate MetSyn have low levels of adiponectin, high leptin, CRP and TNF-α values and normal resistin concentrations without being influenced by the associated endocrinopathy. Weight loss determines an improvement of insulin sensibility. Adipokines' variations cannot be the cause of insulin sensibility reestablishing, being more the result of visceral adiposity reduction.

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Influence of metformin on carbohydrate metabolism in young women with metabolic syndrome

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Introduction
Metformin (MET) is mainly used in diabetes mellitus type 2 (DM2) therapy. Little is known about its effects in patients with metabolic syndrome (MS) and insulin resistance (IR) without DM2.
Aim
To asses the influence of MET on carbohydrate metabolism in young prediabetic women with MS.
Materials and methods
The study population: 30 women with MS aged 25–45 years before (M_1) and after (M_2) MET therapy (1700 mg per day for 4 months). Control group (C): 15 nonobese women. Anthropometric parameters, fasting glucose and insulin concentrations, insulin resistance (HOMA, FIRI) and sensitivity (QUICKI) indexes were estimated. Oral glucose tolerance test was performed. Total glucose (ΣG), total insulin (ΣI), ΣI/ΣG, area under curve (AUC) of glucose and insulin concentrations were calculated. All parameters were evaluated before and after MET.
Results
There were higher glucose and insulin at $0'$, $30'$, $60'$ and $120'$ OGTT, higher ΣG, ΣI, ΣI/ΣG, AUC of glucose and insulin in M_1 than in C group. After therapy ΣG and AUC of glucose did not change significantly, the other parameters decreased nonsignificantly and were still higher than in C. HOMA, FIRI were higher in M_1, showed nonsignificant downward tendency after therapy. QUICKI was lower in M_1, did not change after therapy. Body mass, BMI, waist and hip circumferences decreased after therapy.
Conclusion
Of 4-months MET therapy in women with MS decreased body mass, BMI and waist and hip circumferences. There was nonsignificant tendency to decrease glucose and insulin concentrations and IR indexes values after MET therapy.

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Letrozole normalizes serum testosterone and reduces body weight in morbidly obese 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 in adipocytes. The clinical impact of this sex hormone imbalance is not known.
Aim
To evaluate the clinical effects of aromatase inhibition in obesity-related hypogonadotropic hypogonadism.
Methods
Double-blind, placebo-controlled trial for 6 months in severely obese men (BMI $>$ 35 kg/m^2) with obesity-related hypogonadism (serum total testosterone $<$ 10 nmol/l). Predefined drug regimen: Starting dose 1 tablet/week, subsequent dose escalation up to a maximum of 7 tablets/week or until a serum total testosterone of 20 nmol/l.
Results
So far, 16 patients have completed the study, 8 on letrozole (mean dose: 2 tablets/week) and eight receiving placebo (mean dose: 7 tablets/week). Both groups were well matched for all study parameters. Age (mean $\pm$ S.E.M.) 40.6 $\pm$ 1.6 years, BMI 43.5 $\pm$ 1.3 kg/m^2, serum LH 3.6 $\pm$ 0.4 U/l, total testosterone 7.2 $\pm$ 0.4 nmol/l, free testosterone 214.5 $\pm$ 14 pmol/l, total estradiol 127.5 $\pm$ 11.7 pmol/l. Six months of Letrozole treatment decreased serum estradiol by 53.3 $\pm$ 20.5 pmol/l ($P<0.05$) and increased serum LH by 6.4 $\pm$ 1.6 U/l ($P<0.005$). Total testosterone rose by 12.8 $\pm$ 1.2 nmol/l ($P<0.0001$), and free testosterone by 412 $\pm$ 52 pmol/l ($P<0.0001$), whereas placebo treatment had no statistically significant effects. Placebo-subtracted changes in body weight and abdominal circumference were -7 kg ($P<0.02$) and -5.2 cm ($P<0.005$), respectively. Glucose metabolism, lipid profiles, bone density, and physical exercise capacity did not change. Psychological testing did not reveal any changes during treatment.
Conclusion
Short-term, low dose aromatase inhibition in obesity-related hypogonadotropic hypogonadism normalizes serum testosterone and has beneficial effects on body composition.

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Effect of metformine treatment on serum paraoxonase and oxidative status in obese women

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Objective
To evaluate the effects of metformine newly using in clinical practice to ameliorate insulin resistance on paraoxonase activity and oxidative stress in obese insulin resistant women.
Materials and methods
Sixty-seven obese (BMI $\geq$ 30 kg/m^2) women were enrolled into this study. Serum fasting (F.Glc) and postprandial glucose (P.Glc), insulin, uric acid (UA), paraoxonase (PON1), Arylesterase (AET), malondialdehyde (MDA), cupper-MDA (Cu-MDA) levels and lipid fractions were measured at the commencement and ending of the study. Homeostasis model assessment (HOMA-R) was used to estimate insulin resistance. HOMA-R $\geq$ 2.7 levels were accepted as positive insulin resistance. According to this proposal, insulin resistant (IR+) 32 women were defined as Group I and no insulin resistant (IR$-$) 35 as Group II. Cases in Group I were managed by diet + exercise + metformine (1700 mg/d), cases in Group II were only treated by diet + exercise for 6-month interval. Intra and inter alterations of all parameters were statistically calculated.
Results
Basal PON1, MDA, Cu-MDA and HOMA-R values were considerably higher in Group II than those in Group II. Reduced PON1/AET ratio, HOMA-R, HDL and PON1 values were observed in Group I. The increases in AET/HDL and Cu-MDA were significant. While increases in LDL, Cu-MDA, AET/HDL and HOMA-R values were observed, HDL level reduced in Group II. Decreases in HOMA-R values were further in Group I than in Group II. But increases in Cu-MDA levels were significantly higher in Group II compared to those in Group I.
Conclusions
We thought that metformine treatment with intensive life-style changing is appropriate management in obese, insulin resistant women who have increased propensity for the development of Type 2 DM.

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Serum visfatin concentration in obesity and impaired glucose tolerance – relationship with insulin resistance, blood pressure and proinflammatory factors

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Visfatin, a protein secreted by the adipose tissue, might regulate insulin action. The aim of the present study was to assess serum visfatin concentration in obese women with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) and to estimate the relationships between serum visfatin concentration and insulin sensitivity, blood pressure, proinflammatory and proatherogenic factors.
The study group consisted of 134 women: 30 overweight or obese women with IGT (obese-IGT), 55 overweight or obese with NGT and 49 lean healthy controls. The oral glucose tolerance test was performed and the serum concentrations of visfatin, high-sensitive C-reactive protein (hsCRP) and soluble E-selectin (sE-selectin) were measured in all the subjects. Insulin sensitivity was estimated with euglycemic hyperinsulinemic clamp.
We observed the significantly lower insulin sensitivity and higher serum visfatin concentration in both groups of obese women in comparison to the control group (insulin sensitivity, obese-IGT, $P<0.0001$, obese-NGT, $P=0.0027$; serum visfatin, obese-IGT, $P<0.0001$ obese-NGT, $P=0.016$) and in obese-IGT in comparison to obese-NGT group (insulin sensitivity, $P<0.0001$; serum visfatin, $P=0.0055$). In the whole studied group, serum visfatin concentration was negatively related to insulin sensitivity ($r=-0.29$, $P=0.001$) and HDL-cholesterol ($r=-0.32$, $P=0.00015$) and positively related to systolic and diastolic blood pressure ($r=0.33$, $P<0.0001$ and $r=0.32$, $P=0.00016$, respectively), fasting and postload glucose ($r=0.25$, $p=0.003$ and $r=0.31$, $P=0.00025$, respectively), fasting insulin ($r=0.20$, $P=0.028$), serum triglycerides ($r=0.27$, $P=0.001$), hsCRP ($r=0.25$, $P=0.005$) and sE-selectin ($r=0.33$, $P=0.00013$). In multiple regression analysis, the relationships between serum visfatin and systolic and diastolic blood pressure, and sE-selectin were independent of BMI and insulin sensitivity.
Increased serum visfatin concentration in obesity and IGT is associated with insulin resistance, blood pressure and proinflammatory factors and thus could be linked to an increased risk of type 2 diabetes and cardiovascular disease.

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Intracerebroventricular leptin increase AKT phosphorylation stimulated by insulin in skeletal muscle: the role of adrenergic signal and JAK2 activation

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Leptin is an adipocyte-derived hormone that acts directly in the brain reducing food intake, increasing glucose uptake and modulating the metabolism in adipose tissue. Nevertheless, the central mechanisms related to improve glucose uptake, induced by leptin intracerebroventricular (icv), and the proteins activated in the skeletal muscle are incompletely understood. The aim of this study was to investigate the mechanism by which ICV leptin increase glucose uptake and improve glucose homeostasis. Rats were divided into three experimental groups: i) Control (Saline-ICV); Leptin (Leptin-ICV) and LY (Ly294002 + Leptin-ICV). In all these groups were analyzed glucose tolerance (GTT), AKT, JAK2 activation, stimulated by insulin, in the muscle and hypothalamic AKT, JAK2 and STAT3 activation stimulated by leptin. ICV leptin increased the phosphorylation of hypothalamic JAK2 (80%), STAT3 (160%) and AKT (300%) when compared to control group. Previous ICV LY administration reduced AKT phosphorylation, induced by ICV leptin, but didn't present effect on JAK2 and STAT3 phosphorylation. Besides, ICV leptin improve the clearance of glucose in GTT (50%). Previous administration of propranolol (10 mg/kg bw-IP), but not ICV Ly294002 (1 nmol-ICV), reduced the effect of leptin on GTT. In the *soleus* muscle the AKT phosphorylation, stimulated by insulin, was higher in leptin group (400%) than control group. The previous administration of propranolol (ip) reduced (40%) the effect of ICV leptin on AKT phosphorylation, stimulated by insulin, in skeletal muscle. ICV Ly294002 didn't present effect on AKT phosphorylation, in skeletal muscle. JAK2 phosphorylation was higher in leptin (ICV) group than control group (ICV saline). Our results suggesting that adrenergic signal are activated by central leptin stimulating AKT activation in the skeletal muscle. In the skeletal muscle JAK2 activation is likely a responsible mechanism by AKT activation.

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Evidence of increased cardiovascular risk in patients with non-secreting unilateral adrenocortical adenomas

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Background
Incidentally discovered adrenal adenomas (AA) are associated with increased prevalence of hypertension, obesity, and impaired glucose tolerance, all established risk factors for cardiovascular (CV) morbidity. However, most studies were performed in patients with AA and autonomous cortsisol and/or aldosterone secretion, whereas the presence of cardiovascular risk in patients with non-secreting AA has not been looked into detail.
Methods
Cardiovascular risk factors were studied in 18 patients with non-secreting unilateral adrenal adenoma (AA) (52.5 ± 2.1 years, BMI 27.8 ± 0.8 kg/m^2) (mean $\pm$ s.e.m.) and in 22 healthy age and BMI matched subjects that served as control group (C) (51.5 ± 1.5 years, BMI 26.8 ± 0.9 kg/m^2). Patients with AA had complete inhibition of serum cortisol and aldosterone levels after a low dose DXM suppression test and intravenous NaCl 0.9% infusion test, respectively. Fasting measurement of serum triglycerides, total cholesterol, high and low density lipoproteins, fibrinogen, homocysteine, Lipoprotein(a) (Lp(a)), Apolipoprotein B (apo-B), Apolipoprotein A-I (apo-A) was performed. All patients also underwent a 2 h oral glucose tolerance test (OGTT) and insulin sensitivity was assessed by calculating HOMA((fasting glucose (mmol/l) *fasting insulin (μIU/ml))/22.5) and Matsuda {10 000/square root((fasting glucose*fasting insulin)*(mean OGTT glucose*mean OGTT insulin)} indices. Carotid artery intima-media-thickness (IMT) and brachial artery flow-mediated dilation (FMD) were measured using high resolution linear array ultrasound.
Results
Patients with AA had increased homocysteine (13.3 ± 0.6 vs 10.3 ± 0.4 mg/dl, $P<0.01$), Lp(A) (21.2 ± 7 vs 5.6 ± 0.4 mg/dl, $P<0.05$), fibrinogen (391.7 ± 20.4 vs 310.3 ± 21.7 mg/dl, $P<0.05$) and apo-B/apo-A ratio (0.76 ± 0.04 vs 0.55 ± 0.03, $P<0.01$) compared to C. Patients with AA had higher IMT values than C (0.93 ± 0.05 mm vs 0.78 ± 0.02, $P=0.018$) and lower FMD levels (3.6 ± 0.3 vs 5.9 ± 0.4, $P<0,01$). Compared to C HOMA and Matsuda index were significantly higher (2.67 ± 0.19 vs 1.72 ± 0.14 mmol/mU per l, $P<0.01$) and lower (3.5 ± 0.3 vs 6.6 ± 0.4, $P<0.01$) in AA.
Conclusion
Patients with non secreting AA exhibit increased CV risk factors. Studies examining cardiovascular morbidity and mortality in such patients are further required.

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Metabolic syndrome highly correlates to non toxic multinodular goiter progression: iperinsulinism a way to growth

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Background
Insulin-resistance phenomenon is a key factor in the pathogenesis of Metabolic Syndrome (MS). Several reports focus on the identification of a role of Insulin to trigger thyrocytes proliferation *in vitro*. Thyroid IRS-1 mRNA expression increased progressively during goitrogenesis *in vivo*. In order to individuate the impact of clinical and biochemical markers of MS on the growth-score of non toxic multinodular goiter (MNG) we evaluated a population of 60 subjects with MNG at onset.
Methods
Of 60 patients (mean age: 50 ± 12 years; F/M: 38/22) with MNG at onset were divided into two groups: Group A affected by MS and MNG; Group B affected by endocrine disease alone. To be included subjects had to have: normal thyroid function, negative titers of antithyroid antibodies and no past history of having received any thyroid medications. All procedures were applied in agreement with the ethical guidelines of our institution. In both groups were evaluated BMI, waist circumference (WC), lipids, fasting insulin and glycemia to assess HOMA-ir and by thyroid ultrasound to measure thyroid volume, number and size of nodules.
Results
HOMA-ir in group A vs B was significantly different (4 ± 2.5 vs 1.32 ± 0.5; $P=0.007$). Only in group A the Spearman correlation test demonstrates a positive relationship between: WC and the diameter of the biggest nodule (Rho: 0.45; $P=0.05$); HOMA-ir Index and number of nodules (Rho: 0.48; $P=0.01$) and with

thyroid volume (Rho: 0.473; $P=0.01$); BMI and thyroid volume (Rho: 0.388, $P=0.048$).
Conclusion
These data support the role of insulin-resistance in the pathogenesis/progression of MNG. In this light elevated circulating levels of Insulin cause increased thyroid proliferation. Thus established farmacological approaches in metabolic syndrome, such as insulin-sensitizer, may be useful to prevent the progression of goitrogenesis and to control thyroid nodular growth.

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IGF-1 gene polymorphism in obese patients with insulin resistance

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Objective
Insulin like growth factors (IGFs) are important regulators of pancreatic β cell devolopment, growth and maintanence. Mutations in the IGF genes have been found to be associated with type 2 diabetes, myocardial infarction, birth weight and obesity. These associations could result from changes in insulin secretion. We aimed to investigate IGF- 1 gene polymorphism in obese patients with insulin resistance.
Methods
We included 100 obese patients with insulin resistance who applied to Endocrinology and Metabolism outpatient clinic and 30 healthy subjects to study. At baseline examinations, antropometric measurements were done. Genomic DNA from the patients and controls were prepared Investigated genomic areas were studied using specific primers by PCR methods. Amplified fragments were separated agarose gel electrophoresis and were identified using the u.v. gel documentation system.
Results
Thyroid function tests and serum IGFBP3 levels were similar between patients and controls whereas IGF, GH and cortisol levels were significantly lower in -obese insulin resistant patients. We categorized the IGF-1 $(CA)_{19}$ polimorphism area into 3 group as lower than192-bp(group 1), 192-194 bp (group 2), and higher than 194-bp (group 3). Group 3 was more frequent in both obese and control groups, When all parameters of group 3 were compared between obese (n:71) and control groups (n:28); weight, BMI, waist and hip circumference, fat distrubution, FBG, TG, HDL, LDL, AST, ALT, uric acid, insulin levels were significantly different between two groups. IGF-1 levels were also significantly lower in obese group (138.51 ± 49.3) in than controls (218.14 ± 69.15).
Conclusions
IGF-1 levels were significantly lower in obese than normal people, The most frequent IGF-1 gen polymorphism allel is $>$ 194 bp in both obese insulin resistant patients and controls, IGF-1 levels and the other biochemical and hormonal parameters were similar in different genotype groups. The cause of lower IGF-1 levels in obese patients might be different from IGF-1 gene polymorphism and it may be insulin resistance.

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Familial obesity and the role of nutrition

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Goal
To study the relation between the obesity of children and their parents, and energy intake.
Materials and methods
Of 236 households containing 828 population (356 children age 5–25 years, and the rest their parents) were selected from the subjects of 'Tehran Lipid and Glucose study' (2006–2008) for this study. Food intake of the subjects was studied using FFQ. In this study, energy intake $\geq$75 percentile was regarded as 'high energy intake' among the subjects considering their age and sex. Cut off point for defining overweight among subject age 5–19 years was considered as BMI$\geq$95th percentile of reference diagram for Indian children. The cut off was also considered BMI$\geq$30 for over 20 years old subjects.
Finding
Prevalence of overweight among the subjects from the household with both parents obese, one of the parents obese, and none of them obese were 44.2, 28.8 and 11.6% respectively. There were higher of OR for being over weight among the children with both parents obese (OR=5.1, 1.5–7.7) and even with one parent obese (OR=4.7, 1.6–13.4). There was a significant direct relation between BMI of fathers and mothers separately with BMI of their children, ($r=0.43$, $P<0.001$ and $r=0.51$, $P<0.001$ respectively). The relation between ratio of energy intake to BMR of children with their BMI ($r=0.37$, $P<0.05$) and also 'the ratio of energy intake to BMR' of mothers with BMI of their children ($r=0.3$, $P<0.05$) were significant too.
Conclusion
The findings of this study shows the relation of genetic factors and nutritional habits of parents to their children's obesity.

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Adiponectin and vascular properties in obese patients: is it a novel biomarker of early atherosclerosis?

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Objective
Adiponectin is an adipocyte-derived collagen-like protein, highly specific to adipose tissue and may represent an important link between obesity and atherosclerosis. The present study was designed to investigate a possible association between serum adiponectin levels and early vascular changes in obese patients as determined by intima media thickness (IMT) and arterial pulse-wave contour analysis.
Design
Obese subjects ($n=47$) were evaluated for arterial structure and function, metabolic parameters and serum adiponectin levels.
Measurements
IMT was measured by ultrasound. Arterial elasticity was evaluated using pulse wave contour analysis. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR).
Results
Adiponectin was significantly, inversely associated with mean IMT ($r=-0.369$, $P=0.011$) and significantly positively associated with large artery elasticity index (LAEI) ($r=0.467$, $P=0.001$) as well as small artery elasticity index (SAEI) ($r=0.462$, $P=0.001$). In separate multivariate models, adiponectin remained significantly associated with mean IMT, LAEI, SAEI even after adjustment for cardiovascular confounders. Among metabolic parameters, adiponectin was significantly, positively associated with HDL cholesterol and inversely associated with triglycerides. Adiponectin was significantly, inversely associated with fasting insulin and HOMA-IR. Additionally, a marginally inverse association between adiponectin and ALT was observed.
Conclusions
In the present study, serum adiponectin levels were significantly associated with indices of subclinical atherosclerosis such as IMT and arterial compliance in obese patients. This association was independent of traditional cardiovascular risk factors.

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A comparison of serum leptin levels and metabolic parameters included serum lipoproteins and glucose homeostasis between national wrestlers and healthy males

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Background and aim
Wrestling success depends on power, power endurance, and maximum strength, muscular endurance of short duration, flexibility as well as technique and tactical skills. Weight control and weight loss are also very important issues in the view of wrestling success. In this study, it was aimed to investigate the anthropometric features, serum lipoproteins, glucose homeostasis and leptin levels in Turkish National Senior Wrestlers and healthy sedentary males.
Material and method
Totally, 45 National senior wrestlers in Olympic Camp before Beijing 2008, and sedentary and healthy-volunteers group from university students were selected for comparison. Anthropometric and physiological features including age, body weight, body height and body mass index (BMI) as well as metabolic features including fasting glucose, insulin, leptin and lipoprotein levels with HOMA values were measured and compared with the results of sedentary group.

Results
Wrestlers had significantly higher insulin and triglycerides levels with HOMA values in fasting than healthy males. However, the wrestlers' HDL-cholesterol was found high while it was low in LDL cholesterol. There was only positive correlation between leptin and fasting insulin level.
Conclusion
This study shown that wrestlers have decreased insulin sensitivity including higher fasting insulin and HOMA values with higher triglycerides levels than sedentary males.

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Impact of protein content of low-carbohydrate/high-fat diets upon ketosis, energy expenditure and glucose utilization in rats

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Maintenance of ketosis and increases in resting energy expenditure (EE) are two proposed mechanisms for weight loss observed in humans performing low-carbohydrate/high-fat (LC-HF) diets like the Atkin's diet. To explore these potential mechanisms, we pair-fed isocaloric amounts of regular chow (CH) or two LC-HF diets (carbohydrates ~1%ME) to male Wistar rats for 4 weeks. LC-HF-1 (fat ~66%ME) was matched in protein content to CH, whereas LC-HF-2 was even higher in fat (~94%ME). We analyzed bodyweight (BW) gain, EE, respiratory quotient (RQ), serum ketone-bodies (β-hydroxybutyrat, HBA), free fatty acids (FFA), urea, glucose and insulin. In addition, daily nitrogen balance and urinary energy contents were measured.
BW gain was slightly reduced in the LC-HF-1 group, and clearly reduced in LC-HF-2 ($P<0.01$). Animals on LC-HF-1 accumulated most nitrogen per day; LC-HF-2 the least. Furthermore, daily nitrogen balance was barely positive and serum urea considerably reduced in LC-HF-2. Remaining energy in urine was lower in LC-HF-2 when compared to CH (0.3 ± 0.12 vs 0.93 ± 0.23 KJ/g; $P<0.01$); overall 24-h loss was below 5 KJ in all groups. RQ was ~1 in CH and lower in LC-HF-1 (0.81 ± 0.01) and LC-HF-2 (0.76 ± 0.01, $P<0.001$). Of d24-h EE was significantly lower in both LC-HF groups. Only in LC-HF-2 concentrations of FFA (two-fold) and HBA (four-fold) were significantly increased.
In conclusion, LC-HF-2 diet induced ketosis, but did not increase EE or energy loss through urine. The impaired BW gain in this group was probably caused by the relative protein shortage and resulting inability to acquire muscle mass. Macronutrient composition of LC-HF-1 better reflects human LC-HF diets. Indeed, slight reductions in BW gain were seen. However, LC-HF1 neither induced ketosis nor increased EE. RQ data and unchanged glucose levels suggest presence of gluconeogenesis in this group. Our data in rats do not support the proposed mechanisms for weight loss in humans performing LC-HF diets.

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Thyroid function tests, measures of adiposity and metabolic syndrome in apparently healthy euthyroid individuals

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Objective
Thyroid function tests (TFTs) have been associated with obesity; however associations with the type of adiposity have not been examined. Ultrasound (U/S) was used to assess regional adiposity in euthyroid individuals. Associations of thyroid function with obesity parameters and presence of the metabolic syndrome (MS) were investigated.
Methods
Of 302 apparently healthy slightly overweight individuals (age 42.9 ± 8.8, BMI 19.0–43.3, median 26.2 kg/m^2, 180 women) were examined for indices of the metabolic syndrome (MS). Abdominal subcutaneous (SF) and pre-peritoneal (PF) fat layer was estimated by US. BMI, waist and hip circumference were recorded; TSH, T_3, thyroid autoantibodies, insulin, glucose and lipid levels were measured.
Results
T_3 levels were positively associated with PF ($r=0.245$, $P=0.004$), SF ($r=0.189$, $P=0.019$), BMI ($r=0.257$, $P=0.0004$), waist perimeter ($r=0.324$, $P<0.0001$) and waist-to-hip-ratio ($r=0.363$, $P<0.0001$). TSH levels correlated with SF ($r=0.146$, $P=0.039$). Higher TSH levels were associated with higher total cholesterol and LDL levels ($P=0.008$). HOMA-insulin-resistance-index and BMI were higher among individuals with TSH > 2.5 mU/l compared to those with TSH ≤ 2.5 mU/l, $P=0.048$). TFTs did not differ between those with MS and those without.
Conclusions
Increased subcutaneous and pre-peritoneal fat accumulation is associated with higher T_3 levels in euthyroid slightly overweight individuals; this may represent a compensatory mechanism for the increased abdominal fat accumulation, as has been described in morbid obesity. The positive association of higher levels of TSH with SF may reflect associations with a mild hypothyroid state or possibly resistance to thyroid hormones as has previously been suggested.

P456

25-Hydroxyvitamin D is associated with insulin resistance, obesity, and serum lipids in polycystic ovary syndrome

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Introduction
Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting about 5-10% of women. PCOS is characterized by hyperandrogenism, ovulatory disturbances, and polycystic ovaries. Many women with PCOS present with insulin resistance and central obesity and are at an increased risk for developing diabetes and cardiovascular disorders. Vitamin D deficiency is an important pathogenetic factor in the development of type II diabetes. The aim of the study was to investigate the relationship of 25-hydroxyitamin D (25(OH)D) and metabolic and endocrine parameters in women with PCOS.
Methods
Of 25(OH)D levels were measured by means of ELISA in 40 women affected by PCOS. Metabolic, endocrine, and anthropometric measurements and oral glucose tolerance tests (fasting glucose, 1, and 2 h) were performed. Insulin resistance was determined using HOMA-index (homeostasis model assessment).
Results
Of 25(OH)D levels were negatively correlated with weight ($r=-0.396$), BMI ($r=-0.447$), Waist-to-hip-ratio (WHR) ($r=-0.750$), 1 h-glucose ($r=-0.439$), HOMA-index ($r=-0.357$), and QChol/HDL ($r=-0.369$) and positively correlated with HDL ($r=0.582$) ($P<0.05$ for all). PCOS women with 25(OH)D insufficiency (<25 ng/ml) had higher levels of weight, WHR, BMI, fasting glucose, 1 h-glucose, fasting insulin, HOMA-index, QChol/HDL, and LDL and lower levels of HDL when compared to PCOS women with a sufficient 25(OH)D status of at least 25 ng/ml ($P<0.05$ for all). Lean PCOS women presented with significantly higher 25(OH)D levels than overweight/obese patients ($P=0.01$). No significant correlation was found between 25(OH)D and endocrine parameters such as hyperandrogenism.
Conclusion
Our results suggest that low 25(OH)D levels might be related to metabolic abnormalities that are frequently observed in women with PCOS. No association was found between 25(OH)D status and hyperandrogenism in PCOS women.

P457

Chronic renal disease, hyperfiltration, metabolic syndrome and hypogonadism: is there a link?

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Introduction
Hyperfiltration (HF) is an early marker of chronic renal disease (CRD), which is prevalent in 10–15% of MS patients.. Taking into account the benefit of testosterone replacement towards MS components in men with MS and hypogonadism, we can expect the same benefit on HF.

Objective
To study the role of hypogonadism correction in patients with MS and hyperfiltration.
Methods
Of 71 men aged 35–69 with MS (IDF criteria) and hypogonadism (total testosterone (TT) < 11 nmol/l) were divided into two groups. Of 35 men were treated with testosterone undecanoate (Nebido, Schering) (group 1) and 36 men were the controls (group 2). Glomerular filtration rate (GFR) was estimated at baseline and after 6 months of treatment. GFR was counted by Cockraft–Gault formula with adjustment for standard body surface area (GFRst) and compared to population normal ranges (Nijmegen Biomedical Study 2007), resulting in HF (GFRst > 110 ml/min per 1.73 m^2) or normal filtration (60 < GFRst < 110 ml/min per 1.73 m^2).
Results
Both groups did not differ ($P > 0.1$) by the main prognostic CRD factors such as age (52.5 and 52.8 years), weight (108 and 106 kg), waist circumference (116 and 113 cm), SBP (139 and 136 mmHg), triglycerides (2.3 and 2.2 mmol/l), LDL (3.8 and 3.6 mmol/l), fasting plasma glucose (6.5 and 6.3 mmol/l), GFR (101 and 103 ml/min per 1.73 m^2), as well as TT level (7.9 and 9.4 nmol/l), incidence of HF (31 and 30%) and frequency of ACEI intake (46 and 31%). After 6 months of treatment incidence of HF was lowered by 23% in group 1, and increased to 40% in group 2 ($P = 0.046$).

	Baseline		P	After 6 months		P
	GFR >110 (%)	60<GFR <110 (%)	0.93	GFR >110 (%)	60<GFR <110 (%)	0.046
Group1	31	69		24	76	
Group 2	30	70		52	48	

Conclusion
Our results show the benefit of hypogonadism correction in men with MS towards HF lowering the risk of CRD development.

P458

Changes in serum levels of fetal antigen 1 (FA1/Pref-1/Dlk1) in extreme nutritional states

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Background
Also known as preadipocyte factor 1 (pref-1) or delta-like 1 (Dlk1), fetal antigen 1 (FA1) was originally isolated from amniotic fluid and it is involved in tissue homeostatis during development and tissue regeneration in the post-natal organisms. FA1 may also plays a role in energy metabolism since mice deficient in FA1 are obese and mice overexpressing FA1 exhibit a lipodystrophy phenotype. In our study we determined FA1 serum levels in patients in two extreme nutritional states, anorexia nervosa (AN) and severe obesity.
Methods
We studied 15 women with AN, median body mass index (BMI) 15.0 ± 1.5 kg/m^2, before and after a minor weight change to 15.7 ± 1.3 kg/m^2, and 25 obese patients, median BMI 48.00 ± 7.8 kg/m^2, before bariatric surgery and at defined points of excess weight loss (EWL) of 25, 50 and 75%. Metabolic parameters and FA1 were measured.
Results
FA1 levels were significantly inversely correlated with BMI before and after body weight change in AN patients. In the obese subjects, FA1 levels decreased from 24.2 ± 11.9 to 15.3 ± 5.0 ng/ml after 25% EWL, however, upon the following further weight loss, no significant changes in FA1 levels could be detected. Insulin levels were significantly correlated with FA1 in AN patients before weight change but otherwise not.
Conclusion
In nutritive steady state conditions, FA1 levels were similar in morbid obese and in emaciated AN patietens, however the initial fall in serum FA1 level after restrictive bariatric surgery induced weight loss indicate that FA1 directly or indirectly may be implicated in metabolic adaptation and/or stress following excessive weight loss.

P459

Influence of metformin on selected lipids and apolipoproteins concentrations in young women with metabolic syndrome

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Introduction
Metabolic syndrome (MS) is connected to atherogenic dyslipidemia. Data concerning influence of metformin (MET) therapy on lipid profile in nondiabetic women with MS remains unclear.
Aim
To asses the influence of MET on selected lipid, apolipoproteins AI, B concentrations and atherogenic indexes values in young women with MS.
Materials and methods
The study population: 30 women with MS aged 25–45 years before (M_1) and after (M_2) MET therapy (1700 mg per day for 4 months). Control group (C): 15 nonobese women. Total cholesterol (TC), LDL-C, HDL-C, HDL_2-C, triglycerides (TG), apolipoprotein AI (ApoAI) and B (ApoB), fasting glucose and insulin concentrations were estimated. Atherogenic index of plasma (AIP), Castelli index, LDL-C/HDL-C, TC/HDL-C, apoB/apoAI, HOMA, FIRI, QUICKI indexes were estimated. All parameters were evaluated before and after MET.
Results
In M_1 group we found higher TC, LDL-C, TG, ApoB and lower HDL-C, HDL_2-C, ApoAI concentrations than in nonobese women. HDL-C concentration increased and TG decreased significantly after therapy. Atherogenic indexes values were higher in M_1 than in C, significantly decreased after therapy, except for ApoB/AI, and were still higher than in C. Fasting glucose and insulin concentrations, HOMA, FIRI were the highest and QUICKI was the lowest in M_1, did not change after MET.
Coclusions
MET positively modifies lipid profile and atherogenic indexes values in women with MS.

P460

Correlation of leptin, total body fat mass and body fat distribution in overweight postmenopausal women

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We have investigated the leptin production by total and regional fat mass in 32 overweight postmenopausal women as well as the relation between serum leptin levels, total body fat mass and body fat distribution variables. Body mass index (BMI) was calculated in kg/m^2. Serum concentrations of leptin were evaluated by RIA. Body composition – trunk/total, arms + legs/total + legs/trunk, android/ gynoid ratios were assessed by dual-energy X-ray absorptiometry (DEXA). The relationship between leptin concentrations and body fat distribution variables were investigated by Pearson correlation test. Serum leptin levels at the baseline positively correlated with BMI ($r = 0.701$, $P < 0.0001$), total body fat mass ($r = 0.683$, $P < 0.0001$). No significant correlation was observed between leptin and trunk/total, arms + legs/total + legs/trunk. According to these results we have concluded that total body fat mass have impact on leptin concentrations while the body fat distribution does not affect leptin levels in overweight postmenopausal women.

P461

Diabetic patients with abnormal LFTS

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Objective
Patients with diabetes mellitus often have abnormal liver function tests without any symptoms. Our aim in this study was to evaluate the frequency and cause of abnormal LFTS in diabetic patients.
Methods
150 patients were enrolled from diabetic clinic who had been diagnosed with diabetes (creatinine,fasting glucose,random glucose, GTT were recorded). The following demographic data were recorded from each patients: age,sex, duration of diabetes, BMI, waist circumference, lipid profile, HbA1c, BP (blood pressure). All patients had liver function tests performed and of these patients with abnormal LFTS, underwent full hepatitis screening including ultrasound of hepatobiliary system. If the cause for the abnormal LFTS, was still elusive the patients underwent a liver biopsy and was seen by hepatologist.

Results
54% of patients had abnormal LFTS,of which 61% had hepatitic picture and 28% a mixed hepatitic and cholestatic picture. The remainder 11% had cholestatic picture. The majority of patients with abnormal LFTS had non-alcoholic fatty liver disease (NAFLD) 94%, and 4% had non -alcoholic steatohepatitis (NASH). 0.7% had autoimmune liver disease, 0.2% had viral hepatitis,and 0.1% haemachromatosis and 1% cholelithiasis. In the cohort of patients with NAFLD or NASH there was poor glycaemic control, obesity,abnormal LFTS and hypertension.
Conclusion
Abnormal LFTS in diabetic patients dictate further investigations with treatment of underlying cause.Patients with NAFLD or NASH often have more than one components of the metabolic syndrome and the later patients are usually poorly controlled.

P462

Impact of growth hormone receptor blockade on substrate metabolism during short-term fasting in healthy subjects

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Context and objective
During fasting growth hormone (GH) promotes lipolysis, attenuates glucose oxidation and preserves protein. Previous studies have primarily been conducted in GH deficient patients or during somatostatin-suppression of GH secretion. We aimed to study the impact of the fasting associated increase in GH without concomitant changes in other hormones, by the means of GH receptor (GHR) blockade.
Design
Ten healthy young men participated in a randomized, single-blinded, placebo-controlled, cross-over study with administration of 1) GHR blockade (pegvisomant, 15 mg s.c.) and 2) placebo (Saline, 1 ml) at 20.00 h at the beginning of a 36 h fast. All subjects were studied in the basal state (36–40 h of fasting) and at the end of a hyperinsulinemic euglycemic clamp (2½ h). Main outcome measures: palmitate flux, free fatty acids (FFA), ketone bodies, energy expenditure and oxidation rates, forearm uptake of FFA, insulin stimulated glucose-uptake, IGF-I (total and bioactive).
Results
GHR blockade significantly suppressed the levels and turnover of circulating FFA, ketogenesis, skeletal muscle uptake of FFA as well as lipid oxidation rate. This occurred in the presence of unaltered glucose and protein metabolism and without a detectable decline in IGF-I levels.
Conclusion
1) Stimulation of lipolysis is the primary metabolic effect of GH during fasting. 2) Short-term fasting is a condition where FFA mobilization does not seem to result in either hepatic or peripheral insulin resistance. 3) GHR blockade by pegvisomant constitutes a promising tool for studying the physiological effects of GH on substrate metabolism.

P463

Are hypothalamic oscillators dysfunctional with high fat feeding?

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Circadian rhythms in behaviours and gene expression are driven by the autonomously rhythmic cells of the hypothalamic suprachiasmatic nucleus (SCN). In nocturnal rodents, restricting a daily meal to the lights-on phase, leads to entrainment of behaviour and gene expression in many extra-SCN hypothalamic nuclei and peripheral tissues. This anticipatory activity is independent of the SCN and is likely to arise from the activities of multiple hypothalamic centres including the dorsomedial (DMH). Here, we used digital video recording to monitor the behaviour of lean and 45% High Fat Fed (HFF) Han Wistar rats. Rats were maintained in a 12 h:12 h Light/Dark cycle (lights on at 6am) and were subject to day time restricted feeding (RF) for 28 days (meal provided 09:00 a.m.-13:00 p.m). Hypothalamic neuronal activation was measured by immunohistochemistry for c-Fos protein. Lean RF rats displayed the characteristic increase in cage activity and meal anticipatory hopper approaches, whereas HFF RF rats lacked meal anticipation, as determined by the lack of hopper approaching in the time preceding the scheduled meal. Immunohistochemistry data showed that HFF RF rats had 77% lower c-Fos expression in the SCN and 37% lower c-Fos expression in the DMH compared to lean RF rats. Consistent with other studies, we found a meal anticipatory rise in plasma corticosterone in the chow RF rats, whereas a post-anticipatory peak of plasma corticosterone levels was detected in HFF RF rats. Taken together, these findings suggest that neuronal activation in both the light-entrainable and food-entrainable oscillators are altered by high fat feeding. In addition this loss of meal anticipatory behaviour is associated with an altered regulation of plasma corticosterone.

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Glucose-dependent insulinotropic polypeptide increases glucose-uptake in muscle- and adipocyte cells *in vitro*

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Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone secreted by K-cells of proximal small intestine that is secreted postprandial in response to glucose and fat ingestion. Its effect is mediated through a GIP receptor (GIPR) which is widely expressed in different tissues, e.g. pancreatic islets, adipose tissue, skeletal muscle, adrenal cortex, heart, pituitary and in some regions of the brain. However, only the effect of GIP in pancreatic beta-cells is well-established where GIP stimulates glucose-induced insulin response. The purpose of our studies was to analyze effects of GIP on glucose uptake in differentiated 3T3-L1 cells (murine adipocytes) and RD18 cells (human skeletal muscle). Therefore, cells were incubated with either GIP or insulin or a combination of both.
Glucose-uptake of cells was determined with [^{3}H]-2-deoxyglucose-assay. In 3T3-L1 cells as well as RD18 cells GIP slightly increased glucose uptake (118.2$\pm$12.1% (3T3-L1); 112.3$\pm$6.1% (RD18)). In combination with insulin we observed no additive effects in RD18 cells (158.3$\pm$12.6% ($P<0.001$) versus the negative control) while Insulin alone enhanced deoxyglucose-uptake up to 154.7$\pm$6.4% ($P<0.001$). In differentiated 3T3-L1 cells GIP in combination with insulin increased the deoxyglucose-uptake up to 337.7$\pm$23.6% versus control ($P<0.001$). However, insulin caused a rise of deoxyglucose-uptake to 292.3$\pm$22.6% ($P<0.001$) versus the negative control.
These results reveal further evidence for the physiological importance of GIP in glucose metabolism in extra-pancreatic tissues. GIP is able to influence the glucose-uptake in combination with insulin. However, its effects are small in comparison to insulin.

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Glucose-dependent insulinotropic polypeptide modulates inflammatory markers in adipose tissue

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Background
Glucose-dependent insulinotropic polypeptide (GIP) is a gastrointestinal hormone that is secreted in response to food intake. GIP acts on various tissues, including pancreatic ß-cells, via interaction with its G-protein-coupled receptor. Some studies in rodents suggested that GIP directly links overnutrition to obesity. GIP exerts a physiological role on lipid uptake into adipocytes.
Objectives
To analyse the effect of GIP-Infusions on changes in adipose tissue gene expression at different blood-glucose levels in obese men and also detect different biomarkers and hormone interactions caused by GIP.
Setting and participants
Seventeen healthy overweight men (BMI: 28–40 kg/m^2; age: 30–65 years) with normal glucose tolerance underwent a single-blind intervention study at four different time points with euglycaemic or hyperglycaemic clamps in combination with GIP or saline infusions. Each solution was applied at physiological concentrations for 4 h. Before and after the infusions biopsies from subcutaneous adipose tissue were taken. We isolated total RNA from all fat biopsies and hybridized the RNA to Agilent Whole Human Genome Microarrays. The results were verified by RT-PCR.
Results
Using Agilent Genespring Software and the MetaCore platform we identified several genes being involved in inflammatory processes. Under euglycaemic hyperinsulinaemic clamp conditions in combinations with a GIP-Infusion we find a significant upregulation of chemokine ligand 2 (CCL-2/MCP-1), interleukin-1ß (IL-1ß) and interleukin-6.
Conclusion
GIP may play a role in inflammatory processes in adipose tissue of obese men.

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Glucose RapidSpray™ as adjuvant in diet restriction programmes

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Overweight and obese subjects often suffer from hypoglycaemic symptoms due to hyperinsulinemia. This increases subjects' need to eat excessive amount of food enriched in sugar causing rebound hyperglycaemia which in turn stimulates endogenous insulin release perpetuating a vicious circle that limits weight loss under diet restriction programme. Two previous studies showed that administration of Glucose RapidSpray™ (GRS) in quantity of ~ 0.5 g increases blood glucose levels of approximately 5 mg/dl within few minutes and without stimulating endogenous insulin release, thus increasing compliance to a calorie-restricted diet.
In one of these studies 55 obese subjects were given a 1500 kcal diet (15% proteins, 55% carbohydrates, 30% lipids) and asked to exercise 150 min/week. Of 31 subjects were randomly assigned to make use of GRS, a solution of 100% glucose delivered by buccal spray. Each puff contains 50 mg of glucose. Subjects were asked to spray 10 oral puffs during early symptoms of hypoglycaemia and followed up for 60 days. At the end of follow-up there were no significant differences in body weight, waist circumference, glucose and insulin levels evaluated by OGTT. However, a significant reduction in prevalence of type 2 obesity (BMI 35.0–39.9 kg/m^2), and consequent increase in prevalence of overweight (BMI 25–29.9 kg/m^2) (c2: 7.859–$P=0.01$) was observed in GRS group. These changes were not significant in the control group.
Subjects were followed-up every 4 months for 12 months and after a year. 50% of subjects were lost to follow-up, however 13 subjects taking GRS had significantly lost weight (median -5.4 kg, 95% CI $=-3.073--8.973$ – $P=0.01$) with reduction of BMI – 2.3 (95% CI $=-1.267--3.333$ – $P=0.01$); such reduction was not significant in the control group (12 subjects).
In conclusion, GRS is a useful adjuvant in diet restriction programmes.

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Plasma monocyte chemoattractant protein-1 and macrophage inflammatory protein-1α are increased in patients with polycystic ovary syndrome and associated with adiposity, but unaffected by pioglitazone treatment

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Objective
PCOS patients are often characterized by insulin resistance, abdominal obesity, and low-grade inflammation. Insulin sensitizing treatment reduces the inflammatory state, but the effect on serum levels of chemokines such as migration inhibitor factor (MIF), monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1α have not previously been evaluated in PCOS.
Research design and methods
Plasma chemokine levels (MCP-1, MIP-1α, and MIF) were measured in two study designs. 1) 51 hirsute patients and 63 matched controls and 2) 30 PCOS patients before and after randomized treatment with 30 mg pioglitazone/placebo for 16 weeks. Clinical evaluations and whole body DXA-scans were performed in all participants.
Results
Hirsute patients ($n=51$) had significantly increased MCP-1 (121 (15–950) vs 81 (18–365) pg/ml; $P<0.05$) and MIP-1α (179 (8–4202) vs 103 (4–1598) pg/ml; $P<0.05$) than controls of matched body composition (geometric mean (-2 to $+2$S.D.)). In PCOS ($n=30$), MCP-1, MIP-1α, and MIF correlated positively with central fat mass. A BMI independent positive association was found between MIF-1 and free testosterone ($r=0.49$, $P=0.01$) in PCOS.
Pioglitazone treatment significantly improved insulin sensitivity without affecting testosterone, body composition, MCP-1, MIP-1α, and MIF levels.
Conclusions
Chemokine levels were significantly increased and showed close associations with measures of adiposity in PCOS patients, but were unchanged during insulin sensitizing treatment with pioglitazone. Our data suggests a fat mass independent association between testosterone and MIF-1 levels in PCOS and the effect of antiandrogen treatment on chemokine levels needs to be examined.

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Elevated prolactin serum level in erectile dysfunction cases can be predict by metabolic syndrome

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Objective
Comparing serum pituitary hormones prolactin (PRL), luteinizing hormone (LH) and thyrotropin (TSH) to serum total testosterone (TT) in men with erectile dysfunction (ED) and metabolic syndrome (MetS) versus those having ED without MetS.
Method
We looked for the prevalence of serum high PRL, high LH, high TSH and low TT levels in 24 men (Gr. $A=49.35\pm5.2$ years old) with ED (IIEF-5 questionnaire) and MetS (IDF criteria, 2005) versus those of a control group including 23 cases (Gr. $B=47.35\pm4.9$ years old) also having ED but without MetS. Pituitary hormones levels were correlated to those of serum TT (electro-chemo-luminescence) and compared between the two groups. Borderline values were take in to consideration (PRL = 98–100 μUI/ml, LH = 8.0–8.6 mUI/ml TSH = 4.0–4.2 μg/ml).
Results
IIEF-5 score: Gr. $A=14.3\pm0.8$, Gr. $B=14.7\pm1.1$ ($N\geq21$ points). High PRL prevalence: Gr. $A=9$ cases (37.5%) vs Gr. $B=3$ cases (12.08%): $P<0.05$. High LH prevalence: Gr. $A=7$ cases (29.16%) vs Gr. $B=5$ cases (21.7%). High TSH prevalence: Gr. $A=$ none; Gr. $B=$ none. Low TT prevalence: Gr. $A=10$ cases (41.67%) vs Gr. $B=4$ cases (17.4%). LH values prevalence did not correlate to those of TT. Correlation of high PRL values prevalence to those of low TT: Gr. A, $r=-0.053$, $P<0.05$ vs Gr. B, $r=-0.017$, $P<0.5$.
Conclusion
In ED with MetS versus ED without MetS high serum PRL levels independently correlate to low TT levels, meaning that MetS could predict a PRL enhancement risk in low androgen expressed ED.

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Sexual dysfunction in women with obesity

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Objective
Both overweight and obesity have been identified as risk factors for sexual dysfunction in men, but the relationship between female sexual function and amount of body fat is still obscure. To the best of our knowledge there are few reported studies assessing the relation between female sexual function index (FSFI) and body weight in women. Therefore, the aims of this study were to identify the frequency of sexual dysfunction (FSD) among obese women.
Methods
A total of 45 obese women and 30 age matched voluntary healthy women who served as the as control group were evaluated with a detailed medical and sexual history, including a FSFI questionnaire. Serum prolactin, cortisol, luteinizing hormone (LH), follicle stimulating hormone (FSH), dehydroepiandrosterone sulfate (DHEA-S), testosterone, estradiol, progesterone, sex hormone binding globulin (SHBG), free thyroxin and thyrotropin were measured. These variables were compared statistically between the 2 groups.
Results
Mean ages, FSH, LH, E2, TSH, testesterone, DHEAS, prgesterone between controls and patients did not show statistical difference. The SHBG levels in our patients were significantly lower than our control population ($P=0.00$). The comparison for total FSFI scores between patients and controls showed no statistical difference ($P=0.39$). Since a score of 23 or lower indicated FSD; % 60 of obese patients and % 50 of controls had sexual dysfunction. The mean total FSFI score for obese patients was 22.1 ± 4.3 but the mean total FSFI score for healthy women was 23.1 ± 3.7. FSFI scores were not correlated with any of the antropometric measurements (BMI, WHR, %fat). In the correlation analysis of total FSFI scores and androgen hormones, the levels of total testosterone, DHEA-S showed no correlation with total FSFI scores. There were no correlation between FSFI scores and FBG, insülin, estradiol, FSH, LH, PRL, TSH, cortisol, IGF-1, SHBG levels also. We found a significant negative correlation between BMI and orgasm ($P=0.007$ $r=-0.413$). Satisfaction was also negatively correlated with BMI ($P=0.05$ $r=-0.305$) and weight ($P=0.03$ $r=-0.326$). Tetesterone levels were negatively correlated only with satisfaction among all FSFI scores ($P=0.01$ $r=-0.385$).
Conclusion
In this study we found that % 60 of obese patients and % 50 of controls had FSD. Although obesity does not seem to be a major contribute to sexual dysfunction, it

affects several aspects of sexuality (satisfaction, orgasm). As there are not enough studies investigating female sexual function in obesity, further studies with larger number of patients are necessary.

P470

Effects of green tea consumption on blood pressure, total cholesterol, body weight and fat in healthy volunteers

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Background
Hypertension, obesity and hyperlipidemia are key interlinked features of both metabolic syndrome and cardiovascular disease. Numerous studies have suggested that green tea may reduce blood pressure by activating endothelial nitric oxide synthase and reducing total cholesterol by disrupting the production of apo B and synthesis of chylomicrons and thus have cardio-protective effects. The aim of this pilot study was to investigate the effects of increasing the consumption of green tea-rich catechins on blood pressure (BP), total cholesterol and other body composition parameters in healthy volunteers living in Scotland.
Methods
Following a 2 day green tea free period, participants ($n=12$; 9 females and 3 males) were asked to drink 4 cups of green tea (organic Mao Jian Green Tea from the Zhejiang region of China) for 14 days ($\sim$800 ml green tea infusion containing 600–800 mg total catechins). Fasting total plasma cholesterol, BP, weight, BMI and %body fat were measured at day 0 (baseline), day 7 and day 14.
Results
Mean systolic BP was reduced significantly by 7.1 mmHg ($P<0.0001$) and mean diastolic BP reduced by 7.8 mmHg over 14 days ($P<0.0001$). Mean fasting total cholesterol was reduced significantly by 0.556 mmol/l ($P<0.008$), BMI by 0.34 kg/m^2 ($P<0.001$), body weight by 0.96 kg ($P<0.001$) and body fat by 2.36% ($P<0.005$).
Conclusion
Our results has shown that short term consumption of commercial green tea reduces systolic and diastolic BP, fasting total cholesterol, %body fat and body weight suggesting a role for green tea in decreasing established potential cardiovascular risk factors. This study also suggests that reductions may be more pronounced in the overweight population where a significant proportion are obese and have a high risk of cardiovascular disease. Green tea consumption is cost effective, accepted by patients and has no reported side effects.

P471

Subcutaneous adipose tissue topography and metabolic disturbances in polycystic ovary syndrome

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Objective
Central obesity plays a major role in the pathophysiology of polycystic ovary syndrome (PCOS). However, little information exists concerning the impact of subcutaneous adipose tissue (SAT) on metabolic disturbances in PCOS. The aim of this study was to investigate whether SAT topography influences insulin resistance, impaired glucose tolerance, and metabolic parameters in PCOS women.
Design
Prospective case-control trial.
Subjects
Of 36 PCOS women aged 16–41years and 87 healthy women aged 20–34 years.
Measurements
Lipometry, metabolic and hormonal measurements, oral glucose tolerance test, and hirsutism score. The study protocol was approved by the local ethics committee.
Results
Trunk located SAT measure points correlated significantly positive with HOMA-index (homeostasis model assessment). A negative correlation was seen between calf-SAT and HOMA-index. A multiple regression analysis detected a positive association between HOMA-index and lower abdomen-SAT and upper back-SAT, whereas hip-SAT showed a negative association with HOMA-index. In over-weight/obese PCOS patients lower abdomen and upper back-SAT showed significantly positive correlations with insulin resistance. There was no correlation of SAT topography with insulin resistance in lean PCOS women. Compared to PCOS women with normal glucose tolerance, patients with glucose intolerance had significantly increased trunk obesity and decreased leg fat. Increased trunk SAT layers were related to an unfavorable serum lipid profile whereas increased leg fat correlated positively with HDL cholesterol.
Conclusion
Increased trunk located SAT layers are associated with insulin resistance, impaired glucose tolerance, and an unfavorable lipid profile in women suffering from PCOS. High thickness of leg SAT emerges as being protective against metabolic disturbances in PCOS.

P472

Melanocortin-4-receptor gene variants: hotspot or identical by descent?

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The melanocortin-4-receptor (MC4R) plays an important role in body weight regulation. Mutations in the MC4R gene are the most common genetic cause for obesity. The most frequent Northern European mutation is Y35X, associated with D37V on the same allele. Furthermore, there are two variants with a relatively high frequency: V103I and S127L. In rare cases, we identified the variants V103I and S127L on the same allele. The occurrence of two variants on the same allele makes a founder effect possible but this has yet to be proven. Therefore, we analysed single nucleotide polymorphisms (SNPs) within a range of overall 240 kb up- and downstream of the MC4R gene. Our sample group consisted of a healthy control group and of trios of normal weight and obese patients of Caucasian origin. We analysed 25 mutation carriers of Y35X/D37V, three V103I/S127L carriers and their families and two Arab families each with two homozygous carriers of a pathogenic missense mutation, C271R.
Linkage disequilibrium (LD) analyses show that the investigated SNPs form three different LD-blocks. In a first attempt we focused on those SNPs forming an LD-block that comprises the coding region of the MC4R gene for haplotype analysis. Aside from 11 different haplotypes we found one main haplotype with a frequency of approximately 73% in the control trios. Via analysis of the mutation carriers we could show that the Y35X/D37V mutation is estimated to be always located on this common haplotype making a founder effect highly possible. The double mutation V103I/S127L is located on another haplotype as well as the C271R mutation which indicate identity by decent. In conclusion, we can show that the increased prevalence of certain MC4R mutations, Y35X/D37V and V103I/S127L in Caucasian and C271R in patients of Arab origin, can be attributed to a founder effect in these populations.

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Association between platelet count and metabolic risk factors in over weight and obese women

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Introduction
We aimed to evaluate the relationship between platelet count and metabolic risk factors in over weight and obese women in Turkish women.
Methods
Over weight (BMI>25 kg/m^2) 665 and obese (BMI>30 kg/m^2) 4609 women were enrolled into the study. The patients were divided into three group according to the platelet count (PLT), Group I ($n=1724$) consist of serum PLT$\leq 245\times10^3$, group II ($n=1787$) consist of PLT 245×10^3 to 300×10^3 and, group III ($n=1763$) consist of PLT $>300\times10^3$ µl. The groups were compared for metabolic risk markers.

Results
Body weight, BMI and body fat mass, waist circumference, sagital waist, fasting glucose, insulin, HOMA, A1c, total cholesterol, LDL-cholesterol, triglycerides, hs-CRP, ESR and, systolic and diastolic blood pressures were significantly high in Group III compared to Group I and Group II ($P<0.05$).

Table The comparison of different platelet (PLT) groups.

	Group I ($n=1724$)	Group II ($n=1787$)	Group III ($n=1763$)	*P* value
Age (years)	40.88±12.75	40.18±12.19	39.17±11.87	0.003
Weight (kg)	84.99±18.31	87.57±17.87	91.20±19.38	<0.001
BMI (kg/m^2)	34.06±7.56	35.14±7.23	36.65±7.69	<0.001
Waist circumference (cm)	96.72±14.98	99.05±14.35	102.05±20.64	<0.001
Saggital waist (cm)	23.61±4.11	24.49±4.42	25.50±4.29	<0.001
Glucose (mg/dl)	97.67±26.93	97.52±24.11	99.80±31.44	0.241
HbA1C (%)	5.74±0.88	5.78±0.81	5.85±1.05	0.361
Insulin (uIU/ml log)	12.45±12.00	12.98±9.72	15.20±13.49	<0.001
HOMA (log)	3.11±4.11	3.20±2.82	3.94±5.48	<0.001
LDL-kol (mg/dl)	123.38±38.64	127.79±37.91	128.94±39.04	0.001
Triglicerides (mg/dl log)	137.13± 110.95	143.68± 124.63	151.20± 105.28	0.015
Systolic DB (mmHg)	126.83±24.86	127.55±23.30	130.89±23.51	<0.001
Diastolic DB (mmHg)	80.97±13.55	81.51±13.59	82.91±14.47	<0.001

Conclusion
The data obtained from this study indicates that high PLT count is associated with several metabolic risk markers. Platelet count can be suggested as a risk marker for atherosclerotic disease in overweight and obese women.

P474

The evaluation of the oxidative stress in obese adolescent girls with full or partial metabolic syndrome

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Background
Studies in adults have clearly demonstrated that obesity is a main factor implicated in increased oxidative stress and platelet activation, both predisposing factors for cardiovascular disease leading to increased morbidity and mortality. Furthermore, the metabolic syndrome (MetS), the clustering of abnormalities, such as central obesity, dyslipidemia, impaired glucose tolerance and hypertension, which is an important cardiovascular risk factor in adults, is also highly prevalent among obese adolescents. However, scarce data on the presence of oxidative stress among obese adolescents with full or partial metabolic syndrome exist.
Objective
The aim of this study was to evaluate the oxidative stress in obese adolescent girls with full or partial MetS.
Methods
A total of 53 obese adolescent girls and 20 normal-weight, age matched adolescent controls participated in the study. The obese adolescents were subdivided in two groups, the first one comprising 17 girls with a mean age of 12.12±4.17 years, BMI 35.39±5.01 kg/m^2 and full MetS (group AD-MetS), while the second group included 36 obese girls with a mean age of 12.79±5.12 years, BMI 31.38±3.19 kg/m^2 and partial MetS (group AD-PMetS). The oxidative stress was evaluated by measuring plasma levels of 15-F_2t-Isoprostane (15-F_2t-ISOP), the most reliable biochemical index of the oxidative stress and antioxidant deficiency, using a reliable enzyme – immunoassay method.
Results
Plasma 15-F_2t-ISOP levels in the AD-MetS group were 34.96±7.14 pg/ml versus 13.89±2.68 pg/ml in controls ($P<0.001$). Plasma 15-F_2t-ISOP levels (23.94±4.97 pg/ml) in the AD-PMetS group were significantly lower than in the AD-MetS group ($P<0.05$) but significantly higher than in controls ($P<0.001$). There was a positive correlation between 15-F_2t-ISOP levels and BMI.
Conclusion
The oxidative stress increases in obese adolescent girls, with increasing levels of obesity, being also higher among those with full metabolic syndrome than partial metabolic syndrome. The coexistence of increased oxidative stress and metabolic syndrome constitutes a further unfavorable prognostic set up for cardiovascular risk.

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The role of family and parental factors in childhood obesity

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Poor family functioning will be associated with inadequate parental monitoring and/or regulation of children's eating and activity patterns. We aimed to examine the relationship between a child's weight and a broad rang of family and maternal factors. Our hypothesis was that increasing adiposity in children would be associated with poor general family functioning, lower socioeconomic status, inappropriate parenting style. This cross-sectional study involved 56 obese children (m/f=30/26), mean age11.05±3.50 years and 56 mothers. Obesity was defined as BMI scores at or above the 97th percentile for age and gender. Psychological examination was conducted (Eidemiller test of house education). All the analysis were performed with the Statistica 6.0 software, *P*-value <0.05 was accept as statistically significant. ANOVA test was used for unpaired data. BMI mother's 27.90±5.33 (19.00–41.00) kg/m^2, BMI children's 27.84±4.6 (18.20–39.60) kg/m^2, SD BMI 5.14±1.92.
Results
17.86% mothers had secondary education, 57.14%-higher education and 25%-special education. The full families were observed in 71.43%, incomplete – in 28.57% examined patients. BMI children was not correlated with BMI mothers ($r>0.1$). Differences SD BMI were received depending on mothers education: high – Me 4.77 (3.24–5.44) and secondary-special – Me 5.67(4.68–7.20) ($r=0.0085$). The significantly differences of the following criterion of the test were determined on deflection SD BMI from Me: 'forbid-requirements overweening' ($r=0.1$), 'sanctions overweening' ($r=0.015$), for girls «projection male quality» ($r=0.045$). On the other criteria of the test differences between SD BMI were not revealed ($r>0.1$).
Findings indicated on increase the children BMI under using negative acceptance (raised requirements, prohibitions and punishments) in household education. It was revealed that mothers had difficulties between need of the checking and granting to autonomies child. Level of mothers education in our study was closely connected with obesity and not depended on gender.

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Relationship of serum gamma-glutamyltransferase and adiposity measures with C-reactive protein in severe obese women

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Introduction and aims
Recent epidemiological studies have suggested serum gamma glutamyltransferase (GGT) within its normal range might be an early marker of oxidative stress. Oxidative stress appears to be a key component of many reactions associated with chronic inflammation. Obesity is associated with elevated levels of biomarkers of inflammation and endothelial dysfunction. Oxidative stress and inflammation are also known to play critical roles in the pathogenesis of vascular events. The aim of the study was to investigate association between serum GGT and concentrations of serum C-reactive protein (CRP) in severe obese women and to correlate these parameters with adiposity measures in these women.
Patients and methods
We investigated 31 obese women (mean age 34.60±1.40 years; mean BMI 36.10±0.96 kg/m^2; mean waist 106.33±2.34 cm). CRP (5.06±0.75 mg/l) and serum GGT (24.58±4.32 mg /l), fasting glucose (5.27±0.11 mmol/l), fasting insulin (17.89±1.40 mU/l) were measured in all women.
Results
There was no correlation between GGT and BMI, waist, fasting glucose, fasting insulin. Also, there was no correlation between CRP and fasting glucose, neither

between CRP and fasting insulin, BMI, waist circumference, but there was significant correlation between CRP and GGT ($r=0.735$, $P<0.001$).
Conclusions
Our data confirmed significant correlation between GGT and CRP in obese women. The strong association of serum GGT and CRP in obese women suggests their possible relationship and further studies are necessary to elucidate the association between oxidative stress and inflammation in obesity.

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A retrospective analysis of the relationship of thyroid function with obesity in a single center obesity outpatient policlinic

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The activity of hypothalamic–pituitary axis is increased in obese patients and some hormonal changes can be observed as a consequence. Although the effects of hypothyroidism or hyperthyroidism on weight are clearly demonstrated data regarding the effects of relatively minor defects is limited. There are different results in the literature concerning thyroid function status in obesity, some indicating no change, others elevated serum thyroid stimulating hormone (TSH), free triiodothyronine (fT_3) and free thyroxine (fT_4) levels and some others TSH in the upper normal range as well as fT_4 in the lower normal. Considering the increasing prevalence of obesity and its association with higher rates of morbidity and mortality, the relation between thyroid functions and body mass becomes more important. In the present study, the relation between body mass index (BMI) and alterations in thyroid functions within normal ranges was aimed to be investigated in obese patients. Three hundred and 57 euthyroid obese patients (309 females, 48 males, mean age: 42 years) were included in the study. Thyroid functions, BMI and epidemiological characteristics of the patients were retrospectively evaluated (Table 1). The patients were divided into two groups according to a cut-off BMI value of 40 kg/m^2. No statistically significant difference was detected between the groups in respect of gender, place of birth, place of residence, smoking and family history. Obese patients with BMI $\geq$ 40 kg/m^2 were older and showed lower serum free thyroxine level (fT_4) than obese patients with BMI $<$ 40 kg/m^2 ($P<0{,}01$). Our data indicated that, although thyroid functions were normal in the studied obese population, fT_4 and BMI were related. Minor variations in thyroid functions may importantly influence the prevalence of obesity in a population.

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Relationship between prehypertension and metabolic risk markers in over weight and obese women

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Introduction
Hypertension is a major risk factor for several cardiovascular diseases including coronary heart disease. Prehypertension is defined systolic blood pressure (SBP) between 120–139 mmHg and diastolic blood pressure (DBP) 80–89 mmHg. Recent studies have documented an increased risk of cardiovascular disease in persons with prehypertension. In this study, we evaluated association between prehypertension and the metabolic risk markers in over weight and obese women.
Methods
Overweight (25 $<$ BMI $<$ 30 kg/m^2) 824, and obese (BMI(30 kg/m^2) 2652 women were enrolled into the study. The subjects were divided into two groups: Group 1 ($n=1131$); women with blood pressure $<$120/80 mmHg; normal group, Group 2 ($n=2345$); women with 120 $<$ SBP $<$ 139 mmHg, and 80 $<$ DBP $<$ 89 mmHg; prehypertension group. The groups were compared for metabolic risk markers regards of cardiovascular disease.
Results
BMI, waist, sagital waist, body fat, intra-abdominal fat, HgA1c, fasting insulin, HOMA, triglyceride, cholesterol, LDL-C were significantly higher prehypertension group compared to normal subjects ($P<0.001$). The results are documented on Table.
Conclusion
This study demonstrated that prehypertension was associated with metabolic risk markers in over weight and obese women.It is known that prehypertension is associated with atherosclerosis, including increased coronary atherosclerosis, elevated C-reactive protein, tumor necrosis factor, homocysteine, oxidized LDL, and other inflammatory markers. Successful management can reasonably be expected to reduce CVD morbidity and mortality.

Table Comparison of the groups for metabolic risk markers.

	Group 1 ($n=1131$)	Group 2 ($n=2345$)	*P* value
Age (years)	35.74 $\pm$ 11.14	38.24 $\pm$ 11.58	$<$0.001
Weight (kg)	84.16 $\pm$ 14.69	87.96 $\pm$ 15.85	$<$0.001
BMI (kg/m^2)	33.58 $\pm$ 5.77	35.19 $\pm$ 6.29	$<$0.001
Body fat (kg)	39.25 $\pm$ 15.75	43.64 $\pm$ 17.59	$<$0.001
Waist (cm)	96.64 $\pm$ 22.00	98.99 $\pm$ 12.69	$<$0.001
Saggital waist (cm)	23.71 $\pm$ 3.64	24.55 $\pm$ 3.91	$<$0.001
Intra-abdominal fat (L)	3.13 $\pm$ 1.07	3.38 $\pm$ 1.16	$<$0.001
Cholesterol (mg/dl)	193.70 $\pm$ 40.53	201.74 $\pm$ 40.92	$<$0.001
LDL (mg/dl)	120.98 $\pm$ 34.08	126.21 $\pm$ 35.86	$<$0.001
HDL (mg/dl)	47.99 $\pm$ 11.53	47.85 $\pm$ 11.39	NS
Triglyceride (mg/dl)	124.59 $\pm$ 80.03	141.15 $\pm$ 100.05	$<$0.001
Glucose (mg/dl)	92.89 $\pm$ 18.44	96.61 $\pm$ 27.40	$<$0.001
HbA1c (%)	5.63 $\pm$ 0.61	5.71 $\pm$ 0.81	0.041
Insulin (uIU/l)	11.90 $\pm$ 11.39	13.59 $\pm$ 12.41	$<$0.001
HOMA	2.79 $\pm$ 3.98	3.36 $\pm$ 4.67	0.015

P479

Short term elevation of estradiol concentrations does not affect hepatic VLDL-TG production

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Background
Hormone replacement therapy (HRT) in post-menopausal women carries an increased risk of cardiovascular disease due to an unfavorable effect on lipid profile. Thus, HRT treatment increases concentrations of very-low-density-lipoprotein-triglycerides (VLDL-TGs) which have been demonstrated to possess atherogenic properties. However, the exact mechanisms whereby VLDL-TG concentrations are increased by estradiol are not fully understood. Estradiol acts on both rapid responding intracellular receptors and on slow responding membrane bound receptors. We therefore aimed to study whether or not hepatic VLDL-TG production is acutely increased by a single dose of estradiol.
Materials and methods
In a single blinded cross-over design, eight postmenopausal women (age 57 $\pm$ 5 years, BMI 25 $\pm$ 2 kg/m^2, fat mass 24 $\pm$ 5 kg, lean body mass 40 $\pm$ 4 kg) were investigated twice. Study days consisted of either placebo (CON) or 4 mg of estradiol (EST) administered p.o. 1 h before the study start. VLDL-TG kinetics were determined by a primed-constant infusion of (1-14C)triolein labeled VLDL and body composition by dual X-ray absorptiometry (DXA).
Results
By design, estradiol concentrations were below detection threshold during CON conditions and were significantly increased to $\sim$0.5 nmol/l during EST conditions. No acute changes in VLDL-TG concentrations (VLDL-TG (mmol/l): CON: 0.39 $\pm$ 0.15 versus EST: 0.41 $\pm$ 0.23, $P=0.78$) or total TG concentrations (TG (mmol/l): CON: 1.25 $\pm$ 0.57 versus EST: 1.11 $\pm$ 0.39, $P=0.38$) were observed. Dynamic VLDL-TG kinetic parameters revealed that estradiol does not acutely impact on neither hepatic VLDL-TG production (VLDL-TG production (μmol/min) CON: 20 $\pm$ 12 versus EST: 23 $\pm$ 9, $P=0.52$) nor peripheral VLDL-TG clearance (VLDL-TG clearance (ml/min): CON: 49 $\pm$ 15 versus EST: 64 $\pm$ 29, $P=0.28$).

Conclusion
Acute administration of estradiol does neither affect hepatic VLDL-TG production nor peripheral VLDL-TG clearance. The mechanisms behind estradiol's effects upon circulating lipids remain to be elucidated.

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Serum adiponectin levels and polymorphism of CB1 endocannabinoid receptor (A3813G, A4895G, G1422A) in postmenopausal women

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Recently, low serum adiponectin levels is created as a new risk factor for cardiovascular diseases in climacteric women. Our previous study shows that smoking, serum testosterone levels, blood pressure and BMI have the influence on serum adiponectin levels. Endocannabinoid system plays a role in regulation of food intake and fat accumulation, as well as lipid and carbohydrate metabolism. Menopause transition is related to metabolic disorders (e.g. obesity) and cause the increased risk of cardiovascular diseases. From randomly selected 6000 postmenopausal women, according to the protocol, we selected 360 women (aged 50–65) and finally 348 women was developed according to relation between the endocannabinoid receptor polymorphism and serum adiponectin levels. Standard method was used for DNA genomic isolation from lymphocyte. The polymorphism of endocannabinoid receptor was estimated using minisequenced technique. We have estimated BMI, waist circumference, % fat, gynoid and android fat deposit (using DXA method), blood pressure as well as lipid and carbohydrate metabolism and serum adiponectin levels (using RIA method) in polymorphism A3813G, A4895G, G1422A of CN1 gene. In our study we have found no significant influence of gene polymorphism on serum adiponectin levels, but in G/G genotype (polymorphism A3813G) we have found statistically significant higher BMI, % fat and android fat deposit in comparison to A/A genotype.

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Relationships of TNFα system with glucose and lipid oxidation in subjects with different degree of insulin sensitivity

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One of the key actions of insulin is the regulation of glucose and lipid oxidation. Disturbances in substrate oxidation play an important role in the development of insulin resistance. Insulin action is inversely associated with circulating pro-inflammatory cytokines such as soluble TNF-α receptors (sTNFR1 and sTNFR2). The aim of the present study was to analyze the associations between serum sTNFR1 and sTNFR2 concentrations and glucose and lipid oxidation and non-oxidative glucose metabolism in lean and obese subjects with normal glucose tolerance.
We examined 42 subjects (30 females and 12 males), 22 lean (BMI $<25\ kg\times m^{-2}$, 16 females and 6 males) and 20 with overweight or obesity (BMI $>25\ kg\times m^{-2}$, 14 females and 6 males) with normal glucose tolerance. Insulin sensitivity was measured with the hyperinsulinemic euglycemic clamp technique. Glucose and lipid oxidation was evaluated with indirect calorimetry in the baseline state and during the last 30 min of the clamp. Non-oxidative glucose metabolism in the hyperinsulinemic state was calculated by subtracting glucose oxidation from the total glucose metabolism. Metabolic flexibility was assessed as an increase in respiratory quotient (ΔRQ) in response to insulin.
Serum sTNFR1 concentrations were higher in the obese in comparison with the lean group ($P<0.0001$). Insulin sensitivity was negatively related with serum sTNFR1 ($r=-0.38$, $P=0.014$), whereas the relationship with sTNFR2 was approaching the level of significance ($r=-0.30$, $P=0.057$). Serum sTNFR1 concentration was positively associated with the baseline glucose oxidation ($r=0.32$, $P=0.043$) and negatively – with the increase in glucose oxidation in response to insulin ($r=-0.40$, $P=0.01$) and with non-oxidative glucose metabolism ($r=-0.35$, $P=0.022$). Significant negative correlation between serum sTNFR1 and metabolic flexibility was observed ($r=-0.36$, $P=0.019$). Serum sTNFR2 was positively related to baseline respiratory quotient ($r=0.36$, $P=0.018$).
Our data suggest that TNFα system is associated with multiple metabolic pathways regulated by insulin.

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Variation of daytime melatonin levels

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Melatonin has a key role in circadian timing system. At present, many other functions of melatonin are known. It shows a remarkable functional versatility exhibiting antioxidant (direct free radical scavenging and indirect antioxidant activities), oncostatic, anti-aging, immunomodulatory properties and many other functions. In addition to pineal gland, gastrointestinal tract is the second biggest source of melatonin. Question remains whether changes in endogenous melatonin may be influenced by food intake. In our previous study, melatonin negatively correlated with C-peptide and glucose after food intake during daytime. For the better understanding of the possible role of melatonin as a factor, which can be influence food intake, we monitored levels of melatonin, C-peptide and blood glucose after different food stimulus. Five women (mean age 31.6 ± 2.8 years, mean BMI $23.2\pm2.3\ kg/m^2$ in follicular phase of menstrual cycle) were examined. All of them had standard intravenous glucose toleration test, standard oral glucose toleration test, standard breakfast (standard ratio of fat, carbohydrates and proteins) and non-caloric stimuli (psyllium). Variation in C-peptide levels was followed by changes of melatonin levels and also mechanic stimuli of food intake had the same effect on melatonin levels. Negative relationship between melatonin and C-peptide as well as rapid changes of melatonin levels permits conclusions that melatonin could to be involved in regulation of food intake.
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Aromatase expression in peripheral blood leukocytes from adult and elderly female and male subjects

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Objective
Aromatase, the key enzyme involved in estrogen synthesis, is expressed in a variety of cells and tissues including human peripheral blood leukocytes (PBLs). The present study was designed to evaluate PBL aromatase gene expression in male and female elderly subjects compared to young male controls.
Design
CYP19A1 mRNA and protein were measured in PBLs obtained from young men ($n=13$) aged <35 years, postmenopausal women ($n=13$) and men ($n=13$) aged between 50 and 60 years, as well as elderly men ($n=13$) and women ($n=13$), both aged >70 years. All subjects gave written informed consent to participate in the study.
Methods and results
Aromatase mRNA measured by real-time PCR in PBLs was not significantly different in any group compared to young men mainly due to high inter-individual variation in expression levels within the same group. Immunoblot analysis confirmed the data obtained by real-time PCR showing levels of aromatase protein very variable within the single groups and a scarce correlation between the levels of transcripts and the relative quantity of protein. However subjects with hypertension showed statistically significant higher levels of *CYP19A1* mRNA. No other correlations were found.
Conclusions
CYP19A1 mRNA and protein are not differentially expressed in PBLs from adult and elderly women and men. It is postulated that in these cells aromatase produces estrogens whit paracrine activity on development and maintenance of the immune system. We speculate that increased aromatase transcription in PBLs, can be indicative of the presence of a pathological and/or proinflammatory state, such as a hypertensive state.

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Non-diabetic metabolic syndrome and obesity do not affect serum paraoxonase-1, HDL-paraoxonase and arylestherase activities but affects oxidative stress and inflamation

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Paraoxonase is a high-density lipoprotein-bound antioxidant enzyme that inhibits atherosclerosis. Metabolic syndrome is a combination of several metabolic abnormalities and known to be related with increased cardiovascular events. The most important component of metabolic syndrome which have impact on oxidative stress is diabetes, the most severe form of disturbed carbohydrate metabolism. The effect of metabolic syndrome on oxidative stress and paroxonase-1 (PON-1) activity has been shown be in several studies and PON-1 has been found to be related with accelerated atherogenesis. However, these studies included diabetes in metabolic syndrome. Therefore we aimed to determine the oxidative state and PON-1 activity in non-diabetic metabolic syndrome and obese patients comparing with the controls.
Thirty-three obese patients without metabolic syndrome, 43 non-diabetic, obese or over weight patients and 24 normal control subjects were enrolled in the study. All patients were performed a standard 75 g overnight glucose tolerance test. PON-1 activity, HDL-paraoxonase, arylestherase, total antioxidant status (TAS), high-sensitive C-reactive protein (CRP), lipid peroxides and metabolic parameters were analysed.
PON-1, HDL-paraoxonase and arylestherase activities were not different among the three groups, while total antioxidant status was high in both metabolic syndrome and obese groups compared with the control group ($P<0.001$ and $P<0.05$ respectively). CRP was higher in metabolic syndrome group compared with obese and the control groups ($P<0.01$ and $P<0.001$ respectively). In both obese and metabolic syndrome groups, CRP showed a positive correlation with body mass index.
In conclusion, PON-1 HDL-paraoxonase and arylestherase activities were not different in our metabolic syndrome and obese group. This finding may be due to the absence of diabetes, severely disturbed glucose metabolism.

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Carotid IMT values are related to insulin resistance and visceral disposition of adiposity in obese patients

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The aim of our study was to assess the relation between common carotid intima-media thickness, a marker of subclinical atherosclerosis and other metabolic risk factors, in a group of obese patients.
Patients and methods
Of 142 obese patients (47 male, 95 female, mean age 39.74 ± 11.41 years, and mean BMI 46.88 ± 8.85 kg/m^2) were clinically and biologically evaluated. BMI and waist circumference were measured and a complete patient history regarding cardiovascular and metabolic disease was recorded. Biological tests included insulin resistance assessment (HOMA-IR). The degree of hepatic steatosis and liver right lobe diameter were measured by abdominal ultrasound, while common carotid intima-media thickness (IMT) was evaluated by Doppler ultrasound (UF 850XTD Tellus) and was expressed as the mean value of the bilateral measurements
Results
We found a significant correlation between IMT values and waist circumference, independent of BMI level ($r=.307$, $P<0.001$). IMT values were higher in previously known hypertensive (0.64 ± 0.16 vs 0.53 ± 0.13 cm, $P<0.01$) and in diabetic patients (0.66 ± 0.13 vs 0.57 ± 0.15 cm, $P<0.05$), despite non-significant differences in BMI level. In non-diabetic patients, IMT positively correlated with HOMA-IR ($r=0.234$, $P<0.05$) and with liver right lobe diameter ($r=0.299$, $P=0.01$). Obese patients with severe steatosis (diagnosed by the ultrasonographic aspect – bright liver- assessed by the same independent investigator) had higher mean IMT values compared to those with apparently normal structure or lesser degrees of steatosis (0.63 ± 0.16 vs 0.55 ± 0.14 cm, $P<0.05$).
Conclusions
High IMT values, a feature of subclinical atherosclerosis, correlate with markers of visceral obesity (waist circumference) and ectopic adiposity (right liver lobe diameter and steatosis). The positive relation between all these parameters and HOMA-IR suggest a possible common pathological pathway, with insulin resistance playing a central role.

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The effect of weight loss on serum mannose binding lectin levels

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Background
High levels of mannose-binding lectin (MBL), an activator of complement, have been associated with increased mortality and risk of albuminuria in patients with type 2 diabetes. It is not known if MBL is synthesized in human adipose tissue and the effects of weight loss and changes in insulin sensitivity on MBL levels have been poorly elucidated.
Methods
Of 36 nondiabetic obese subjects received a very low-calorie diet (VLCD) of 800 kcal/day for 8 weeks. Fasting blood samples were obtained at baseline and after 8 weeks of VLCD and concentrations of MBL, glucose, and insulin were measured. Insulin resistance was assessed using the HOMA-IR method. Furthermore, to investigate if MBL is synthesized in adipose tissue MBL real-time RT-PCR was performed on human adipose tissue compared to liver tissue.
Results
After 8 weeks the mean body weight was reduced by 13.5 kg (106.3 ± 2.6 kg (Se) vs 92.8 ± 2.4 kg, $P<0.0001$). Insulin resistance was reduced by $45.4\pm7.0\%$, $P<0.0001$. Median MBL at baseline was 746 μg/l (Iqr 316-1190) vs 892 μg/l (IQR 336-1511) at 8 weeks, $P=0.23$. No correlations were found between weight loss and changes in MBL ($r=-0.098$, $P=0.57$) nor between changes in insulin resistance and MBL ($r=-0.24$, $P=0.15$). MBL real-time RT-PCR showed no expression of mRNA in adipose tissue but as expected good expression in liver tissue.
Conclusions
Serum MBL levels do not seem to be related to weight or insulin resistance, and the concentrations are not affected by weight loss and changes in insulin resistance. MBL is synthesized in human liver tissue, but not in human adipose tissue. Inter-individual differences in MBL depend primarily on MBL genotype, and may not be modifiable by lifestyle interventions.

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Insulin resistance and anemia markers

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Aim
In our study, in regard with the women cases of obesity, we have aimed at determining the correlation that existed among the anemia markers, insulin resistance (IR) and fact of Metabolic syndrome (MetS).
Method
We have conducted our assessment 67 women who had resorted to our clinic due to their obesity complaints and were in the premenopausal and mean age 36.7 ± 8.8 years. We have put forward the demographic data and measured the biochemical (fasting and postprandial blood glucose, uric acid, insulin, lipid profile, leptin, visfatin, hCRP) and hematologic (hemogram, ferritine, folate, vitamin B12) parameters. Such cases were included in three different groups; HOMA-IR>2.2 and HOMA-IR<2.2 and BMI of overweight-obese-morbid obese and whether or not a diagnosis, was established as MetS.
Results
The correlation analysis which was performed on whole group served to indicate that ferritine had a correlation with factors such as weight, fat%, leptin, visfatin, h-CRP. In the grouping process which was performed according to HOMA-IR, weight, waist circumference, fat%, fasting-postprandial glucose, triglyceride were found significantly high in the HOMA-IR>2.2. In the grouping process which was performed according to obese-morbid obese systolic blood pressure(SBP) and h-CRP were found significantly in favor of morbid obese group. The comparison of persons who were overweight and morbid obese the SBP, HOMA-IR, leptin, h-CRP were found significantly high in the group of morbid obese. In the grouping process which was carried out according to the

diagnosis of MetS the SBP, weight, waist circumference, WHR, fasting-postprandial glucose, uric acid, triglyceride, HOMA-IR MetS group.
Conclusion
The existence of correlations that is determined in all cases by the ferritine has a unique nature as to support the hypothesis which is brought forward in the course of some studies whether or not the ferritine might be considered a parameter of MetS. However, the statistical data obtained from the comparison of groups that are formed according to the values of BMI, MetS and IR, have not enabled the continuance of such hypothesis. The assessment to be conducted in the future groups wherein the number of cases would be rather high, will enable us to crystallize our data on this subject.

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Vasomotor dysfunction and alterations of adipokines in metabolic syndrome patients with insulin resistance

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Aim
To evaluate relationships between adipokines (adiponectin, resistin, leptin, interleukin-6, tumor necrosis factor-alpha), insulin resistance and cutaneous vasomotor responses in metabolic syndrome (MS) patients with insulin resistance. MS patients with insulin resistance were divided into two groups: 18 patients with type-2 diabetes mellitus (without insulin therapy and pronounced diabetic complications) (DM) and 18 patients without DM. Of 18 healthy subjects were selected as controls (C). The study groups were matched for age and sex. Insulin resistance was measured by HOMA-IR method (IMx Abbott analyzer) and adipokines were measured by xMAP technology (Luminex-200 analyzer). We recorded changes in laser Doppler flux (LDF; PeriFlux 4001, Perimed) in the foot. The following variables were measured: basal LDF (b-LDF), postocclusive hyperemia (m1-LDF), vasoconstrictor response (v-LDF) to deep inspiration on the pulp of the toe; and heat (+44 °C; PeriTemp 4005) induced hyperemia (m^2-LDF) on the dorsum of the foot.
Results
Only the patient group with diabetes demonstrated a significant diminution in v-LDF compared to the group of healthy subjects ($P<0.05$). m1-LDF was decreased in both patient groups in comparison with the group of controls ($P<0.05$), but only in diabetics the decrease of m2-LDF was significant ($P<0.05$). Adipokines levels were changed ($P<0.05$) in diabetic patient group. Our findings show that MS patients with insulin resistance have significant cutaneous vasomotor dysfunction but diabetics (with insulin resistance and MS) have also changed adipokines levels.

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Effect of pharmaceutical intervention on serum levels of advanced glycated end products in women with polycystic ovary syndrome

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Background
Polycystic ovary syndrome (PCOS) is associated with obesity and insulin resistance, two major contributors to cardiovascular risk. Even in the absence of detectable insulin resistance, lean, non-diabetic PCOS women demonstrate increased circulating levels of advanced glycated end products (AGEs), known proatherogenic molecules. This adverse cardiovascular risk profile should be considered in the syndrome's management.
Objective
To investigate whether oral contraceptives (OCPs) or metformin, the commonest pharmaceutical treatments of PCOS, affect serum AGEs levels in PCOS women.
Patients-methods
Of 48 lean, nondiabetic PCOS women were randomized to the following treatments for 3 months:
Group A: 16 patients (mean age: 22.5 years, mean BMI: 21.76 kg/m^2) received an OCP containing 30 μg ethinylestradiol plus 3 mg drospirenone.
Group B: 16 patients (mean age: 21.19 years, mean BMI: 21.09 kg/m^2) received an OCP containing 35 μg ethinylestradiol plus 2 mg cyproterone acetate (CA).
Group C: 16 patients (mean age: 20.75 years, mean BMI: 21.68 kg/m^2) received metformin (1700 mg/day).
Serum AGEs levels were determined before and after 3 months of treatment.
Results
The three groups had similar age, BMI and AGEs levels at baseline. The BMIs remained unaltered in all treatment groups. Post treatment mean serum AGEs levels were not significantly altered in Groups A (pre vs post: 9.383 ± 0.22 vs 8.900 ± 0.5, $P=0.66$) and B (pre vs post: 9.980 ± 0.15 vs 10.270 ± 0.20, $P=0.22$), while they significantly decreased in Group C (pre vs post: 9.310 ± 0.33 vs 8.840 ± 0.32, $P=0.02$).
Conclusions
For the first time, OCPs are shown to lack significant effects on circulating AGEs in PCOS women, at variance with metformin which is confirmed to reduce AGEs levels, in accord with previously published data. Thus, metformin may be superior to OCPs in alleviating the cardiovascular risk associated with PCOS.

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Insulin-resistance and abnormal glycaemia characterize the metabolic syndrome of polycystic ovary syndrome but not of matched controls

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Background
The incidence of metabolic syndrome (MetS) is higher in polycystic ovary syndrome (PCOS) patients than in the general population. However, it is still unclear if MetS is qualitatively different in PCOS.
Aim
To compare the distribution of MetS diagnostic criteria and of insulin-resistance in subjects with MetS (PCOS versus matched controls).
Patients
Of 97 patients with PCOS (Rotterdam criteria) and 31 age- and BMI-matched eumenorrheic, non-hirsute women were recruited at the Institute of Endocrinology, Bucharest, Romania. All subjects were examined and waist circumference, BMI and blood pressure (BP) were recorded. Blood lipids, glycemia and insulinemia were measured after an overnight fast and a standard oral glucose tolerance test (OGTT) was performed. All samples were collected during the early follicular phase of the menstrual cycle.
Results
According to IDF criteria, MetS was present in 33% of PCOS patients and in 29% of controls (P=NS). No significant differences were found between the PCOS patients with MetS (group A, $n=32$) and controls with MetS (group B, $n=9$), regarding serum triglycerides, HDL-cholesterol, BP values and waist circumference. Impaired fasting glucose (IFG) or type 2 DM was more frequent in group A than group B (40.63% versus none, $P<0.05$).
Insulin-resistance (HOMA-IR> 90th percentile of a Romanian female population reference) was more frequent in group A than group B (55.17 vs 12.5%, $P=0.04$). Insulin-resistance was strongly associated with MetS ($P<0.0001$, χ^2) within the whole PCOS group, but not in the control subjects. Furthermore, PCOS patients with MetS had higher 2hr-glucose during OGTT than patients without MetS (125.2 ± 6 vs 97.8 ± 4.1 mg/dl, $P<0.001$; mean$\pm$S.E.M).
Conclusions
The incidence of each MetS criterion is not different between MetS subjects with PCOS and matched MetS controls, except for IFG / type 2 DM. In PCOS, insulin-resistance is more frequent and correlates strongly with MetS.

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Regulation of novel metabolic regulator fibroblast growth factor-21 by body adiposity and hypercortisolemia: studies in patients with obesity and Cushing's syndrome

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Fibroblast growth factor-21 (FGF-21) is a novel regulator of metabolic homeostasis that improved diabetes compensation and dyslipidemia in diabetic

mice and monkeys. However, little is known about its regulation in humans. Cushing's syndrome (CS) is characterized by endogenous hypercortisolism and is often associated with numerous metabolic abnormalities. The objective of this study was to test the hypothesis that CS is associated with altered levels of FGF 21 that may in turn contribute to some metabolic disturbances and altered endocrine function of adipose tissue in these patients.
Biochemical, hormonal and anthropometric parameters, plasma levels of insulin, FGF-21, adipocyte fatty acid binding protein (AFABP) and adiponectin were measured by standard laboratory methods and commercial RIA (insulin) and ELISA kits (FGF-21, adiponectin, AFABP) in 16 patients with active CS, 20 patients with simple obesity (O) and 50 healthy controls (C).
BMI, insulin levels, HOMA index and AFABP were significantly higher in O and CS groups relative to C while serum adiponectin levels were decreased in both O and CS groups relative to C. Plasma FGF-21 levels were significantly higher in CS group relative to C (483.5 ± 121.5 vs 197.3 ± 36.6 pg/ml, $P = 0.002$) but they did not significantly differ from O group (246.8 ± 35 pg/ml). In a combined population of all three groups FGF-21 levels significantly positively correlated with waist circumference and percentage of truncal fat mass, blood pressure, triglyceride levels, HOMA index, insulin, glycated hemoglobin, leptin and AFABP levels and were inversely associated with plasma protein, albumin, HDL-cholesterol and free triiodothyronine levels.
We conclude that both obesity and CS are associated with paradoxically increased FGF-21 levels suggesting a possibility that resistance to FGF-21 rather than its deficiency may contribute to some metabolic disturbances in these patients.
Acknowledgements
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The influence of weight lose on immune system

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Aim
The prevalence of overweight and obesity is increasing and becoming a global health problem. Obesity is the cause of increased cardiovascular morbidity and mortality and psychosocial problems. There are several reports which mentioning the unfavourable effects of aggressive weight loss regimes on the immune system. This study was designed to search for the effect of a straightforward, controlled and permanent weight loss program on the immune system.
Material and method
Overall 76 patients who have body mass indexes (BMI) higher than 30 kg/m^2 were enrolled. The number of the control subjects was 139. Any chronic systemic illness, chronic drug intake, smoking and alcohol intake were the exclusion criteria. The venous samples were taken both before and after the standard diet and exercise program. The percentage of the lymphocyte subgroups (CD3, CD4, CD8, CD19, CD3, CD16, CD56) were measure by flow cytometry.
Results
According to the results the CD3+ and CD8+T cells were significantly lower and the CD19+B cells were significantly higher in the obese patients when compared to the controls. After a 3 months duration of diet and exercise 26 patients of the total group lost about a 5% of their total body weight. After the weight loss the CD3+ and CD8+T cells, CD19+B cells, CD4/CD8 ratio increased, and CD8+T cells decreased. When the patients who lost weight were compared with the healthy controls, the CD8+T cells and CD3-CD16+56+ NK cell ratios were lower and the CD19 B+ cells were higher.
Discussion
According to the results it can be inferred that the adaptive component of cellular immunity is decreased with a compensatory increase in the humoral immune response in patients with obesity. Weight loss does not improve the number of cytotoxic T cells and cause a further decrement in the other cytotoxic CD3-CD16+56+ NK cells

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Plasma levels of uric acid in the patients with fatty liver

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Backgraound
The liver occupies a central position in lipid metabolism. Free fatty acids (FFAs) are taken up by the liver to join the hepatic pool of FFA, a portion of which the liver synthesizes. Fatty liver occurs when lipid accumulation exceeds the normal 5% of liver weight. In the macrovesicular type, large fat droplets balloon the liver cell, displacing the nucleus to the periphery of the cell, like an adipocyte. Triglyceride accumulates most commonly because it has the highest turnover rate of all hepatic fatty acid esters. We investigated the plasma levels of uric acid in the patients with fatty liver and to correlate it with ultrasonographically measured liver echogenity.
Methods
Fasting plasma levels of cholesterol and triglycerides were detected in the 91 patients with fatty liver and 47 healthy subjects. Liver echogenity was measured by 3.75 mHz ultrasound probe and was graded by comparison with renal paranchymal echogenity.
Results
Fasting plasma levels of was detected in the ninetyone patients with fatty liver and fortyseven healthy subjects. In the fatty liver group, plasma levels of uric acid was 5.3 ± 1.8 (1.8–11.0) mg/dl. The grading of liver echogenity was 1.9 ± 0.6 (1–3) in the fatty liver group. In the control group, plasma levels of uric acid was 4.4 ± 1.3 (2.1–7.3) mg/dl. The grading of liver echogenity was 1.0 ± 0.1 (1.0–1.0). Plasma levels of uric acid were higher in the fatty liver group those of the control group (P(0.05).
Conclusions
Hyperuricemia has been found 80 per cent in hypertriglyceridemic patients. However, hypertriglyceridemia has been found 50–75 per cent in patients with gout. Several studies have been detected hyperuricemia in patients with hyperlipidemia and fatty liver. We observed that there is hyperlipidemia and hyperuricemia in the patients with fatty liver.

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The atherogenic index in the fatty liver

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Backgraound
Fatty liver can relate with arteriosclerosis. We investigated the atherogenic index in the patients with fatty liver.
Methods
Ninety-one patients with hyperlipidemia and 47 healthy subjects were included in the study. Fasting plasma levels of lipids were detected. Liver echogenity was measured by 3.75 mHz ultrasound probe and was graded by comparison with renal paranchymal echogenity. Atherogenic index was calculated as the ratio of plasma levels of cholestrol to plasma levels HDL – cholesterol.
Results
In the hyperlipidemia group, plasma levels of cholesterol were 253.5 ± 41.0 (161–440) mg/dl, plasma levels of triglycerides were 231.8 ± 74.4 (45–493) mg/dl, grading of liver echogenity was 1.9 ± 0.6 (1–3) and atherogenic index was 4.9 ± 1.1 (2.8–8.5). In the control group, plasma levels of cholesterol were 173.4 ± 19.1 (122–207) mg/dl, plasma levels of triglycerides were 110.5 ± 39.3 (40–185) mg/dl, grading of liver echogenity was 1.0 ± 0.1 (1.0–1.0) and atherogenic index was 3.4 ± 0.7 (1.6–5.5). There were significant differences between plasma levels of lipids, liver echogenity and atherogenic index. In addition, there was a significant correlation between atherogenic index and liver echogenity in the fatty liver group ($r = 0.4$, P(0.0001).
Conclusions
We found that atherogenic index was higher in the fatty liver group than the control group. In addition, there was a significant correlation between atherogenic index and liver echogenity in the fatty liver group.

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Energy and nutrients intake among overweight/obese school children in Tehran

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Introduction
Overweight and obesity are being emerged as one of the most prevalent nutritional problems among children in developed and developing countries.

To decrease the rate of them there is need to know more about effective factors.
Objective
This study aimed to determine association between energy and nutrients intake with overweight / obesity in male and female school children of Tehran.
Methods
A sample of 761 school students (378 from first graders & 383from grades 2nd to 5th) was randomly selected using a multistage cluster sampling method, from all 19 educational districts in Tehran. Weight and height of the children were measured and data on food consumption were also collected by a 24-hour recall. Overweight and obesity was evaluated using body mass index (BMI) centiles for age and sex. Obesity was defined as BMI $>$ or $=$95th percentile and overweight was $>$ or $=$85 to $<$95th percentile of the sex-specific BMI-for-age growth charts of CDC, 2000. Overweight and obese children were named 'overweight' versus other students who were named 'normal'.
Results
Energy intake was positively correlated to fat intake and BMI of first graders ($r=0.76$, $r=0.15$, $P<0.01$; respectively). Energy was also positively correlated to fat intake and BMI of others ($r=0.75$, $P<0.01$ & $r=0.10$, $P<0.05$; respectively). Overweigh first graders had lower intake of calcium and higher intake of riboflavin ($P<0.05$). Other overweight schools had higher intake of fat ($P<0.01$). Male and female overweight first graders were not different in energy or nutrients intake but female overweight children of other grades had higher intake of energy ($P<0.01$). Rate of overweight was not significantly different in girls and boys.
Conclusion
This study confirms other data which indicate over consumption of energy and fat may contribute to childhood obesity. Energy intake seems to be unhealthy among girls in this study.

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Influence of finasteride treatment on metabolic profile of men with androgenetic alopecia

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Androgenetic alopecia, not only caused psychological distress, but also is the risk factor of cardiovascular diseases, glucose metabolism disorders, benign prostate hyperplasia and prostate carcinoma and suspected to present the sign of male equivalent of polycystic ovary syndrome. Finasteride, used for treatment of androgenetic alopecia in dose of 1 mg/day, is the first 5α-reductase type II inhibitor. The 5α-reductase is enzyme responsible for the reduction of testosterone to dihydrostestosterone, progesterone to dihydroprogesterone and deoxycorticosterone to dihydrodeoxycorticosterone. Following recent observation, dihydrotestosterone plays the role in visceral fat metabolism. The aim of the study was to assess the effect of 1 year treatment of 1 mg finasteride on hormonal levels, lipid spectrum and insulin sensitivity in men with premature androgenetic alopecia. The study included 30 men with premature hair loss (defined as grade three vertex or more on the alopecia classification scale of Hamilton with Norwood modification) starting before 30 years of age. In all individuals, the levels of total testosterone, androstenedione, dehydroepiandrosteron sulfate, dehydroepiandrosterone, epitestosterone, allopregnanolone, dihydrotestosterone, and further reduced androstane metabolites, cortisol, estradiol, SHBG, prolactin, TSH, LH, FSH, index of free testosterone, cholesterol, HDL, LDL, triacylglycerols and insulin tolerance test were determined. Finasteride in the daily dose of 1 mg was administered for 12 months. The same hormonal profile and lipid spectrum was monitored after 4, 8 and 12 months of the treatment and insulin tolerance test was repeated after 12 months of the treatment. Besides the decrease of dihydrotestosterone level after treatment, the alteration in further 5alfa- steroids metabolites was found. However the metabolic state remained unchanged. The study was supported by grant No. NR/8525 – 5 of the IGA MZCR.

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Prevalence of hypo- and hypermagnesemia in an urban population

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Background
Magnesium is the fourth most abundant action in the body and plays an important role in metabolic processes. Association between magnesium deficiency and clinical condition including hypertension, diabetes, insulin resistance, hyperlipidemia, and atherosclerosis. Although magnesium deficiency is associated with a variety of medical conditions, the prevalence of hypo- and hypermagnesemia have not been reported in general population.
Methods
This cross-sectional study was performed on 1558 (754 males and 804 females) with the mean age of 39.9 ± 14.3 years, who were selected by a multistage cluster random sampling method. Serum magnesium level was measured by flame atomic absorption spectrophotometry. The reference range for serum magnesium was 0.75–0.95 mmol/l.
Results
The prevalence of hypomagnesemia was 4.6% (95% CI, 3.6–5.6) in total population. Hypomagnesemia was more prevalent in females (6.0%) compared to males (3.2%), ($P<0.01$). Prevalence of hypermagnesemia was 12.2% (95% CI, 10.6–13.8) and was more prevalent in males (13.9%) than females (10.6%), ($P<0.01$).
Conclusion
Our data show a relatively high prevalence of abnormal levels of serum magnesium among general population; which may contribute to the pathophysiology of some diseases.

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The effect of α-lipoic acid on myostatin and myosin heavy chain isoform profile of skeletal muscle of OLETF rats

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Aims
To investigate the mechanism of α-lipoic acid (ALA) on preventing diabetes, we evaluated the effect of ALA on growth regulation and composition of skeletal muscle.
Methods
OLETF and LETO rats, aged 24 weeks, were treated with or without ALA (100 mg/kg body weight/day in drinking water) and / or insulin (insulin glargine, 2 U/kg body weight/day; subcutaneous injection) for 8 weeks.
Results
The treatment of ALA with or without insulin reduce body weight significantly and save relatively larger amount of skeletal muscle mass from gastrocnemius compared with insulin-alone treated group and control group of OLETF rats. Myostatin gene expression in skeletal muscle was decreased in ALA-alone and insulin alone treated group compared with control group of OLETF and LETO rats. The isoform profile of MHC was significantly increased in MHC I, MHC IIa and MHC IId/x in ALA-alone treated group compared with the control group of OLETF rats.
Conclusion
The short-term treatment with ALA showed preventing weight gain, preserving skeletal muscle mass, decrement of myostatin gene expression and increment of both MHC I and IIa gene expression.

P499

Comparing the activity of orlistat and sibutramine on obesity treatment

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Introduction
In this study, we aimed to examine the weight alterations after the medical treatment and compare the effects of medical treatment in obese patients who applied to Endocrinology Policlinic.
Material and method
We examined the patients who were diagnosed as obesity and initiated medical treatment at Endocrinology and Metabolism Diseases Policlinic in Inonu University Medical Faculty between Agust 2005–May 2008. Patients were divided into two groups. In the first group, we applied 120 mg orlistat three times per day before each meal. In the second group, we applied 15 mg sibutramine

once a day before breakfast. We evaluated the patients at the beginning and at the end of the third month of the treatment.
Inventions
Out of the total 342 patients, 172 patients were treated with orlistat and 170 patients were treated with sibutramine. The mean values of age, height, weight and BMI were similar at the initial evaluation ($P>0.05$). Mean values of weight, BMI of the patients at the initial and the third month controls who were treated with orlistat (group 1) and sibutramine (group 2) were demonstrated on Table 1. There was no significant difference between two groups in means of third month values after the treatment.
Conclusion
Both of the drugs were found as effective in losing weight. There was no significant difference between these two drugs in means of weigt loss.

Table 1 The values of Group 1 and Group 2 at the initial evaluation and the third month control.

	Orlistat (Group1, $n=172$)			**Sibutramine** (Group 2, $n=170$)		
	Initial	3 months	*P* value	Initial	3 months	*P* value
Weight (kg)	96.56± 14.48	84.15± 12.86	<0.01	94.55± 12.57	81.96± 10.61	<0.01
BMI (kg/m^2)	37.60± 5.19	33.81± 5.11	<0.01	37.39± 4.72	33.96± 6.51	<0.01
Waist (cm)	113.28± 11.20	102.45± 10.91	<0.01	111.66± 13.89	101.80± 13.33	<0.01

P500

Normal metabolic flexibility despite insulin resistance in lean and obese women with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is heterogeneous disorder, where insulin resistance might be involved in the development of endocrine and metabolic abnormalities. Insulin resistance is associated with the so-called metabolic inflexibility i.e. an impaired switch from lipid to glucose in response to insulin. The aim of the present study was to estimate glucose and lipid oxidation, metabolic flexibility and non-oxidative glucose metabolism in lean and obese PCOS patients.
The study group consisted of 72 women with PCOS (28 lean and 44 overweight or obese) and 26 healthy, normally menstruating women (13 lean and 13 overweight or obese). Euglycemic hyperinsulinemic clamp and the measurements of serum sex hormones were performed. Glucose and lipid oxidation was evaluated with indirect calorimetry in the baseline state and during the last 30 min of the clamp. Non-oxidative glucose metabolism in the hyperinsulinemic state was calculated by subtracting glucose oxidation from the total glucose metabolism. Metabolic flexibility was assessed as an increase in respiratory quotient (ΔRQ) in response to insulin. To evaluate the impact of obesity and PCOS on the studied parameters and the interaction between both conditions, general linear models were constructed.
Both PCOS ($P<0.0001$) and obesity ($P=0.0045$) were associated with lower insulin sensitivity. No significant interaction between PCOS and obesity was found. Similarly, PCOS ($P=0.00078$) and obesity ($P=0.009$) independently predisposed to the lower non-oxidative glucose metabolism. Obese women had lower glucose oxidation ($P=0.0097$) and higher lipid oxidation ($P=0.0001$) in insulin-stimulated conditions whereas PCOS had no effect on these parameters. Metabolic flexibility was impaired in obese ($P=0.0015$) but not in PCOS women. Our data indicate that lean and obese PCOS women have normal metabolic flexibility, which could suggest a distinct pathophysiological mechanism for insulin resistance in this group.

P501

Lipid levels in anorexia nervosa patients

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Anorexia nervosa (AN) is a chronic disorder characterized of profound reduction in body weight and amenorrhoea. Despite emaciation and avoidance of cholesterol-rich food, hypercholesterolaemia is a common feature. The aim of the study was to investigate the changes in lipid metabolism with weight gain in 17 severe AN patients aged 23.8±1.6 years (DSM-IV criteria). They were compared with age-matched cohorts of 11 healthy controls (22.5±3.9 years) before and after weight gain. For a 12 weeks period, AN patients were on a special hypercaloric diet 2500 cal/24 h. After overnight fast serum cholesterol, HDL-cholesterol, LDL-cholesterol (cholesterol-oxidasa method) and triglyceride (glycerol 3 phosphat oxidasa method) levels were measured. Relevant parameters are presented as mean±S.E.M. Although AN patients increased body weight (from 42.3±1.6 to 53.3±0.9 kg, $P<0.05$) and BMI (from 14.3±0.4 to 19.5±0.4 kg/m^2, $P<0.05$), no significant decrease (although a slight fall) were found in serum cholesterol levels (from 5.9±0.6 to 5.5±0.3 mmol/l), HDL-cholesterol (from 1.8±0.4 to 1.5±0.7 mmol/l), LDL-cholesterol (from 4.1±0.9 to 4.0±1.2 mmol/l) and triglyceride (from 1.0±0.9 to 1.2±0.5 mmol/l). AN patients had lower body weight and BMI at the beginning of the study compared with healthy females (42.3±1.6 vs 56.8±2.9 kg, 14.3±0.4 vs 21.7±2.9 kg/m^2, $P<0.01$). After weight gain, there were no significant differences in body weight and BMI between AN patients and controls (53.3±0.9 vs 56.8±2.9 kg, 19.5±0.4 vs 21.7±2.9 kg/m^2, $P>0.05$). There were no significant differences in serum lipid levels between AN patients (before and after weight gain) and healthy controls ($P>0.05$).
Conclusion
Our results favour relationship between body weight and cholesterol value, as it is showed that cholesterol levels fall after increase in body weight. So it is considered that patients with AN and hypercholesterolemia do not need treatment with lipid-lowering drugs.

P502

Prevalence of the metabolic syndrome in men with late-diagnosed Klinefelter syndrome

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Introduction
Klinefelter syndrome (KS) is the most common sex-chromosome disorder with a prevalence of 1 in every 660 men, but only 25% of the expected number of patients are diagnosed. It is also one of the most common endocrine disorders, and takes the third place after diabetes mellitus and thyroid gland diseases. KS is one of the most frequent causes of hypogonadism in men. It is known that hypogonadism in men has been found to be an independent risk factor for development of abdominal obesity and metabolic syndrome (MS).
Objective
To study the prevalence of MS in men with KS.
Materials and methods
Twenty-six men (16–37 years old) with KS (karyotype 47,XXY) diagnosed in the age of 15–37 years were examined for presence of MS (IDF criteria).
Results
Ten from 26 patients with KS (38.5%) had MS (IDF criteria). Moreover, 8 from this 10 patients with MS (80%) were late-diagnosed (diagnosis of KS was determined after 24 years old).
Conclusion
MS is frequent in men with KS and associated with late diagnosis of this syndrome and late beginning of testosterone-replacement therapy. According to the high prevalence and poor diagnosis of this disorder these patients often remain without timely treatment.

P503

Metabolically healthy but obese individuals

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Introduction
Obesity is a heterogenous disorder. One of its subtypes that has been recently described is termed the metabolically healthy, but obese (MHO) individuals. Preliminary evidence suggests that this could be due to lower visceral fat levels and earlier onset of obesity.
Objectives
To determine the prevalence of MHO in a sample of obese patients. To evaluate whether body mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR) are significantly different between MHO and obese non metabolically healthy (NMHO) individuals.
Methods
The authors present a retrospective analysis of 254 obese patients (223 women and 31 men) evaluated in the obesity outpatient clinic of Hospital São João. Anthropometric variables, blood pressure (BP), fasting plasma levels of glucose (FPG), HDL cholesterol (HDL-C), triglycerides (TG) were measured. Insulin resistance (IR) was evaluated by the following index: homeostasis model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI) and insulin sensitivity index-Matsuda (MATSUDA). MHO was defined by TG $<$ 150 mg/dl, HDL-C $\geq$ 40 mg/dl (men) or $\geq$ 50 mg/dl (women), FPG $<$ 100 mg/dl, BP $<$ 130/85, HOMA-IR $<$ 2.5, QUICKI $>$ 0.33 and MATSUDA $>$ 5. Pearson's correlation coefficient, Student's *t*-test and Fisher's exact test were used for the statistical analysis.
Results
Patients had mean age of 39.7 $\pm$ 11.2 years and mean BMI of 45.1 $\pm$ 6.6 kg/m^2. About 8.3% of these patients were MHO when all the above criteria are considered. If IR wasn't considered, the percentage increased to 15.7%. Patients classified as MHO had a significantly lower BMI, WC and WHR. Their age wasn't significantly different from NMHO individuals. BMI was positively correlated with all the IR index, WC ($r = 0.61$; $P < 0.00001$) and WHR ($r = 0.14$; $P < 0.05$).
Conclusions
In this study, only a small percentage of the obese patients were MHO. It almost doubled when IR was not considered, suggesting that this parameter is of early onset.

P504

Effects of stress on kidney: a histochemical study on rat model

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Objective
Stress, which is seen prevalently among people, causes a general situation of tension via damaging the balance of the body. During stress, it is known that, at the first, central systems, like nerve system, heart and blood circulation system, nutritive system, genital systems and adrenalin tissues are also affected. In this study, kidney was histopathologically examined on the rat model in order to investigate how and in what level the kidneys are affected from the stress.
Methods
Eighteen adult Sprague Dawley rats were used in this study. Rats were grouped into the 4 group as the control male group ($n = 4$), stress male group ($n = 4$), stress female group ($n = 4$) and control female group ($n = 4$). Chronic mild stress (CMS) model of depression was performed to the stress group animals during two weeks. At the end of the test, rats were anesthetized with ketamin HCI. Their kidneys were removed with opening the abdomens and kidney volumes were measured with the water immersion method. After routine histological processing, samples were histopathologically examined under light microscope.
Results
When compared with the control groups, volumes of stress performed kidneys did not change in female rats ($P > 0.05$; independent samples *t*-test) but kidney volume was significantly decreased in males ($P < 0.05$; independent samples *t*-test). In the sections of the test group, epithelial outpouring and degeneration and also luminal extension were observed in kidney tubules. Cytoplasmic bulge and vacuoles in epithelial cells of tubule were defined. Once again, when both groups were compared with control groups, puckered glomerulus and extended Bowman space were observed. Also in the epithelium of loop of Henle; cell bulge, vacuolization and degeneration were observed.
Conclusions
In the light of our findings, it is concluded that stress has a negative effects on kidney structure. In this sense, it is thought that functions of kidney can be disordered in stressful people.
Acknowledgement
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P505

Anthropometric parameters and non alcoholic hepatic steatosis in type 2 diabetes

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Aim
The aim of the study was to assess the association between anthropometric parameters and non alcoholic hepatic steatosis in type 2 diabetes.
Methods
Computed tomography imaging was used to assess hepatic fat content in 80 men and women with type 2 diabetes. Inclusion criteria included a confirmed diagnosis of type 2 diabetes ($\geq$1 year of duration), without history of hepatic disease or daily consumption of alcohol drink. Clinical and biochemical variables were examined with univariate and multivariate analysis. Receiver operating characteristic (ROC) curves were used to identify the sensitivity and specificity.
Results
The global prevalence of hepatic steatosis was 30% (24/80). Body mass index and waist circumference were significantly higher in diabetes with steatosis (respectively 33.3 $\pm$ 6 vs 29.5 $\pm$ 7.4 kg/m^2 $P = 0.038$ – 109 $\pm$ 12.4 vs 109 $\pm$ 12.4 cm $P = 0.0004$). BMI $\geq$ 30 kg/m^2 and WC $\geq$ 94 cm were significantly associated with an increased risk of hepatic steatosis (respectively: OR = 4 CI 95% 1.4–12.5 $P = 0.005$ – OR = 5.6 CI 95% 1.2–26.6 $P = 0.017$).
Conclusion
Obesity and visceral fat distribution are a high risk factor of hepatic steatosis in type 2 diabetic patient. Life style intervention must be intensifying in obese diabetic patient to improve insulin sensitivity.

Endocrine Disruptors

P506

Reproductive and haematological toxicity of Nurelle D 220 EC in male rats

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The present study was carried out to investigate the possible toxic effects of different doses of a two component of Nurelle D 220 EC (200 g of chlorpyriphos-ethyl and 20 g cypermethrin per 1 l) on blood hematology, testosterone and thyroxin levels, and semen quality of male rats. As well as histological examination of testis and epididymis. The investigation covered four groups of 8 rats each: three experimental groups and one served as control. The mixture liquid was given to rats by oral route at different doses in the form of a water solution. Experimental groups received 5, 10 and 15 mg/kg bw/day of the insecticide for 6-weeks. At the end of the experimental period, the animals were sacrificed and blood samples were collected for measuring the hematological parameters and serum levels of testosterone and thyroxin. Testes and epididymes were removed for measuring semen quality and histology. The obtained results showed that all three doses of Nurelle D caused significant decrease in the body weight gains. Weights of testes, epididymes and seminal vesicles were significantly decreased, while weights of liver and kidney were significantly increased in rats receiving 15 mg/kg only. Hematological study showed that the insecticide Nurelle D caused a pronounced change in blood parameters especially in the highest dose. Similarly, the dose of 15 mg/kg induced significant decrease in spermatids number, sperm count, motility and daily sperm production (DSP), while dead and abnormal sperm, and sperm transit rate were significantly increased. This was proved histopathologically by the pronounced alteration of architecture of epididymes and testes with dramatically reduce of spermatozoa

produced in lumen of testes accompanied by a significant reduction of tubular diameters. Such observations were coupled with a reduction in plasma testosterone levels and an increase in plasma free thyroxin (FT4) levels compared to control. It is, therefore assumed that treatment with Nurelle D up to 15 mg/kg bw alters both hematological and reproductive parameters in rats, and subsequently affects fertility.

P507

The association between organochlorine compounds, iodine intake and thyroid hormones during pregnancy

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Objective
An adequate thyroid function during pregnancy is essential for the normal brain development of the foetus, and some organochlorine compounds (OCs) can interfere with the thyroid system. The objective of the present study was to evaluate the association between exposure to some OCs and thyroid function in pregnant women from two different areas in Spain, as well as to investigate the potential confounding effect of iodine intake.
Methods
Thyroid hormones (free T4 and T3) and TSH concentrations, and levels of polychlorinated biphenyls (PCB congeners 118, 138, 153 and 180), hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), dichlorodiphenyl dichloroethylene (p′p′-DDE) and dichlorodiphenyl trichloroethane (p′p-DDT) were measured in 1187 pregnant women from two population-based cohort studies. Urinary iodine concentrations (UIC) were analyzed in spot urine samples and iodine intake from diet, iodized salt and supplements were estimated from a food frequency questionnaire. The association between OCs (log-transformed) and thyroid parameters was assessed using linear regression models adjusted for potential confounders.
Results
Levels of HCB, β-HCH and PCBs (congeners 138, 180 and 153) were related to lower total T3 levels (adjusted coefficient (P value): -3.8 ($P<0.001$), -1.8 ($P<0.05$), -3.3 ($P<0.01$), -4.0 ($P<0.001$), and -4.1 ($P<0.001$), respectively) and higher free T4 levels (adjusted coefficient (P value): 0.014 ($P<0.001$), 0.010 ($P<0.05$), 0.010 ($P<0.05$), 0.018 ($P<0.001$), and 0.016 ($P<0.001$) respectively). The associations with total T3 were homogenously observed in both cohorts. Iodine intake was not related to OCs exposure.
Conclusions
Total T3 appears to be the main target of the toxicity of OCs during pregnancy. Moreover, iodine intake may not affect the association between OCs and TH.

P508

Histopathologic changes of exocrine and endocrine pancreas in stress-exposed female rats

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Objective
Stress has been linked to several diseases. In the course of stress; blood sugar. cortisol and catecholamine levels increase. Visceral hypersensitivity will increase due to catecholaminergic discharges leading to an over-induction of the intrapancreatic secretion. A chronically stress has a role in the induction of insulin resistance in different tissues and pancreas begins to release excessive insulin causing burned out pancreas. For this reason, it becomes insulin insufficiency, elevated blood sugar and risk of type 2 diabetes.
Aim
In this study, our aim was to investigate the histopathologic influence of stress on the pancreas at light microscopic level on the rat model.
Methods
Animals were divided into two groups, stressed and control ($n=4$/group). Rats of the stressed group were exposed to Chronic mild stress (CMS) model of depression for 2 weeks. During the experiment, rats were given food and tap water ad libitum. At the end of the test, rats were slept with ketamin HCI and sacrificed. The pancreas was totally removed with opening the abdomens and fixed with 10% formaldehyde for histopathological evaluation. Tissue samples were blocked in paraffin blocks. The prepared 5-μ thickness sections were stained with haematoxylin–eosin and examined by light microscopy.
Results
Our histological observations have showed disruption of normal configuration of serous acini in pancreatic tissue sections of the stress-exposed animals in comparation with the control group. The borders of the acini were broken down and there were cytoplasmic vacuolations in aciner cells. Additionally, shrunken, apoptotic and necrotic acini were seen together. In stressed animals, sinusoidal dilatation and cellular degeneration were observed in the islets of Langerhans. Mononuclear cell infiltrations in the perivascular areas and vacuolations in the vascular wall were determined in the interlobular connective tissue of the pancreas.
Conclusions
According to our data, CMS can lead to evident damage in the microscopic structure of pancreas. At the same time, this case may effect negatively pancreas physiology and cause to the onset diabetes.
Acknowledgement
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P509

The effect of feeding of aerial part of *Vaccinium myrtillus* on blood glucose and lipids of diabetic rats

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Aim
Use of medicinal plants for attenuation of hyperglycemia and restoration of lipids to normal level is very important. The effect of oral administration of Vaccinium myrtillus (VM) on serum glucose and lipids in diabetic rats was investigated.
Material and methods
Female Wistar rats were divided into 4 groups, i.e. control, VM-treated control, diabetic, and VM-treated diabetic groups. The treatment groups received oral administration of plant-mixed pelleted food (6.25%) for 4 weeks. Serum glucose, triglyceride, total cholesterol, LDL- and HDL- cholesterol levels were determined before the study, and at 2nd and 4th weeks after the study.
Results
Serum glucose level in diabetic group increased 2 and 4 weeks after the experiment as compared to data one week before the study ($P<0.001$) and VM treatment of diabetic rats did have a significant hypoglycemic effect ($P<0.01$). In addition, triglyceride level in diabetic group increased 4 weeks after the experiment in comparison with related data one week before the study ($P<0.05$) and there was a significant lower level of triglyceride in VM-treated diabetic rats ($P<0.05$). Furthermore, there was no significant changes regarding serum total cholesterol, HDL- and LDL- cholesterol levels in treated diabetic group as compared to untreated diabetic group.
Conclusion
Oral administration of VM has a significant hypoglycemic effect and leads to an appropriate changes only in triglyceride level.

P510

Effect of silymarin on nerve conduction velocity, hyperalgesia and oxidative stress in experimental diabetic neuropathy

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Aim
Neuropathy is one of the potentially serious late complications of diabetes that occurs in certain tissues as a consequence of long-term hyperglycemia. Oxidative stress has been implicated to play an important role in the pathogenesis of diabetic neuropathy. In the present study, we have investigated the effect of silymarin, as a potent free radical scavenger in streptozotocin (STZ)-induced diabetic neuropathy in rats.

Material and methods
The rats were randomly divided into six experimental groups; i.e. control, vehicle-treated control, silymarin-treated control, diabetic, vehicle-treated diabetic and silymarin-treated diabetic. Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg) dissolved in cold 0.9% saline immediately before use.
Results
After 8 weeks of diabetes induction by STZ, rats showed significant deficit in motor nerve conduction velocity (MNCV) and mechanical, chemical and thermal hyperalgesia, indicating development of diabetic neuropathy. Antioxidant enzyme superoxide dismutase level was reduced and malondialdehyde (MDA) level was significantly increased in diabetic rats as compared to control rats. This indicated the involvement of oxidative stress in diabetic neuropathy. The pre-treatment of diabetic rats, 1 h before diabetic induction (200 mg/kg, i.p.) for 8 weeks post-treatment (100 mg/kg, i.p., daily) with silymarin significantly ameliorated the alteration in MNCV, hyperalgesia, MDA levels and antioxidant enzyme in diabetic rats.
Conclusion
Results of the present study suggest the potential of silymarin in treatment of diabetic neuropathy.

P511

Testosterone serum level in haemodialysed male patients positively correlates with adequacy of dialysis

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Introduction
Hypogonadism in men is the increasing problem of the present medicine, specially concerning patients suffering from chronic diseases, e.g. diabetes, chronic kidney diseases. However, there are no enough data referring to haemodialysed patients and correlation with adequacy of dialysis was not found.
Aim
The aim of the study was to assess the gonadal status of male patients receiving dialysis in our ward with reference to parameters of adequacy of renal replacement therapy.
Material and methods
Gonadal status of 51 male patients aged from 31 to 80 (mean 60.55 ± 14.3) was studied. All of them were receiving haemodialysis (3 times a week, 4–5 h for each procedure). Non-fasting plasma was analysed for testosterone, tPSA and luteinising hormone (LH). Dependence of androgens and tPSA levels upon the age and Kt/V was analysed.
Results

Table 1 Outcome Spearman rank correlation for analysed parameters.

Parameters	*N*	*R* Spearman	*T* (*N*−2)	*P* value
Kt/V & testosterone	41	0.320755	2.11486	0.040885
Kt/V & LH	41	0.011416	0.07130	0.943527
Kt/V & tPSA	41	−0.112646	−0.70798	0.483168
Age & tPSA	43	0.357105	2.448000	0.018734

Discussion
Complications observed in haemodialysed patients are results of persisting of renal failure or insufficient renal replacement therapy. This is the reason, that the laboratory tests in these patients must be analysed in reference to adequacy of dialysis. It is possible, that the results in various centres may be different, due to kind of renal disease, dialysis regime et ethnic factors.
Conclusion
In our group of haemodialysed male patients testosteron level is positively correlated with adequacy of dialysis.

P512

Effects of permethrin on sexual behaviour and plasma concentrations of pituitary–gonadal hormones in adult male NMRI mice

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Background and aim
Pyrethroids are commonly used as insecticides for both household and agricultural purposes, and recently have been shown to have detrimental effects on endocrine system. Permethrin is a type I pyrethroid which is used widely in Iran. In the present study the effects of permethrin on sexual behaviour and plasma level of PG (pituitary–gonadal) hormones of adult male mice were investigated.
Material and methods
Mice received daily Intra peritoneal injection of permethrin (10, 15, 20 mg/kg) for 5 weeks. Using receptive females, permethrin-treated male mice exhibited reduced sexual behavior (i.e. decrease in the number of sniffing, following, mounting and mating).
Results
The concentrations of plasma testosterone, LH and FSH were measured by means of ELISA method. Serum testosterone levels were reduced significantly ($P < 0.05$) in the experimental group versus control group, whereas FSH and LH values were not altered significantly.
Conclusion
The results of this study indicate permethrin can have detrimental effects on plasma testosterone level and sexual behavior. In regard to considerable use of this insecticide in Iran, it's necessary to restrain its use and extent of human contact to prevent hazards of this insecticide on human body tissues.

P513

Hyperprolactinaemia in men

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The hyperprolactinemic disorder is a syndrome of prolactin hypersecretion. The syndrome is more prevalent in women than in men, but lately accidence of the syndrome disease tends to increase in men. Hyperprolactinemic disorder mostly affects men at the age range 40–59 years. Patients at the physician's consultation have following complaints: erectile dysfunction, infertility, libido reduction, rarely gynecomastia.
Sixteen patients were examined at the Endocrinology department of the medical faculty of Iv. Javakhishvili Tbilisi State University, clinical and laboratory evaluation showed hyperprolactinemic disorder. The average age of research participants was 19–62 years. The purpose of our research was to study etiological factors of the disease, clinical setting and the optimal choice of treatment. The following tests and investigations were performed: 1) plasma prolactin 2) plasma free testosterone; 3) plasma ACTH and cortizol; 4) TSH and FT4; 5) liver function tests; 6) ultrasound densitometry; 7) MRI. Patient examination and anamnesis revealed following etiologic factors: In 4 cases the hyperprolactinemia was linked to primary hypothyroidism, in 3 cases hyperprolactinemia was caused by pharmacologic agents (2 – drug materials and 1-Ca channel blockers), in 1 case previous to hyperprolactinemia there was an onset to infectious diseases, in 4 cases adrenal gland hyperplasia was diagnosed, in 2 events – there was a trauma damage, in 2 cases of hyperprolactinemia etiological factors were not established. The choice of the treatment made was decreed on the bases of anamnesis and clinical–laboratorial investigations. Long term treatment reported positive results in 14 cases.

P514

Association of CTLA4 + 49 A/G polymorphism with type 1 diabetes in Tunisian population

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Type 1 diabetes (T1D) is a complex autoimmune disease. Several genetic loci have been implicated in the susceptibility to this illness. Evaluated was the role of the CTLA4 exon 1 A49G polymorphism and its role as a risk factor for T1D in our population. DNA from 120 patients with T1D and 96 control individuals were genotyped for CTLA4 exon 1 polymorphism by polymerase chain reaction (PCR) amplification-restriction enzyme analysis and PCR amplification that used sequence-specific primers, respectively. Patients were nonobese and <26 years old. The CTLA4 G allele was found to be more frequently present in patients with T1D (36.4%) as compared with its frequency in control individuals (18.5%). The GG genotype was also significantly higher among patients (17.6%) than in controls (7.2%). χ^2 analysis and family-based association studies were performed and

suggested the association of CTLA4 exon 1 G polymorphism with T1D ($P=0.02$). This study suggests that CTLA4 is a candidate susceptibility gene for T1D.

P515

Why does Klinefelter syndrome often remain undiagnosed?

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Introduction
Underdiagnosis and delayed diagnosis of Klinefelter syndrome (KS) is a major problem. KS is revealed only in 25% of the expected amount of patients. Early diagnosis facilitates prevention of the long-term consequences of hypogonadism and permits to begin testosterone-replacement therapy opportunely. The 'prototypic' patient with KS has traditionally been described as tall, thin, with long hips, narrow shoulders, having gynecomastia, small testes, sparse body hair and azoospermia. A less similar phenotype, however, has been described.
Objective
To study the phenotypic differences of patients with KS (karyotype 47,XXY).
Materials and methods
Twenty-six men with KS (karyotype 47,XXY) diagnosed in the age of 15–37 years were examined for height, weight, waist circumference, BMI, testes volume and presence of gynecomastia. Spermograms of 6 patients complaining on infertility were examined.
Results
Height of 14 men with KS (53.8%) was within the limits of 180–189 cm. Eight patients (30.8%) were higher than 190 cm. Height of 4 patients (15.4%) was less than 180 cm, that is the medium height of men in Russian Federation. Twelve patients with KS (46.1%) had abdominal obesity (waist circumference more than 94 cm). These were the patients with late diagnosed KS (24 years and older) and, therefore, late start of testosterone-replacement therapy. Gynecomastia was found in 16 patients with KS (61.5%). All patients had small testicular volume (from 1 till 12 cm^3) but different density of testes (from very firm till soft in 3 men and even flabby in 2 patients). We examined spermograms of 6 patients. Four of them had azoospermia and 2 had oligozoospermia of high degree.
Conclusion
The phenotype of KS patients significantly varies. All the patients with testes volume, less than 12 ml, or primary hypogonadism should pass karyotype analysis.

Paediatric Endocrinology

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Short stature in pediatric Cushing's syndrome: effectiveness of hypercortisolism cure

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Cushing's disease (CD) is the most common cause of endogenous Cushing's syndrome in children and adolescents and represents a rare cause of short stature. A 14-year-old boy came to our attention for progressive weight gain and short stature. Birth length and weight were normal; clinical history was negative for use of glucocorticoids. At examination, height was 140 cm (3th centile), weight was 37.7 kg (10th centile). Tanner stage was: G2, PH 3, testis 3 ml. Hypothyroidism and growth hormone deficiency were excluded. A marked increase of urinary free cortisol, a nonsuppressible serum cortisol after Liddle 1 test and an elevated ACTH value confirmed the diagnosis of ACTH dependent Cushing's syndrome. Pituitary MRI showed a left microadenoma and an other right focal area of lesser enhancement. Therefore, bilateral inferior petrosal sinus sampling (BIPSS) with CRH stimulation was performed to obtain an accurate preoperative localization of the adenoma: the interpetrosal sinus ACTH gradient indicated lateralization of ACTH secretion to the left side. The patient underwent transsphenoidal surgery with selective microadenomectomy, with an immediate ACTH decline in the postoperative phase. Histology confirmed the diagnosis of corticotrophic pituitary adenoma. Glucocorticoid replacement therapy was instituted. Clinical examination demonstrated a rapid catch-up growth (10th centile), with a normalization of body mass index and an adequate pubertal development.
This is a rare case of pediatric Cushing disease: one of the most reliable indicators of hypercortisolism in these patients is growth failure associated with weight gain while laboratory data and pituitary MRI are very important tools to confirm the clinical suspicion. In our case, BIPSS was necessary to lateralise the site of ACTH production, because of the co-existence of an ACTH secreting microadenoma and a pituitary 'incidentaloma'. Transsphenoidal surgery allowed a successful remission of hypercortisolism, with a dramatic improvement of auxological parameters.

P517

Serum nitric oxide metabolites and clustering of metabolic syndrome components in paediatrics: an exploratory factor analysis

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Objective
To determine risk factor pattern of the metabolic syndrome (MetS) and its association with serum nitric oxide metabolites (NO_x) in children and adolescents.
Subjects and methods
A cross-sectional study was carried out in 409 male and 442 female children and adolescents aged 4 to 19 years. The ethical committee of our institute approved the study. The MetS was defined according to modified ATPIII criteria and factor analysis was used to examine the risk factor pattern of the MetS in entire population and within strata of sex, MetS, and obesity.
Results
The prevalence of MetS was 10.8 and 10.0% in males and females respectively. Subjects with MetS had higher age-and-sex adjusted NO_x compared to those without MetS (25.2 vs 27.9, $P=0.04$). Age-and-sex-adjusted odds ratio of having MetS was significantly higher in the upper quartile of NO_x compared to the lower quartile (2.2, 95% CI: 1.1–4.7, $P=0.029$). In the entire study population, three factors were identified including blood pressure/obesity, lipid/obesity, and glucose/NO_x; factors that explain 59.9% of the total variance in the data. After stratifying analyses for sex, again three factors were retained in both genders however, NO_x was loaded in two factors in males. In subjects without MetS and those who had normal weight, NOx constituted a separate factor while in subjects with MetS and those who were overweight or obese, it loaded with FPG and/or BMI.
Conclusions
Serum NO_x was associated with MetS in children and adolescents; in addition, serum NO_x was loaded with other MetS components especially fasting glucose in the cluster analysis of metabolic risk factors and it may have a unifying role in clustering of MetS components, at least in male subjects.

P518

A common deletion in the uridine diphosphate glucuronyltransferase (*UGT*) *2B17* gene is a strong determinant of androgen excretion in healthy pubertal boys

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Testosterone (T) is excreted in urine as water soluble glucuronidated and sulphatated conjugates. The ability to glucuronidate T and other steroids depends on a number of different glucuronidases (UGT) of which UGT2B17 is essential. The aim of the study was to evaluate the influence of *UGT2B17* genotypes on urinary excretion of androgen metabolites in pubertal boys.
Study design
A clinical study of 116 healthy boys aged 8 to 19 years. *UGT2B17* genotyping was performed using quantitative PCR. Serum FSH, LH, T, estradiol (E2) and SHBG were analysed by immunoassays, and urinary levels of androgen metabolites were quantitated by gas chromatography/mass spectrometry in all subjects.
Results
Ten out of 116 subjects (9%) presented with a homozygote deletion of the *UGT2B17* gene (del/del), while 52 and 54 boys were hetero- or homozygous carriers of the *UGT2B17* gene (del/ins and ins/ins), respectively. None of the reproductive hormones were affected by *UGT2B17* genotype. In all subjects, mean urinary T/E ratio was 1.56 (1.14 (S.D.); 0.1–6.9 (range)) and unaffected by age or pubertal stage. Subjects with homozygous deletions of *UGT2B17* had

significantly lower urinary levels of T, and 5α- and 5β-Androstanediol. Mean urinary T/E was significantly reduced in del/del subjects (0.29 (0.30); 0.1–1.0 (range), $P<0.0001$).
Conclusion
In pubertal boys, a common homozygous deletion in the *UGT2B17* gene strongly affected urinary excretion pattern of androgen metabolites, but did not influence circulating androgen levels.

P519

Sex hormone-binding globulin levels predict insulin sensitivity, disposition index and cardiovascular risk during puberty

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Objective
Early puberty is associated with increased risk of subsequent cardiovascular disease. Low sex hormone-binding globulin (SHBG) levels are a feature of early puberty as well as conditions associated with increased cardiovascular risk. The aim of the present study was to evaluate SHBG as predictor of glucose metabolism and metabolic risk during puberty.
Research design and methods
Cross-sectional study on 132 healthy Caucasian children and adolescents evaluated by oral glucose tolerance test, dual energy X-ray absorptiometry scan, direct oxygen uptake measurement during cycle ergometry and fasting blood samples.
Results
SHBG levels declined with advancement of puberty in both boys ($P<0.001$) and girls ($P=0.019$). SHBG was statistically significantly positively associated with insulin sensitivity in boys ($P<0.001$) and girls ($P<0.001$). In addition, SHBG was a strong predictor of insulin sensitivity ($P=0.001$) and the only predictor of the disposition index ($P=0.031$) after adjusting for puberty, fat mass and aerobic fitness. SHBG was significantly negatively associated with metabolic risk ($P=0.032$) independent of fat mass as well as hypersensitive CRP levels ($P=0.004$) independent of fat mass and insulin sensitivity.
Conclusions
SHBG was a strong predictor of insulin sensitivity and metabolic risk during puberty. Thus, we hypothesize that SHBG integrates the marked metabolic and body compositional changes that occur during pubertal transition.

P520

Mild hypothyroidism in children with congenital heart malformations

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Congenital hypothyroidism is frequently associated with congenital cardiac malformations (CCM). Studies in knock-out mice showed that heart and great vessels organogenesis share some nuclear transcription factors with the embryonic thyroid, suggesting that thyroid defects may have a higher prevalence in children with CCM. The present study investigated thyroid function and morphology in 280 children (145 M/135 F, aged 0.3–12 years), affected by CCM (septal defects, ductus arteriosus, Fallot tetralogy, valvular stenosis). Patients with Down syndrome, recent administration of iodinated contrast agents or receiving amiodarone were excluded. Hypothyroidism was diagnosed in 35 children (12.5%): two were identified at neonatal screening, while the remaining 33, though normal at neonatal screening, showed high serum TSH with normal free hormones levels (fT4 0.9–1.8 ng/dl (nv 0.8–1.9); fT3 3.0–4.0 pg/ml (nv 1.5–4.1)) in absence of low T3 syndrome, consistent with mild or subclinical hypothyroidism. Increased TSH levels were confirmed six months later. No relationship between hypothyroidism and type of CCM as well as age were detected. FreeT4 levels were lower in hypothyroid children compared with 69 age and sex-matched euthyroid children with inter-atrial defects (1.37 ± 0.22 vs 1.46 ± 0.18 ng/ml, mean$\pm$S.D.; $P=0.04$). Thyroid autoimmunity was present in only 2 hypothyroid children (5.7%). Thyroid ultrasound revealed normal morphology and ecogenicity in all hypothyroid children except in one case with emiagenesia. The mean HSDS (height standard deviation score) was lower in hypothyroid than in euthyroid children (-0.23 ± 1.3 vs 0.26 ± 1.3; $P=0.04$). This difference was not related to the CCM severity, as it was confirmed when we compared 12 hypothyroid with 93 age and sex-matched euthyroid children with inter-atrial defects and ductus arteriosus (HSDS-0.68 ± 0.2 vs 0.40 ± 1.4; $P=0.017$). In conclusion, a mild hypothyroidism frequently occurs in children with CCM and is rarely related to thyroid autoimmunity or dysgenesia. Moreover, the subclinical hypothyroidism seems to be associated with a relative stature deficit.

P521

Increased intra-erythrocyte magnesium is associated with gamma-glutamyl transferase in obese children

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Objective
To determine the association between markers of hepatic injury and serum, urinary, and intra-erythrocyte magnesium concentrations and dietary magnesium intake in obese children.
Methods
In a case–control cross-sectional study, we studied 42 obese children and adolescents and 42 sex- and puberty-matched lean controls. Serum, urinary, and intra-erythrocyte magnesium levels, indexes of insulin sensitivity, and liver enzymes were measured. Dietary magnesium intake was assessed using a food frequency questionnaire.
Results
Obese children exhibited insulin resistance as determined by a higher fasting insulin and the HOMA-IR ($P<0.001$) and lower QUICKI indices ($P=0.001$); in addition these children had significantly higher intra-erythrocyte magnesium (IEM) concentration than non-obese ones (3.99 ± 1.05 vs 3.35 ± 1.26 mg/dl of packed cells, $P=0.015$). Serum, urinary, and dietary magnesium levels were comparable between groups. Among liver enzymes only gamma-glutamyl transferase (GGT) was significantly higher in obese than in non-obese subjects (22.7 ± 9.4 vs 17.1 ± 7.9 U/l, $P=0.002$). Positive association was found between GGT and IEM in both groups; however in multivariate analysis, in obese subjects, only GGT ($\beta=0.375$; $P=0.026$, model $R=0.38$) and, in non-obese subjects, only age ($\beta=-0.466$; $P=0.006$, model $R=0.47$) remained as significant predictors of IEM.
Conclusions
Increased IEM concentration was seen in insulin resistant obese children; furthermore, serum GGT was associated with IEM independently of body mass index and HOMA-IR.

P522

Iodine levels and thyroid hormones in healthy pregnant women and birth weight of their offspring

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Introduction
The fetus is the most vulnerable to severe iodine deficiency and hypothyroidism during pregnancy. Severe iodine deficiency and hypothyroidism during pregnancy have long been known to be associated with neurologic deficits and mental retardation The effects of mild iodine deficiency and subclinical hypothyroidism are poorly known. The present study assesses the association between thyroid hormones and urinary iodine concentration (UIC) in healthy pregnant women and the birth weight of their children. The effect of iodine supplementation during pregnancy was also examined.
Methods
Six hundred and fifty-seven pregnant women were recruited in the city of Sabadell and followed until delivery. The association between thyroid hormones during the first trimester, UIC during the first and third trimesters and birth weight or small size for gestational age (SGA) was studied in 557 (85%), 251 (38%) and 528 (80%) mother–newborn pairs, respectively, using linear and logistic regression models adjusted for potential confounders. Six percent of newborns were classified as SGA.
Results
The median UIC was 95 and 104 μg/l during the first and third trimesters, respectively. Women with third trimester UICs between 100 and 149 μg/l had lower risk of having an SGA newborn than women with UICs below 50 μg/l (adjusted OR (95% CI): 0.15 (0.03–0.76)). There was no significant reduction in SGA among mothers with higher UICs. Lower free T4 and higher TSH levels during the first trimester were not associated with birth weight or SGA.
Conclusions
The present study suggests that iodine status during pregnancy may be related to prenatal growth. Further studies should be undertaken to assess the effects of moderate-to-mild iodine deficiency and iodine excess during pregnancy, as well as to validate the current guidelines in relation to reproductive and long-term outcomes in both iodine-deficient and iodine-sufficient areas.

P523

Development of multiple pituitary hormone deficiency (MPHD) in pediatric patients originally diagnosed with isolated GH deficiency (IsGHD)

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Patients originally diagnosed with IsGHD may develop additional pituitary hormone deficiencies later in life. This study aimed to identify factors that predict development of MPHD and to characterize the time course of specific hormone deficiencies. Pediatric patients with IsGHD were from an observational study (GeNeSIS). Additional hormone deficiency during follow-up was accepted, if indicated by check box on the case report forms, by an adverse event or by starting replacement therapy. Baseline characteristics of patients with at least 2 year follow-up ($n=2161$) suggested more severe GHD in those who developed MPHD vs. those who did not ($n=108$ vs 2053; mean (S.D.), P by ANOVA): age at diagnosis (8.2 (4.2) vs 9.1 (3.6) year, $P=0.019$), height SDS (-3.1 (1.4) vs -2.5 (0.9), $P<0.001$), stimulated GH peak (median (Q1; Q3)) (2.6 (1.0; 5.5) vs 7.4 (4.7; 9.9) μg/l, $P<0.001$). Limiting the window of observation to patients with at least 3.5 years follow-up or development of MPHD within 4.5 years revealed significantly different proportions of patients who developed MPHD in the various diagnostic sub-groups: all GHD (116/1058, 11%), idiopathic (60/785, 8%), congenital (29/128, 23%), acquired (19/48, 40%), abnormal pituitary development (20/71, 28%). Deficiency of TSH was the most frequent (75%), followed by LH/FSH (16%), ADH (10%) and ACTH (10%); no PRL deficiency; 90% had 1 additional hormone deficiency and 10% had 2. In the entire population the time (years) from diagnosis of GHD to the additional hormone deficiency (median (Q1; Q3)) was 0.9 (0.4; 2.8) for ADH, 1.4 (0.8; 3.1) for TSH, 1.8 (1.4; 2.7) for ACTH, and 5.4 (2.5; 8.6) for LH/FSH. Logistic multiple regression modeling identified the following significant ($P<0.001$) predictors for development of MPHD (odds ratio(95% CI)): organic cause of GHD (3.6 (2.2–6.0)) and low stimulated GH peak (0.5(0.4–0.6)).
Conclusion
In patients with IsGHD additional pituitary hormone deficiencies may develop especially in those with organic cause and severe GHD. Therefore, patients with IsGHD require continuous monitoring for development of MPHD.

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Clinical and genetic features of type 1 diabetes mellitus and autoimmune thyroiditis combination in Belarusian Children

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Background and aims
High correlation is revealed between type 1 diabetes mellitus (DM1) and autoimmune thyroid pathology in children. Several candidate-genes including CTLA, PTPN, Ins-23Hphl could be associated with the combined autoimmune endocrinopathy. The aim of this study was to define whether polymorphisms of CTLA 49 A/G, PTPN22-1858 C/T, Ins-23Hphl ?/? genes contribute to DM1 and autoimmune thyroiditis (AT) combination development.
Material and methods
Twenty-nine DM1 patients (group 1) and 22 DM1 + AT (group 2) with mean age of 9.95 (3.9–16.2) and 14.3 (9.8–16.7) years accordingly were genotyped for our investigation. Mean age at DM1 onset and the disease duration in the 1st group was 9.15 (2.48–14.97) years and 1.1 (0–4); 8.85 (4.93–14.93) and 4.2 (0.56–10.37) ($P>0.05$) in 2nd group correspondingly. AT criterions: typical ultrasonography signs (in 100%), antibodies to thyroidperoxidase (in 83.4% patients >100 U, in 26.6% – $>50<100$ U). AT manifestation preceded DM1 in 8.3% of children, started simultaneously in 25% and manifested later in 66.7%. Euthyroidism was observed in 22.7%, subclinic hypothyroidism – in 27.35%, hypothyroidism – in 50% cases. Polymorphism analysis was performed by the PCR method with the specific primers and endonuclease processing of amplificated fragments.
Results
We ascertained PTPN22-1858 T risk-allele high frequency occurrence in DM1 children with the increase of heterozygous carriers (5.7% T/T and 56.6% C/T) in comparison with Belarusian population sample (4.4% T/T and 29.3% C/T, $P<0.01$). A significant difference in locus Ins-23Hphl genotype rates was discovered in DM1 children: 86% AA, 7% AT, 7% TT and in the population sample: 52.9% AA, 35.2%AT, 10.7%TT ($P<0.01$). Significant differences between groups 1 and 2 weren't revealed in connection with latest AT onset.
Conclusion
The increased risk-allele frequency of PTPN22-1858 T and Ins-23Hphl ? genes was observed in Belarusian children with combined autoimmune endocrinopathy.

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LEOPARD syndrome and pilocytic astrocytoma: a random association?

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Leopard syndrome (LS) is a rare autosomal dominant disease of variable penetrance and clinical expression. LEOPARD is an acronym for the major features of the disorder: lentigines, ECG conduction abnormalities, Ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth, and deafness. LS is caused by different mutations in PTPN11 gene (protein-tyrosine phosphatase, nonreceptor-type, 11), allelic with Noonan syndrome (NS). The diagnosis is established if multiple lentigines are present in association with at least two other cardinal features. To date, approximately 200 cases have been reported but the real frequency may be underestimated. We present the case of a male patient who was referred to the endocrinological department at the age of 18 for short stature (-3 S.D.) and delayed puberty (Tanner III). The association of multiple lentigines, echocardiographic abnormalities including large pulmonary stenosis, trivalvular insufficiency, and hypertelorism, suggested the diagnosis of LS, which was confirmed by a heterozygous substitution mutation detected in exon 13 of the PTPN11 gene. Other less frequent features, as triangular face, cafe-au-lait spots, and retractile testis were also present. He also presented right spastic hemiparesis and left central facial palsy, and brain IRM identified a large tumour located mainly in the cerebellum. Partial surgery was performed with improvement of the neurological symptoms. Pathology confirmed pilocytic astrocytoma. To our knowledge, this is the first report of a LS associated with astrocytoma. Tumours as neuroblastoma, choristoma and malignant melanoma have been described in few cases. Dysregulations of the RAS/MAPK (RAS/mitogen activated protein kinase) cascade seems to be the common molecular base for congenital syndromes as LS, NS, type 1 neurofibromatosis (which has an increased risk for astrocytoma). More than that, recent studies implicate aberrant activation of MAPK pathway as a molecular pathogenesis in astrocytoma. Therefore, we which suggest that LS-astrocytoma may be more than a random association.

P526

Genetic characterization of children with isolated growth hormone deficiency in Turkish population

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Background
Isolated growth hormone deficiency (IGHD) is a condition associated with the growth failure of children due to deficient growth hormone (GH) production and action. IGHD occurs in 1/4000 to 1/10 000 births and the most of cases are sporadic and idiopathic. Between 5 and 30% show familial pattern, suggesting a genetic etiology of disease. Mutations on GH-1 gene lead to growth failure and cause IGHD disease.
Objective
Purpose of our research was to characterize mutations on GH-1 gene in children with IGHD in Turkish population.
Methods
Seventy-five Turkish children who were diagnosed to have IGHD were included in this study. DNAs were isolated from patient and specific exon and exon/intron regions of GH-1 gene were amplified with PCR using specific primers. The PCR products for exons and exon/intron boundaries for GH-1 gene were sequenced.
Results
We analyzed the GH-1 gene for mutations in seventy-five patients with IGHD. We previously reported five mutations on GH-1 gene. Furthermore, we defined three more mutations on the GH-1 gene and these are GAAA insertion in the intron 1 and deletions of +83 C residue in the intron 1 and TTC codon-encoding F166 at exon 5 of GH-1 gene. These mutations are heterozygote and also novel.
Precise conclusions
One insertion (GAAA) and two deletion mutations were detected in Turkish population and these mutations are novel.

P527

Growth hormone receptor (GHR) mutations in Turkish children with Laron syndrome

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Background
Laron syndrome (LS) is an autosomal recessive disease characterized by severe postnatal growth failure, short stature, normal or elevated serum GH, and low levels of IGF-I and IGF binding protein-3 (IGFBP-3). The disorder is caused by dysfunction of the growth hormone receptor resulted from mutations in GHR gene.
Objective
Purpose of this research was to describe mutations on GHR gene in five children with Laron syndrome.
Methods
Five children who were diagnosed as Laron syndrome according to the clinical and biochemical test results. Genomic DNAs were isolated from their blood by salting out method. The exons and exon/intron boundaries of GHR for each patient were amplified by PCR using specific primers. The PCR products of the exons for GHR were run on agarose gel electrophoresis, purified and sequenced by forward and reverse primers.
Results
We determined one splice site mutation (70+G->A), two missense mutations (I526L and S40L) and exon 3 deletion polymorphism in GHR from Laron children and they are homozygote. Splice site mutation was caused by substitution of G residue of GT consensus sequence in donor splice site to A residue disrupting consensus sequence at intron 2 of GHR. I526L mutation was created by changing of A residue of ATC codon to C residue leading CTC codon encoding to leucine in one child at exon 10 and S40L mutation occurred by changing of C residue of TCA codon encoding serine to T residue at exon 4 leading TTA codon encoding Leucine. However, no mutation in GHR was found in the two children and they are under investigation for mutations in genes located at more downstream of GHR.
Precise conclusions
One splice site mutation and two missense mutation were detected in three laron patient from five children with Laron in Turkish population.

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Childhood obesity and bone age

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Obese children frequently present with accelerated growth and early puberty.
Objective
To examine the degree of bone maturation in children with simple obesity.
Patients and methods
One hundred eighteen boys with mean chronological age (CA) 9.9±2.2 years (3–13 years) and 102 girls with CA 8.4±2.0 years (3–12 years) with simple obesity (BMI>97th centile for age and sex) were studied. Ninety-five children were prepubertal and 125 were pubertal. Pubertal stage, body mass index (BMI), height SDS (HtSDS) were recorded. Bone age (BA) was estimated according to Greulich-Pyle's standards. The difference between BA and CA (BA–CA) was calculated.
Results
Mean HtSDS was greater than average in both sexes (0.93±1.0 for girls and 0.74±1.1 for boys). BA was significantly greater than CA in both sexes (11.1±2.2 vs 9.9±2.2, $P<0.001$ for boys and 9.7±2.4 vs 8.4±2.0, $P<0.001$ for girls). There was a statistically significant correlation between BMI and BA for both sexes ($r=0.4$, $P<0.001$ for boys and $r=0.5$, $P<0.001$ for girls). Prepubertal and pubertal children had the same degree of bone age acceleration (BA–CA prepubertal 1.0±0.9 versus BA–CA pubertal 1.2±0.6 for boys and BA–CA prepubertal 1.1±1.1 versus BA–CA pubertal 1.3±0.9 for girls. Thirteen percent of girls and 2.1% of boys had precocious adrenarche.
Conclusions
BA is significantly accelerated in obese prepubertal and pubertal children, and is positively correlated with BMI. Obese children tend to be taller than average but as their bone maturation is more advance than CA, final height will not surpass their genetically predicted height.

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The effect of one year of therapy with rhgh on growth velocity in patients with growth hormone deficiency (GHD)

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The authors studied the effect of one year of therapy with rhGH on growth velocity in patients with growth hormone deficiency (GHD). We analyzed 120 patients (85 boys and 35 girls), 6–21.5 years of age (mean 14.2±3.0) treated in Department of Endocrinology and Diabetology for Children and Adolescents, Medical University of Wrocław. Patients received rhGH in a dose of 0.7IU/Kg/week. Partial GHD was diagnosed in 71 cases (52 boys and 19 girls), complete GHD was diagnosed in 49 patients (34 boys and 15 girls). The therapy was started at 11.7±2.9 years. The mean height velocity in the first year of treatment was 8.72±2.27 cm per year; 8.77±1.76 cm per year in girls and 8.58±2.27 in boys. The mean height velocity in patients with complete GHD was pretreatment height was 8.94±2.56 cm per year; 8.85±1.63 cm per year in girls and 8.51±2.20 per year in boys. The mean height velocity in patients with partial GHD was 8.52±2.07 cm per year; 8.70±1.85 cm per year in girls and 8.62±2.27 per year in boys.
The present study shows that there is no statistically significant difference between studied groups.

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The goiter etiology in children of the south west of Romania

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Aim
To study the etiology and the treatment of the non endemic goiter, in our region.
Material and methods
The study group consisted of 67 children (F/M: 48/19; age: 4–17 years old) with non endemic goiter admitted in our hospital during the period, 2003–2008. The diagnostic procedures were represented by: the clinical examination (including the anthropometric measures and pubertal stages after Tanner's criteria), laboratory data (serum cholesterol, triglycerides, urinary iodine), the thyroid function tests (TSH, freeT4, free T3), immunologic parameters (antiTPO and antithyroglobulin antibodies); thyroid ultrasonography (thyroid volume and aspect). The bone age and thyroid MRI were performed in selected cases. All the cases were followed over 36 month under treatment.
Results and discussions
According to the thyroid functional tests, the patients were divided in four groups. Group I: 28 euthyroid patients were treated with iodine and after 1 year the goiter disappeared in 25 patients (89.25%). Group II: 22 hypothyroid patients, (20 with chronic autoimmune thyroiditis (CAT) 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 III: 9 patients with elevated serum TSH level (subclinical hypothiroism); only the children with high levels of the serum cholesterol were treated with L-Thyroxin. Group IV: 4 cases with autoimmune hyperthyroidism were treated, initially, with thiamazol and, subsequently, the children developed hypothyroidism and were treated, with L-Thyroxin.
Conclusions
1. The goiter in childhood may have variable causes;In our region head especialy an autoimmune etiology.
2. The treatment depends on the etiology and thyroid function.

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Influence of the exon 3: deleted polymorphism of the GH receptor on glucose and lipid metabolism in GH treated subjects with GH deficiency: results of a preliminary study

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GH has contra-insulin actions and exogenous GH can reversibly reduce insulin sensitivity in patients treated with GH. It has been recently reported that the exon 3 – deleted (d3) isoform of the GH receptor (GHR) appears to be preventive for type 2 diabetes mellitus in adult subjects (GH&IGF Res 2007;17:392). Aim of this study was to investigate possible influences of the GHR-d3 polymorohism on glucose metabolism, lipid profile and BMI in children treated with GH for GH deficiency (GHD). We studied 26 GHD subjects (12 male). Mean age (S.D.) was 20.3 (1.0) years. All had been treated with GH at a mean dose of 0.33 mg/kg per week until final height for 3 to 6 years. Patients' genotype at GHR-exon 3 locus was determined by simple multiplex PCR. Fasting glucose, insulin, total and HDL-cholesterol, triglycerides, oral glucose tolerance test (OGTT), QUICKI and HOMA-R indexes, systolic and diastolic blood pressure were evaluated at treatment start, each year during treatment and at the end of it. The study protocol was approved by the local Ethical Committee and informed consent was obtained from the subjects and/or subjects' parents where appropriate. The full-length (fl) GHR exon 3 polymorphism was found in 13 subjects in homozygosity (group fl); d3 was found in 11 subjects in heterozygosity and in 2 in homozygosity (group d3). No differences in the above mentioned parameters were found comparing the two groups at treatment start, during and at the end of treatment. Furthermore, final height (SDS) did not differ between the two groups. On the basis of these preliminary data, d3 does not seem to influence glucose and fat metabolism during GH treatment in GHD subjects.

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'Hidden' congenital adrenal hyperplasia: case report

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Introduction
Congenital adrenal hyperplasia is a group of autosomal recessive diseases, caused by mutations in the enzymes implicated in the synthesis of cortisol. In females, the classical pattern is characterized by progressive virilisation, short stature and in severe cases, by salt wasting in the newborn.
Results
We present the case of a 14 years old female patient, who was referred to our clinic for primary amenorrhea. Her personal and family history was unremarkable. The patient's height was normal (166 cm), Tanner stage was B2P3, pubarche occurred at age 7–8 years, external genital organs and blood pressure were normal and she presented only very mild hirsutism on her upper lip (Ferriman-Gallwey score = 2). The pelvic ultrasound revealed a normal-for-age uterus and ovaries. The X-ray of the hand revealed closed growth cartilages. A triptoreline stimulation test showed a pubertal increase in the serum LH levels. Serum pituitary and adrenal hormonal levels were within normal range, except increased androgens: 17 OH-progesterone = 64.70 ng/ml (normal range 0.07–1.7), dehydroepiandrosterone sulphate = 368 μg/dl (45–270), androstendione = 19.5 ng/ml (0.3–3.5), testosterone = 1.49 ng/ml (0.14–0.76). The abdominal computed tomography showed bilateral adrenal hyperplasia, which was probably due to CYP 21A2 deficiency (genetic testing pending).
Conclusion
Despite high levels of 17 OH-progesterone and adrenal androgens, this adolescent girl with congenital adrenal hyperplasia and amenorrhea had almost no signs of virilisation and a normal height (based on the mid-parental target height), although she had advanced bone age. These features may be suggestive for a concurrent partial peripheral resistance to androgens.

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Prevalence of autoimmune thyroiditis in children with diabetes mellitus

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Introduction
Type 1 diabetes mellitus (DM1) is frequently associated with other autoimmune diseases. Among children and adolescents, thyroid disease is the most common autoimmune endocrinopathy. The possibility of occult thyroid disease should be considered at diagnosis and when a patient is assessed at the annual review.
Objective
The aim of our study was to determine the prevalence of autoimmune thyroiditis among children and adolescents with type 1 diabetes, followed in our hospital, during one year.
Material and methods
We reviewed the medical records of 66 diabetic patients. The following parameters were analysed: gender, age, age at diagnosis, duration of DM1, hemoglobin A1c (HbA1c), thyroid peroxidase antibodies (TPO Ab), antithyroglobulin antibodies (Tg Ab), thyroid-stimulating hormone (TSH) and free thyroxine (FT4).
Results
The study included 66 patients, 47% were girls and 53% boys. Mean age 12.9 ± 3.8 years (max: 19; min:4). The mean age at diagnosis was 7.9 ± 4 years and the mean disease's duration was 5 ± 3.2 years. HbA1c levels averaged 9.5 ± 1.8%. Eleven children had positive antithyroid antibodies (17%): 11 were positive for Tg Ab, 7 for TPO Ab. Among children with positive antithyroid antibodies, 4 were in euthyroidism, 7 in hypothyroidism, subclinical in 3 and clinical in 4 patients.
Comments
Our data shows indeed the great prevalence for thyroid disease in DM, with 6% of our type 1 diabetic children with hypothyroidism (in the literature, approximately 3.9%). This emphasizes the need to evaluate the thyroid function in diabetic children.

Growth and Developmental Endocrinology

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Characterization of growth hormone (GH) mutants R77C and D112G found in patients with retarded growth

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Introduction
Two heterozygous missense mutations R77C and D112G have been identified in GH-1 gene of patients with short stature by Takahashi *et al.* These patients had high serum immunoreactive GH concentrations but low IGF-1 concentrations, indicating bioinactivity of their GH. Separation of GH in patients' serum by isoelectric focusing revealed the coexistence of mutant and wild type (wt) GH. In order to understand the molecular mechanism of the isolated GH deficiency of these patients, we have studied the biological activity of the two mutants *in vitro*.
Material and methods
The mutations R77C and D112G were produced by site-directed mutagenesis and the cDNA of wt and mutant GH gene were subcloned into the expression vector pcDNA3.1. Human embryonic kidney cells (HEK-293) were transfected with these plasmids, and secreted wt or mutant GH in supernatants was harvested for the experiments. The mutants were studied in comparison to wt GH by fluorescent immunoassays with different monoclonal antibodies to GH, a binding assay with recombinant GH receptor extracellular domain (GHBP), a BaF-B03 cell proliferation assay and a STAT5 transcription assay.
Results
The mutants could be secreted similarly as wt GH from HEK-293 cells. The binding affinity to GHBP was only 25% of wt GH for mutant D112G and 80% for R77C. However, both mutants achieved about 80% of the maximal biological activity induced by wt GH in BaF-B03 cell proliferation assay (R77C and D112G) and in STAT5 transcription assay (D112G).
Conclusion
Receptor binding is much more diminished due to the D112G mutation, which is located close to the receptor binding site 2, than to the R77C mutation, whose position is far away from both binding sites. Both mutants display a modest reduction in their biological activity in comparison to wt GH, which may contribute to the retarded growth of the patients.

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The effect of fetal hypothyroidism on carbohydrate metabolism during adulthood in rats

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Introduction
Thyroid hormones have major effects on regulation of metabolism and function of most cells. A number of prevalent diseases during adulthood have been attributed to the intrauterine status during fetal life. In this study, the effect of fetal hypothyroidism on the carbohydrate metabolism during adulthood investigated.
Subject and methods
After mating, the pregnant rats were divided in two, the fetal hypothyroidism (FH) and the control (C) groups. During the gestation period propylthiouracil (PTU) dissolved in drinking water (100 ppm) was administered to the FH group, while the C group consumed tap water. After delivery, the weight of male neonates was measured periodically until the adulthood; adult animals were anesthetized and intravenous glucose tolerance tests (IVGTT) were performed, for which catheters were inserted into the femoral vein and artery and after obtaining the first arterial sample of zero, the glucose solution (0.5 g/kg) was injected and samples were obtained 5, 10, 15, 20, 30 and 60 min. Plasma glucose and insulin concentration were measured using the glucose oxidase and ELISA methods respectively.
Results
Plasma glucose concentration at 5 min after glucose administration in the FH group (239.2 ± 15.6 mg/dl) was significantly higher ($P < 0.05$) than the C group (190.1 ± 4.5 mg/dl). There was no significant difference in plasma insulin concentration of the groups. Daily water consumption during the gestational period in PTU administered mothers was significantly lower compared to the C group ($P < 0.05$). The body weight of animals throughout the study period was significantly ($P < 0.05$) lower in the FH group compared with the C group.
Conclusions
Fetal hypothyroidism can alter carbohydrate metabolism during adulthood, which may contribute to the diabetes development.

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Development of specific monoclonal antibodies and highly sensitive immunoassays for 20 kDa and 22 kDa human growth hormone (hGH)

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The physiological and pathophysiological significance of hGH isoforms remains to be fully elucidated. In order to study the two most abundant hGH isoforms 20 and 22 kDa hGH, we have generated monoclonal antibodies (mAbs) against 20 and 22 kDa hGH. The mAbs against 20 and 22 kDa were characterized for their specificity and epitopes with different binding assays as well as by Western blot. The mAb 1G12 against 20 kDa with lower than 0.05% cross reactivity to 22 kDa hGH combined with the detection mAb 5C4 were chosen to construct the time resolved fluorescence sandwich assay for 20 kDa hGH. The assay has a working range of 0.02 to 20 ng/ml and the cross-reactivity to 22 kDa hGH is $<0.2\%$. The intra- and inter-assay CVs are 3.5–4.6 and 10.7–16.6% respectively. The recovery is 102.9% and the linearity is 96.3%. The mAb 5E1 against 22 kDa hGH with lower than 0.01% cross reactivity to 20 kDa hGH combined with the detection mAb 8B11 were chosen for the 22 kDa sandwich assay construction. The assay has a working range of 0.02 to 50 ng/ml and the cross-reactivity to 20 kDa hGH is $<0.1\%$. The intra- and inter-assay CVs are 4.6–6.7 and 4.2–9.4%, respectively. The recovery is 99.1% and the linearity is 91%. Spiking recombinant growth hormone binding protein (hGHBP) to the hGH samples reduced the concentration measured by both 20 and 22 kDa hGH assays as hGHBP concentration increased. However, the ratios between 20 and 22 kDa hGH remained stable ($<15\%$ for hGHBP from 0 to 2 nM). There is a good correlation between 20 and 22 kDa hGH concentrations in the serum samples from 105 healthy donors ($20\ \text{kDa} = 0.041 + 0.189 \times 22\ \text{kDa}$, $R^2 = 0.935$), indicating the ratios between 20 and 22 kDa hGH are quite constant.

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The effect of gestational hypothyroidism on insulin secretion from isolated islets of adult offspring in male rats

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Introduction
Any abnormalities during pregnancy can affect the growth and development of the fetus and may have a long lasting effect presented during adulthood (intrauterine programming). In this study, the effect of maternal hypothyroidism on insulin secretion from the isolated islets of offspring has been investigated in male rats.
Materials and methods
In Test group maternal hypothyroidism was induced using 0.02% propylthiouracyl in drinking water throughout the pregnancy period while the control animals consumed drinking water alone. Islets were isolated from offspring by collagenase digestion method. Islets were incubated in Krebs ringer solution containing different glucose concentration (5.6, 8.7 and 16.7 mM) in static condition for 60 min. Insulin was measured with rat specific ELISA Kit.
Results
The results indicate that the insulin secretion from isolated islets of the offspring from hypothyroid mothers were significantly reduced when were stimulated with

different glucose concentration ($P<0.05$). Thyroid hormone measurements show that the offspring were not hypothyroid at the time of the insulin secretion assessment.
Conclusion
From the results of this study, it appears that maternal hypothyroidism can affect insulin secretion capacity and therefore its impact on carbohydrate metabolism and possible role on diabetes induction should be considered.

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Short stature in betathalassemia major: a multifactorial condition
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Short stature is a frequent finding among patients with beta-thalassemia major, with a major impact on the quality of life and a multifactorial aetiology.
Aim
To study the relationship between growth retardation of thalassemic patients and associated sistemic conditions and endocrine complications.
Subjects and methods
Cross-sectional study on 59 patients with βthalassemia major who did not reach their final height, with a mean age of 17.37 ± 6.53 years. All patients were evaluated by clinical, biochemical and hormonal parameters.
Results
Short stature, defined as height more than 2.5 s.d. below the mean for chronological age was found among 62.7% of the patients. Growth failure was significantly associated with lower serrum Hb levels ($P<0.005$), higher mean ferritin values ($P<0.05$), higher mean transaminase levels ($P<0.0001$ and $P<0.05$ for AST and ALT respectively) and early form of hypogonadism (delayed and arrested puberty) ($P<0.05$). All hypogonadic patients had hypogonadotropic hypogonadism.
IGF1 was measured in a subgroup of 19 thalassemic patients. We found that patients with short stature had significantly lower values of IGF1 compared with those with adequate height (58.61 ± 24.1 vs 207.4 ± 124.5, $P<0.005$). Six of the patients with impaired growth were also evaluated for GH reserve by provocative tests and all of them had subnormal GH responses (peak GH values <10 ng/ml). In our study group 15.5% of the patients presented mild types of primary hypothyroidism (mean TSH 5.8 ± 1.24 μU/ml) without any significant association with short stature.
Conclusions
Our results support the involvement of chronic anemia, iron overload, hepatic dysfunction and early form of hypogonadism as pathogenic factors of short stature in thalassemic patients. Although data regarding GH reserve and IGF1 levels are provided by a small group of patients, they suggest that impaired GH–IGF1 axis may be a major contributor to impaired growth.

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Histopathologic changes in pregnant rat pancreas
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Objective
Placental lactogen (PL) produced by the placenta stimulates lipolysis and fatty acid metabolism in the mother. PL is an anti-insulin hormon. It adjusts the metabolic state of the mother during pregnancy to make easy the energy supply of the fetus. It also reduces maternal tissue sensitivity to insulin, leading to gestational diabetes. A moderate hypertriglyceridaemia due to uncontrolled diabetes may be noticeably worsen by the gestation. As a result, acute hypertriglyceridaemic pancreatitis may occur in the pregnancy. In this study, pancreas was histopathologically examined on the pregnant rat model in order to investigate how and in what level the pancreas is affected from the gestation.
Methods
Eight adult female Sprague Dawley rats were used. Rats were divided into two equal-sized groups as a control and a pregnancy group. The precence of vaginal plug was checked to determine the first day pregnancy in the female rats mated with adult males. In the second week of pregnancy, rats were sacrificed. Pancreases removed from the control and pregnant rats were fixed in 10% formalin and embedded in parafin. The 5 μm tissue sections were stained with hematoxylin–eosin for light microscopic examination.
Results
Islets of Langerhans surrounded by extensively acidophilic stained acini were observed in the pancreas of the pregnants, but not in that of controls. Necrotic changes such as cytoplasmic swelling and nuclear condensation were present in some of these acini. Large clear vacuoles were demonstrated in some aciner cells. Additionally, pancreatic acini that were densly acidophilic stained and shrunken were also seen, suggesting apoptosis. Prominent disruption of the cellular architecture were found in such acini, indicating the cytoplasmic digestion leading to possible pancreatit. Cytoplasmic vacuolization of endocrine cells and sinusoidal dilatation were prominent in the islets of Langerhans.
Conclusions
Our data suggested evident cellular injuries in both endocrine and exocrine pancreas in the pregnant rats. In conclusion, we have thought that these histopathological changes may lead to acute pancreatit and possible gestational diabetes.
Acknowledgement
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Growth Factors

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IGFBP3 and IGFBP2 negatively and positively modulate IGFs autocrine effect in lung cancer cell lines
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Insulin-like growth factors (IGFs) play an important role in the pathogenesis of several neoplasias and the IGF-binding proteins (IGFBPs) may have a role as autocrine/paracrine factors in regulating the local actions of the IGFs. In the present study we investigated IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 production in cultured media from three human lung cancer cell lines (Calu-3, Calu-6, A549) and in human neoplastic and normal lung tissue samples obtained at surgery from 8 patients. Calu-6 cells secreted much more IGF-II than Calu-3 and A549 (190, 25, and 5 ng/10^7 cells respectively) and much less IGF-I (0.7, 13, and 5 ng/10^7 cells). Conversely, IGFBP-1 and IGFBP-3 were most abundant in media conditioned by CALU-3 (13 and 120 ng/10^7 cells respectively) and least abundant in CALU-6 (<1 ng/10^7 cells). Molar ratio between IGF-I + IGF-II and IGFBP-1 + IGFBP-3 was much higher in Calu-6 which is also the most actively replicating cell line. Regarding IGFBP-2 we found a higher concentration in Calu-6 than in Calu-3 and A549 conditioned media. In consideration of the enhancing action of IGFBP-2 on IGF bioactivity this finding further supports the high replication rate of Calu-6 cell line. Regarding tissue we found a significantly higher concentration of IGF-I in neoplastic (18 ± 3.6 ng/g of tissue) than in normal (7.8 ± 1.5 ng/g of tissue) lung tissue in all subject studied. IGF-II concentration was higher than IGF-I, but the difference between neoplastic and normal tissue was not significant (90.3 ± 15.5 and 61.1 ± 11.8 ng/g of tissue respectively). Neither the normal nor the neoplastic lung tissue produced significant amounts of IGFBP-1. IGFBP-2 and IGFBP-3, as evaluated by immunoassays and immunoblot were predominantly expressed in neoplastic tissue as compared to normal. These data raise the possibility that IGFBPs are important modulators of lung cancer proliferation by inhibiting (IGFBP-3) or stimulating (IGFBP-2) the autocrine mitogenic effects of IGFs.

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Exogenous growth hormone administration effects organ weights but does not markedly change serum IGF-I levels in common mice
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Mice overexpressing growth hormone (GH) have about two-fold higher IGF-I and clear increases in body and organ weights. Moreover, administration of GH to hypophysectomised or dwarf mice has been shown to increase IGF-I. Data on the short term effects of exogenous administration of rhGH in normal rodents remain scarce.
Therefore, we measured IGF-I before and after one week of GH-treatment in 10-week-old female mice, injected daily with two different doses of rhGH: 0.5 mg/d (GH-1) and 0.125 mg/d (GH-2). Controls (C) received equal volumes of 0.9% NaCl.
Liver, heart, kidneys, adrenals, perirenal and abdominal fat pads were excised and weighed.
After one week, mice were heavier with both doses of GH. Bodyweight (BW) increased by 6% under high dose GH-administration ($P<0.05$), whereas control mice did not show any BW-gain. Liver weights were significantly higher in both GH-groups compared to control groups (GH-1: 1.27 ± 0.06 g; GH-2: 1.39 ± 0.12 g; C: 1.15 ± 0.03 g $P<0.001$). Weights of kidney, heart and adrenals were higher under both GH doses compared to controls, but only increases in heart weight of GH-2 reached statistical significance ($P<0.05$). No differences were present in perirenal fat pad weights, whereas abdominal fat pad weights were dramatically lower by about 50% in GH-1 when compared to controls ($P<0.05$). Surprisingly, neither GH-1 nor GH-2 showed significant change in IGF-I levels, when compared to control animals (GH-1: 445 ± 104 ng/ml; GH-2: 576 ± 54 ng/ml; C: 435 ± 136 ng/ml; GH-1 and GH-2 versus C $P=0.9$ or 0.57, respectively). In GH-1 group, serum IGF levels were unchanged before and after one-week of GH treatment ($P=0.46$).
In conclusion, administration of supraphysiological doses of GH to healthy adult mice led to profound changes in BW, liver weight and visceral adipose tissue. However, despite these effects we could not detect any change in IGF-I. Our data suggests that IGF-I is not a suitable pharmacodynamic marker of GH-action in ordinary rodents.

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Immunohistochemical analysis of VEGF and CD34 in adrenal cortical tumors

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The aim of the study was investigating of microvessels density (MD) and expression of vascular endothelial growth factor (VEGF) in adrenocortical tumors.
Under investigation there were 23 adrenocortical tumors: adenomas – 13, 'border' tumors – 4, and carcinomas – 6. Immunohistochemical studied were carried out using monoclonal antibodies to both VEGF and endothelial marker CD34 (Dako). VEGF expression was estimated as a weak one (+), moderate (++), and intensive (+++).
The degree of vascularization of adrenocortical adenomas was high. In this case, the distribution of capillaries through the tumor tissue was relatively even and they surrounded small cell groups or sometimes single cells. VEGF expression in these tumor cells was either indefinable or weak (+), approximately 50% of cells like little granules localized predominantly along cellular membrane. In the 'border' tumors, an extremely uneven MD was noted. In this case, in addition to narrow-lumen capillaries, significant amount of sinusoids were revealed which prevailed here and there. Microgranular or diffuse VEGF expression was observed in the tumor cellular cytoplasm (++), predominantly in the foci of hypervascularization. Adrenocortical carcinomas had a little number of vessels within cell clusters. Usually, there were not numerous narrow-lumen capillaries or vascular 'buds' consisting of endothelial cell strands. Thin-wall sinusoids divided tumor lobes; in connective layers and pseudocapsule, they formed narrow-wall angioma-like structures. Intensive diffuse VEGF expression was determined in the tumor cell cytoplasm both in the central areas of the tumor (++) and in the peripheral growth areas where its intensity reached '+++'.
The outcome obtained is indicative of the fact that VEGF expression in the adrenocortical tumor cells is inversely related to the degree of their vascularization: the higher the VEGF level in tumor cells, the lower the MD, and vice versa. Biological significance of this fact is unclear. However, one can consider that VEGF expression and estimation of MD (reaction with CD34) in adrenocortical tumors might serve an additional criterion for evaluation of their malignant potential.

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Effects of intraperitoneal gibberelic acid (GA3) on IGF-1 and growth hormone (GH) in the rats

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Plant growth factors are used commonly in agriculture. The effect of these factors on human health is unknown. Gibberellins are the most commonly used growth factors that have the structure of Diterpenoid acid. One of the gibberellins (GA3) induces tumor formation in mice but the mechanism of action is not known. We here examined changes in levels of GH and IGF-1 and examined whether there is a correlation between growth factors' levels and weight changes.
Male Wistar Albino rats were used and all the injections were made i.p. We had five experimental groups. Single high dose of GA3 was given to group 1 (20 mg/kg); Single injection of solvent was given to group 2; 2 mg/kg GA3 was given to group 3 for 30 days; 20 mg/kg GA3 was given to group 4 for 30 days and lastly solvent of GA3 was injected to group 5 for 30 days.
At the end of the experiment, levels of GH and IGF-1was measured in serum, liver and kidney. Although GH and IGF-1 levels were not changed in the serum, GH levels in liver was decreased significantly in group 1 ($P<0.05$). IGF-1 levels in liver decreased in group 3, but this effect was returned to control levels in group 4. In renal tissue IGF-1 levels decreased in group 1 ($P<0.05$), but increased significantly in group 3 and 4 ($P<0.05$). Weight of the animals increased significantly in group 3 compared to group 5 only at the second week, while it increased continuously ($P<0.05$) in group 4 compared to group 5. This is the first report demonstrating that GA3 alters GH and IGF-1 levels.

Neuroendocrinology, Pituitary and Behaviour

P544

The effects of selective serotononin reuptake inhibitors on the thyroid axis in perimenopausal depression

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Introduction
There is a great interest in the association of thyroid hormones and mood. Although it appears to be important interactions between the central regulation of mood and the thyroid axis, the effects of antidepressants and especially of selective serotonin reuptake inhibitors (SSRIs) remain ambiguous. We investigated the thyroid function in perimenopausal women suffering from depression.
Material and methods
We examined 102 perimenopausal women. Twenty-three of them had depression without taking any medication, 20 of them had depression and were on treatment with SSRIs and 59 were normal. All women were between the ages 40 and 55 and presented with a history of menstrual cycle irregularity of at least 6 months duration but not longer than 1 year of amenorrhea. We measured plasma levels of T3, FT4 and TSH.
Kruskal-Wallis test was applied to evaluate the relationship between plasma hormone levels and the use of SSRIs.
Results
Depressed women using SSRIs had a higher level of T3 than the other two groups ($P<0.03$). Serum TSH and FT4 were similar in the three groups.
Conclusions
Our results demonstrate that depressed perimenopausal women who take SSRIs have a higher serum concentration of T3. It is known that T3 is used as an augmentation therapy in treatment resistant depression. Subsequently, we could suppose that the rise of T3 levels is one mechanism of SSRIs to fight depression.

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Pre-treatment IGF-I concentrations predict radiographic osteoarthritis in acromegalic patients with long-term cured disease

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Objective
To identify factors influencing the development of osteoarthritis during long-term control of acromegaly, focusing on disease specific parameters, growth hormone (GH) and insulin-like growth factor I (IGF-I) concentrations and duration of disease, adjusted for the well-known determinants of primary osteoarthritis.
Design
Follow-up study.
Methods
We studied 67 patients, with adequate biochemical control of acromegaly for a mean of almost 13 years. Study parameters were the results of radiological assessment of the spine, hip, knee, and hand. Osteoarthritis was defined as radiological osteoarthritis using the scoring system developed by Kellgren and Lawrence (K&L). Correlations between potential factors of influence and osteoarthritis were performed by analysis of covariance and adjusted for age, gender and body mass index (BMI).
Results
Patients with pre-treatment IGF-I standard deviation (S.D.) scores in the highest tertile had an almost four-fold increased risk for radiological osteoarthritis of the hip when compared with patients in the lowest tertile. After adjustment for age, gender, BMI, and disease duration, pre-treatment IGF-I S.D. predicted radiographic osteoarthritis in all joint sites. Osteoarthritis was not predicted by other factors including pre-treatment GH levels, type of treatment, and duration of follow-up.
Conclusion
The severity of acromegaly at diagnosis reflected by the height of pretreatment IGF-I concentrations is a predictor of radiographic osteoarthritis in acromegalic patients also after with long-term disease control.

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The prevalence of diabetes mellitus in 9776 adult patients with childhood- and adult-onset growth hormone (GH) deficiency before GH replacement: a KIMS analysis

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Background
Untreated GH-deficiency (GHD) in adults has been suggested to be associated with an increased prevalence of type 2 diabetes mellitus (DM). Proposed causative factors are the tendency to overweight and the insulin resistance due to central adiposity.
Patients and methods
The KIMS (Pfizer International Metabolic Database) was used to evaluate the prevalence of DM in both childhood-onset (CO; $n=1977$; 56% males) and in adult-onset (AO; $n=7799$; 48% males) GHD patients before start of GH replacement as adults. In adulthood, mean age at initiation of GH treatment in CO was 28 (S.D.: 11) and in AO 49 years (S.D.: 13). Observed/expected prevalence ratios (PRs) were based on 5-year age-groups. Expected prevalence was calculated with published age-specific estimates on global prevalence of diabetes (Diabetes Care, 27(5):1047–1053, 2004).
Results
In CO-GHD, 42 patients (2.12%; 50% males) presented with DM vs 1.34% expected (PR=1.6 95% CI 1.15–2.15). PR was elevated in the younger age groups (10.5, 4.2 and 1.7 in the age-groups of 20–24, 25–29, and 30–39 years, respectively). For older age groups PRs were similar to expected. Impaired fasting glucose (IFG) was found in 60 patients (3.0%; 63% males). Mean BMI was 33.5 in DM, 26.9 in IFG, and 26.5 kg/m^2 in non-diabetics.
In AO-GHD, 631 patients (8.19%; 43% males) presented with DM vs expected 6.57% (PR=1.25; 95% CI 1.15–1.35). PRs were elevated in age groups below 50 years (18.6, 7.0, 5.5, 2.7, 1.7 and 1.3 in age-groups of 20–24, 25–29 30–34, 35–39, 40–44, and 45–49 years, respectively) and similar to expected in ages above 50 years. IFG were found in 355 patients (4.6%; 46% males). Mean BMI was 33.2, 31.0 and 29.2 kg/m^2 in DM, IFG, and non-diabetic patients, respectively.
Conclusions
Untreated GHD is associated with an increased prevalence of DM in both CO- and AO-GHD, predominantly in younger patients.

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Effects of 3 years growth hormone (GH) replacement in adult-onset growth hormone deficiency (GHD) due to controlled Cushing's disease (CD)

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Objective
Our study evaluates the contribution of untreated GHD to the phenotype in controlled CD, by comparing patients with GHD due to CD ($n=322$) and those with non-functioning pituitary adenoma (NFPA $n=748$) before and after 3 years of GH treatment.
Methods
The patient cohorts were obtained from KIMS (Pfizer International Metabolic Database) and matched for age and gender. Duration between pituitary disease onset and GH start was 9.7 (CD) and 6.6 years (NFPA) ($P<0.001$).
Results
At baseline, there were no differences in IGF-I SDS, BMI, body composition, triglycerides or HDL-cholesterol. Total cholesterol (5.7 ± 1.17 mmol/l vs 5.9 ± 1.23 ($P<0.05$)), LDL-cholesterol (3.5 ± 1.03 mmol/l vs 3.8 ± 1.09 ($P<0.01$)), glucose (4.7 ± 1.21 mmol/l vs 4.8 ± 0.97 ($P<0.01$)) were more favourable in CD than NFPA. QoL-AGHDA (14.6 ± 6.64 vs 11.3 ± 7.57 ($P<0.001$)) indicated poorer quality of life (QoL) in patients with CD. The mean starting GH dose was 0.22 mg/day in both groups; maintenance doses were 0.39 (CD) and 0.37 mg/day (NFPA). Mean IGF-I SDS increased similarly (by $\sim$2 SDS).
After 3 years of GH, BMI increased only in CD (0.3 ± 3.9 kg/m^2 $P<0.01$) while waist circumference decreased only in NFPA (-1.2 ± 6.6 cm $P<0.001$). There was a reduction in total (-0.6 ± 1.2; -0.5 ± 1.1 $P<0.001$) and LDL-cholesterol (-0.6 ± 1.0; -0.5 ± 0.9 $P<0.001$) in CD and NFPA, respectively, while HDL-cholesterol and triglycerides were unchanged. Glucose and HbA1C increased similarly in both groups ($P<0.001$). Improvement in QoL (a decrease in QoL-AGHDA scores) was observed in both groups (-6 ± 6 in CD and -5 ± 6 in NFPA), with greater improvement in the CD group ($P<0.01$). No other changes were significantly different between the groups.
Conclusions
Patients with CD and GHD had better metabolic profile and poorer QoL than those with NFPA before GH replacement and experienced greater QoL improvement with GH treatment. Untreated GHD may contribute to the phenotype of controlled CD.

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The cost-effectiveness of growth hormone (GH) treatment (Genotropin®) in adult patients with growth hormone deficiency (GHD)

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Objective
To evaluate cost-effectiveness of GH treatment (Genotropin) in adult patients with GHD by comparing health status and costs in patients who received GH treatment with those who did not.
Methods
Direct costs included health care costs related to morbidity (stroke, cardiovascular events) and GH costs. Indirect costs were determined by sick-leave and mortality. A model (Markov-type) was constructed to simulate differences in morbidity and mortality and corresponding costs between treated and untreated patients. Separate simulations for gender-, age- and QoL-AGHDA- groups were performed and gains in life-years and QALYs (quality adjusted life years) were calculated. Incremental cost-effectiveness ratios (ICER) were assessed for each subgroup. The overall ICER was calculated as the weighted mean of all subgroup ICERs. Data on untreated GHD patients ($n=2135$) were obtained from Swedish national registers and on GH treated patients ($n=550$) from the KIMS database (Pfizer International Metabolic Database). All patients had GHD due to non-functioning pituitary adenoma. The cost-effectiveness is presented as incremental costs per QALY gained.
Results
Direct costs for GH-treated patients compared with non-treated patients were higher by SEK421851 (€44 405) and SEK534694 (€56 284) for men and women respectively. This difference was smaller when indirect costs were included: in men SEK410974 (€43 260) and in women SEK500263 (€52 659). Mean incremental life-years gained was 3.4 (men) and 2.7 (women). Mean gains in QALYs were 3.0 (men) and 2.8 (women). Excluding indirect costs, the overall ICER were SEK139047 (€14 637) and SEK205850 (€21 668) for men and women, respectively. Including indirect costs, corresponding values were SEK125683 (€13 230) and SEK150588 (€15 851). Key determinants of the results were improvement in quality of life, increased survival and GH costs.
Conclusions
GH treatment (Genotropin) in adult patients with GHD is cost-effective in relation to informal Swedish thresholds where incremental cost per QALY gained between 100 000 (€10 526) and 500000SEK (€52 631) is considered moderate.

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High TSH levels in healthy pregnant women are related to a decrease in motor development of their children at 14 months of age

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Objectives
The fetus is dependent on maternal thyroid hormones during pregnancy and an adequate thyroid function during pregnancy is essential for the normal brain development. The effects of subclinical hypothyroidism during pregnancy are poorly known. The present study assesses the association between thyroid hormones and thyrotropin in healthy pregnant women from the general population, and the neurodevelopment of their children at the age of 14 months.
Methods
A total of 555 pregnant women were recruited in Sabadell (Spain) and levels of thyroid hormones (free thyroxine (free T4) and total triiodothyronine (total T3)) and thyrotropin (TSH) in serum were measured at first trimester of pregnancy. Those women with thyroid pathology were excluded from the analysis. Mental and motor development of their children were assessed using Bayley Scales of infant development at 14 months of age. We used multivariate regression adjusted for potential confounders to evaluate the association between TH and TSH, and the neurodevelopment of the children.
Results
TSH levels at first trimester of pregnancy were negatively associated with psychomotor developmental indice (PDI) (adjusted-coefficient: -1.05 P value$=$ 0.038) of their children at 14 months of age. No association was found between free T4, total T3 and TSH and mental developmental indice (MDI).
Conclusions
High TSH levels in healthy pregnant women from the general population have an adverse effect on motor development at 14 months of age. There is a need to establish thyroid hormones and TSH reference ranges during pregnancy in relation to developmental outcomes of the children.

P550

Growth hormone receptor polymorphism and the effects of pegvisomant in acromegaly

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Clinical trials have demonstrated that pegvisomant therapy is highly efficacious, normalizing serum IGF-I levels in the majority of patients with acromegaly. Multiple factors could influence the dose of pegvisomant required to normalize IGF-I, that ranging from 10 to 40 mg/day. However, the determinants of this variability are unknown and, to date, there is no specific recommendation to adjust the dose to the type of patient. Lack of exon 3 of the Growth Hormone receptor (d3-GHR) has been associated with increased responsiveness to GH therapy and with a more morbid acromegalic clinical and biochemical picture. Aim of our study was to assess whether the presence of polymorphism of GH receptor may have a role in predictive dose regimen and responsivity to pegvisomant in acromegaly. We studied a cohort of 19 acromegalic patients with active disease after unsuccessful neurosurgery and somatostatin analogs therapy. All patients started treatment with pegvisomant at 10 mg daily and then increased during a 12-months follow-up until normalize IGF-I levels. The genotype of the GH receptor was determined from peripheral blood. The patients carriers of d3-GHR genotype required a significant lower dose and shorter treatment time to normalize IGF-I. In conclusion, we demonstrated that in acromegaly the GHR genotype could be useful in predicting dose and individual response to pegvisomant in acromegaly.

P551

The low-dose ACTH stimulation test in the assessment of outcome of pituitary surgery for Cushing's disease

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Objective
To evaluate the results of the early postoperative low-dose (1 μg) ACTH (adrenocorticotropin) stimulation test in patients with Cushing's disease (CD) in order to predict long-term outcome of transsphenoidal surgery.
Methods
We reviewed the serum cortisol response to 1 μg synthetic ACTH (1–24) in the second week after pituitary surgery in 40 patients with Cushing's disease. Median follow-up was 48.5 months (range 6–106).
Results
Eighty-eight percent of patients in sustained remission (cure) recorded peak cortisol concentrations below 774 nmol/l (28.0 μg/dl) after stimulation with 1 μg synthetic ACTH. All patients with recurrent disease after initial remission (relapse) also showed ACTH-stimulated peak cortisol levels below 774 nmol/l. All patients with persistent Cushing's disease after surgery (failures), except one, noted absolute peak cortisol levels greater than 774 nmol/l in response to ACTH stimulation.
Conclusion
The postoperative low-dose ACTH stimulation test can be useful in testing the integrity of the pituitary–adrenal axis after pituitary surgery. In patients with Cushing's disease, the low-dose ACTH stimulation test has a sensitivity of 93% and a specificity of 88% in predicting immediate remission after pituitary surgery. Successful resection of a corticotroph adenoma causes a sudden drop in circulating plasma levels of endogenous ACTH. Subsequent down-regulation of ACTH-receptor expression in the adrenal cortex might explain the relative hyporesponsiveness to exogenous ACTH stimulation in patients in remission after pituitary surgery compared to patients with persistent Cushing's disease.

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The D3 GH receptor polymorphism is associated with osteoarthritis, especially of the hip, in patients with long-term cured acromegaly

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Objective
To evaluate the impact of the genomic deletion of exon 3 in the growth hormone receptor (d3GHR) on co-morbidities of acromegaly in a well-characterized cohort of patients with long-term remission of acromegaly.
Design
Cross sectional study.
Methods
The presence of the d3GHR polymorphism was assessed in 86 acromegalic patients and related to clinical outcome, i.e. anthropometric parameters, osteoarthritis, and the metabolic syndrome (MS), after long-term disease control.
Results
Fifty-one patients had two wild-type alleles (59%), whereas 29 patients (34%) had one allele, and 6 patients (7%) had two alleles encoding for the d3GHR isoform. The presence of one allele of the d3GHR was associated with an increased prevalence of osteoarthritis, especially of the hip (52 vs 26%, $P=0.03$), and remained significant when adjusted for age, gender, BMI, and duration of active disease. Other factors representing long-term clinical outcome, i.e. cardiovascular risk factors like hypertension, high body mass index, abdominal obesity and spinal disc degeneration were not significantly different between patients with and without the d3GHR genotype.
Conclusion
The d3GHR polymorphism is associated with the development of osteoarthritis, especially of the hip, but not with other co-morbidities, in patients with long-term cured acromegaly.

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Ghrelin as new provocative test for the diagnosis of GH deficiency in adults

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ITT is the test of reference for the diagnosis of adult GH deficiency (GHD), but it is recognized that also GHRH in combination with arginine (ARG) or GH secretagogues (GHS) are equally reliable tests. It has also been suggested that testing with GHS would represent a potent stimulus exploring also the integrity of hypothalamic pathways controlling somatotropic function. We therefore aimed to clarify the diagnostic reliability of testing with ghrelin, the natural GHS. We studied the GH response (every 15 min from -15 to $+120$ min) to acylated ghrelin (1 μg/kg i.v. at 0 min) in 42 patients with history of pituitary disease (HYPOPIT, 34 M, 8 F; age (mean$\pm$S.D.): 49.3$\pm$19.3 years; BMI: 26.9$\pm$5.6 kg/m^2). As gold standard for the diagnosis of GHD we assumed the lack of GH response to GHRH + ARG and/or the lack of GH response to ITT. We tried to identify the best GH cut-off to ghrelin test, defined as the one with the best sensitivity and specificity, using the Receiver-Operating Characteristic Curve (R.O.C.) analysis. The GH response to ghrelin in GHD patients was lower than that in noGHD (3.0$\pm$4.7 vs 18.9$\pm$12.9 μg/l, $P<0.00005$). The GH response to ghrelin was similar to that after GHRH + ARG both in GHD (3.7$\pm$2.8 μg/l) and in noGHD (19.9$\pm$9.6 μg/l) but clearly higher than that elicited by ITT (GHD: 1.3$\pm$1.3 μg/l, $P< 0.05$; noGHD: 10.4$\pm$8.7 μg/l). The best GH cut-off to gherlin test was 3.2 μg/l, with a sensibility and specificity value of 80.6 and 90.9%, respectively, with a diagnostic accuracy of 83.3%. In conclusion, these preliminary results indicate that testing with acylated ghrelin would represent a reliable diagnostic tool for the diagnosis of adult GHD.

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Screening for neuroendocrine dysfunction in patients after spontaneous subarachnoid hemorrhage

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Spontaneous subarahnoid hemorrhage (SAH) is a recently identified risk factor for hipopituitarism, especially growth hormone (GH) and corticotroph deficiencies. The aim of this study is to perform the initial screening procedure to identify the patients with increased risk of neuroendocrine dysfunction after SAH in the chronic phase and to identify the possible predictor(s) for neuroendocrine dysfunction. Ninety-one patients (30 males, 61 females), age between 19 and 69 years (48.0$\pm$1.1 years), with BMI 24.7$\pm$0.5 kg/m^2 and good outcome (GOS 4.6$\pm$0.6) were tested 1.8$\pm$0.2 years after SAH. Some patients experienced vasospasm (VS, $n=18$) and/or hydrocephalus (HDC, $n=9$) during acute SAH. At baseline, serum samples for insulin-like growth factor I (IGF-I), thyroxine (T4), TSH, FSH, LH, testosterone (in males), estradiol (in females), prolactin and cortisol were taken.
During screening procedure, according to the baseline hormonal evaluation, 42 of 91 (46.2%) had normal pituitary function. Eleven SAH patients (12.1%) had multiple pituitary hormone abnormalities, with two pituitary axes affected in 10, and three pituitary axes affected in one patient. Thirty-eight SAH patients (41.7%) were diagnosed as isolated pituitary hormone abnormality. A total of 25 SAH patients (27.5%) exhibited low IGF-I level, indicating the need for GH provocative testing for diagnosis of GH deficiency (GHD); in fifteen as an isolated abnormality and in additional 10 patients combined with other pituitary hormone abnormalities. Eighteen SAH patients (19.8%) exhibited low morning cortisol level (in 12 patients as an isolated abnormality). Low TSH and T4 levels were seen in two patients. Two of 30 males and three of 61 female patients had gonadotroph deficiency, in one female combined with other pituitary hormone abnormalities. Hyperprolactinemia was diagnosed in 3 patients. The VS and HCT during acute phase of SAH were related to abnormal pituitary hormonal testings – VS with low IGF-I levels and HCT with low cortisol levels.
In summary, during initial screening procedure, neuroendocrine dysfunction was identified in a substantial portion of patients with previous SAH and some predictor factors for these abnormalities were identified. Further pituitary function testings are mandatory in these patients.

P555

Characterisation of PARS, a novel putative pituitary regulator of hormone secretion

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We present data on open reading frame 62 encoded on human chromosome 6 (C6orf62), a gene whose protein expression and function has not been described to date. The gene product of C6orf62 was designated pituitary associated regulator of hormone secretion (PARS).
Initial gene expression screens showed that *PARS* transcription is differentially regulated in secreting and non-secreting pituitary adenomas, as well as in normal pituitary tissue. Inspired by these findings, C6orf62 was amplified by PCR from HEK293 cDNA using specific primers. To investigate the gene product of C6orf62, it was ligated into an expression vector with an N-terminal His-tag and expressed in *E. coli*. Western blotting using an anti-His antibody confirmed the expression of a 30 kDa recombinant His-tagged protein from C6orf62.
The open reading frame of PARS is comprised of five exons, which are encoded by 689 bp. The resulting protein consists of 229 amino acid residues. PARS is highly conserved throughout species, with 100% similarity to predicted mouse and rat homologues and consistently high conservation in zebrafish and chicken. However, no sequence similarity to proteins with known function was found. The PARS promoter contains numerous consensus binding sites for transcription factors, including CREB (cAMP response element binding), AP-1 (activator protein 1) and GR (glucocorticoid receptor).
Real-time PCR analysis revealed that PARS mRNA is highly expressed in pituitary tissue, and significantly down-regulated in pituitary adenomas, including somatotrophinomas, corticotrophinomas and prolactinomas, with particularly low expression in non-functioning pituitary macroadenomas. Its expression negatively correlates with tumour size. Ongoing experiments are investigating the role of PARS in pituitary hormone secretion and cell proliferation.

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Combined treatment for acromegaly with long-acting somatostatin analogues and pegvisomant: long-term safety up to 4.5 years of follow-up in 86 patients

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Background
We previously reported on the efficacy, safety and Quality of Life of long-acting somatostatin analogs (SSA) and (twice) weekly pegvisomant (PEG-V) in acromegaly and improvement after the addition of PEG-V to long-acting SSA.
Objective
To assess the long term safety in a larger group of acromegalic patients over a larger period of time; 29.2 (1.2–57.4) months (mean (range)).
Design
Pegvisomant was added to SSA monotherapy in 86 subjects (37 females), to normalize serum IGF-I concentrations ($n=63$) or to increase the Quality of life (QoL). The median dosage was 60.0 (20–200) mg weekly.
Results
After a mean treatment period of 29.2 months, 23 patients showed dose independent PEG-V related transient liver enzyme elevations (TLEE). TLEE occurred only once during continuation of combination therapy, but discontinuation and re-challenge induced a second episode of TLEE. Ten of these patients with TLEE also suffered from diabetes mellitus (DM). In our current series DM had a 2.28 (odds ratio)(CI 1.16–9.22; $P=0.03$) higher risk for developing TLEE. During the combined therapy, a clinical significant decrease in tumor size by more than 20% was observed in 14 patients. Two of these patients were previously treated by pituitary surgery, 1 with additional radiotherapy, all other patients received primary medical treatment.
Conclusion
Long-term combined treatment with SSA and (twice) weekly PEG-V up to more than 4 years seems to be safe. Patients with both acromegaly and DM have a 2.28 higher risk of developing TLEE. Clinical significant tumor shrinkage was observed in 14 patients during combined treatment.

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Impact of long-term growth hormone (GH) substitution on lipid metabolism and bone mineralisation (BMD) in pituitary insufficient patients with growth hormone deficiency (GHD)

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Introduction
Growth hormone (GH) is a lipolytic hormone with pleitropic metabolic functions. The effects of long-term GH substitution in pituitary insufficient patients with growth hormone deficiency (GHD) on lipid metabolism and bone mineralisation (BMD) have yet to be ascertained.
Methods
We measured fasting total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, glucose and insulin concentrations in 52 GHD on constant hormone replacement for pituitary insufficiency (21f/31m, median age 51.5 years (27–82)). Twenty-two GHD were additionally on constant GH substitution (GH-Sub) for at least 2 years (median 10 years (2–42 years)). Thirty age- and BMI-matched GHD had not been substituted for at least 2 years (non-Sub). Five GH-Subs and 4 non-Subs received medical treatment for lipid metabolism (GH-Sub: 4 statine, 1 fibrate, non-Sub: 3 statine, 1 fibrate). One non-Sub was on bisphosponate therapy for treatment of osteoporosis. BMD was measured by Dual-Energy-X-Ray-Absorptiometry. Osteoporosis was defined according to the World-Health-Organization.
Results
Total cholesterol, LDL, HDL and triglycerides were not significantly different between GH-Sub and non-Sub (total cholesterol 214 mg/dl (162–295) vs 205 mg/dl (149–309), LDL 133 mg/dl (85–218) vs 129 mg/dl (65–218), HDL 57 mg/dl (31–84) vs 48 mg/dl (14–93), triglyceride 123 mg/dl (55–292) vs 134 mg/dl (41–923)). Glucose was significantly lower for GH-Sub than non-Sub (87 mg/dl (71–103) vs non-Sub 89 mg/dl (71–113), $P<0.05$), whereas insulin did not differ significantly (10 μE/ml (4–42) vs non-Sub 10 μE/ml (4–63)). Furthermore, BMD and *T*-scores did not differ significantly between the two groups (BMD: GH-Sub: 1.18 g/cm^2 (0.97–1.39) vs 1.14 g/cm^2 (0.92–1.32), *T*-score: GH-Sub: −0.3 (−2.4–2) vs −0.2 (−2.7–1.3)). The percentage of patients having osteopenia was higher in GH-Sub compared to non-Sub (36 vs 0%), but more non-Sub had significant osteoporosis (20 vs 7%).
Conclusion
Long-term GH substitution alone does not seem to significantly impact on lipid metabolism and BMD in patients with pituitary insufficiency.

P558

Effect of intracerebroventricular infusion of bombesin in glucose dependent insulinotropic peptide on conscious dogs

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Several studies have pointed to a neural regulation of glucose dependent insulinotropic peptide (GIP) secretion. Bombesin is a neuropeptide found in the amphibian brain and gastrointestinal system – homologous to the C terminus of gastrin-releasing protein (GRP) in mammalians, which displays a wide range of metabolic actions.
Aim
Aim of this study was to investigate the effect of intracerebroventicular infusion of bombesin in a centrally mediated regulation of GIP secretion in dogs.
Methods
Twenty-four adult dogs were used in this study. In group 1, the animals received a bolus or a continuous i.c.v. infusion over a 3-hour period of bombesin or an equivalent amount of artificial cerebrospinal fluid (aCSF). In group 2, the continuous infusion was repeated with a simultaneous intraduodenal infusion of a glucose load. Blood samples were taken from cannulation of a hind limb vein and plasma levels of glucose, insulin and GIP were assayed.
Results
Bolus i.c.v. infusion of bombesin evoked a strong clinical reaction in the animals, which had all the characteristics of sympathetic stimulation. The levels of glucose and GIP demonstrated a significant increase without however, a respective increase in plasma insulin levels in both bolus and continuous i.c.v. infusions. In the combined experiment there was a significant increase in all parameters.
Conclusions
Intracerebroventricular administration of bombesin increases GIP secretion in a nutrient independent manner, while inhibiting GIP and glucose induced insulin secretion. However in the presence of intraduodenal glucose load insulin secretion escapes bombesin's inhibitory effect, demonstrating an alternative regulation of the entero-insular axis, which is influenced by neural or neurohormonal mediators.

P559

Plurihormonal cells are more frequent in early embryofetal stages of the human pituitary

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Our initial studies suggested that plurihormonal cells are constantly found in early embryonic stages of human pituitary but are very rare later.
Aim
A quantitative evaluation and morphological characterization of these plurihormonal cells.
Methods
Pituitaries from therapeutically aborted human fetuses of 8–24 weeks, gestational age were fixed (with ethical permission) by immersion in 4% buffered formaldehyde. One-μm-thick sections of LRGold-embedded tissue and 12-μm-thick cryostat sections were incubated for immunocytochemical labeling with *anti-rat GH* and anti-human FSH or anti-LH primary antibodies. Primary antibodies were located with either fluorescein- or Texas red-linked secondary antibodies. Confocal microscopy was to analyze the sections. All possible combinations of primary antibodies, produced in mouse, rabbit and monkey were tested, and secondary antibodies coupled to FITC, Texas red or rhodamine, biotinylated or not. Cell density counting, digital densitometric analysis, gray level measurement, and 3D digital analysis were performed.
Results
The density of plurihormonal cells (GH with both or either FSH and LH) is maximal at 15–16 weeks (2.87 ± 1.96 cells per microscopic field) and decreases toward 20–24 weeks (0.14 ± 0.55) (mean ± S.D.) ($P<0.005$). Colocalisation with GH at the early stage (8–10 weeks) was detected in 15–20% of the gonadotropin-immunoreactive cells. 3D digital analysis and gray levels confirmed specific patterns of colocalisation.
The plurihormonal cells showed occasionally cytoplasmic extensions and bridges suggesting a polyhedral lattice. High magnification of colocalising cells showed distinct immunoreactive spots for the gonadotropins and GH, suggesting that subcellular colocalisation was rarely complete. In 8–10 weeks pituitary the colocalizing cells were adjacent to peripheral blood vessels.

Conclusion
These data show that colocalisation/coexistence of GH with FSH/LH occurs in pituitary cells early during normal human ontogenesis but decreases markedly up to 20 weeks gestation. We suggest that these might be progenitor cells. The potential for colocalisation could be reactivated in adulthood during tumoral transformation.

P560

Ovariectomy causes hippocampal neuronal loss and cognitive dysfunction and restored by Ginkgo biloba extract EGb761 in rats

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Combination of ovariectomy (OVX) and chronic restraint stress causes cognitive dysfunction and reduces hippocampal CA3 neurons in female rats and that estrogen replacement suppresses the OVX/stress-induced behavioral and morphological changes. Aim of this study is to exsamine the effect of *Ginkgo biloba* extract (EGb 761) on the cognitive dysfunction and neuromorphological change in OVX/stress-subjected rats. Female Fisher 344 rats were randomly divided into three groups: vehicle-treated ovariectomized, EGb 761 (50 mg/kg)-treated ovariectomized and vehicle-treated sham-operated control groups. Two months after ovariectomy, all animals received restraint stress for 21 days (6 h/day), and were then subjected to a novel object recognition test followed by morphological examination by Nissl staining. EGb 761 was orally administered once daily until the behavioral analysis was done. Treatment with EGb 761 improved memory impairment and neuronal loss of hippocampus in the OVX/stress-subjected group in the same ways as 17-estradiol. On the other hand, EGb 761 did not affect the loss of bone mineral density and increase in body weight after OVX, although 17β-estradiol attenuated them. These results have important implications for neuroprotective and cognition enhancing effects of EGb 761 in postmenopausal women and suggest that the effects are mediated by a different mechanism from estrogen.

P561

Glucose tolerance and somatostatin analogues treatment in acromegaly: a 12 month open, prospective study

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Objective
The impact of first-line somatostatin analogues (SSA) on glucose tolerance in acromegaly was investigated during a 10 year period.
Patients
One hundred and twelve patients treated with depot SSA.
Outcome measures
Primary outcome measures were fasting glucose levels. Data were analyzed according with baseline glucose tolerance and disease control.
Results after 12 months SSA treatment
In the 63 patients with normal glucose tolerance (NGT) at baseline, fasting glucose levels did not change but in the 26 controlled patients were lower than in the 37 uncontrolled ($P=0.019$). All controlled patients had NGT while 13 of uncontrolled patients were receiving treatment with metformin. In the 24 patients with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) at baseline, fasting glucose levels were lower than pre-treatment levels ($P=0.0023$) and in the 14 controlled patients were significantly lower than in the 10 uncontrolled ($P=0.012$). Eight patients remained IFG or IGT at study end (33.3%), 16 patients had NGT (66.6%) (8 because of metformin treatment). In the 25 patients with diabetes mellitus at baseline, fasting glucose levels were lower than pre-treatment ($P<0.0001$) and in the 14 controlled patients were significantly lower than in the 11 uncontrolled ($P=0.007$). At study end, 74 patients had NGT (66.1%), 11 had IGT (9.8%) and 21 had DM (18.7%). Changes in glucose tolerance (improvements in 20 patients (17.8%) and worsening in 13 patients (15.2%)) were correlated with achievement or not of disease control. Mean GH levels were the most important predictors of 12 months fasting glucose ($t=2.73$; $P=0.0076$).
Conclusions
This study demonstrated a non significant worsening of glucose tolerance 12 months after SSA treatment given as first-line. Deterioration glucose tolerance was associated with uncontrolled acromegaly during treatment.

P562

Heart rate variability of young men with hypogonadotropic hypogonadism

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Objective
There is little data available regarding the effects of male sex hormones on cardiac autonomic function. The aim of this study is to evaluate the association between male sex steroids and cardiac autonomic function by comparing heart rate variability (HRV) parameters of young male hypogonadotropic hypogonadism patients to those of healthy controls.
Design
The study consisted of 22 male hypogonadotropic hypogonadism patients (mean age 20.8 ± 1.2 years) and the same number of age-matched healthy male controls (mean age 21.0 ± 1.5 years).
Methods
A 24-hour holter monitoring was performed to assess the following HRV parameters; SDNN, SDANN, SDNN5, RMSSD, pNN50, and HRV triangular index (TRIA). Serum levels of FSH, LH, testosterone, estradiol, progesterone and prolactin were measured.
The HRV parameters of patients and control groups were compared, and possible associations between levels of tested hormones and HRV parameters were evaluated.
Results
SDNN, SDANN and SDNN5 values of patients were significantly lower compared to those of controls (147.47 ± 56.16 vs 193.63 ± 40.89; 138.31 ± 57.64 vs 190.15 ± 43.94 and 67.89 ± 21.46 vs 84.63 ± 24.35, respectively; $P<0.05$ for all). Significant negative correlations were observed between serum FSH, LH and testosterone levels and most of the HRV parameters.
Conclusions
Male sex hormone deficiency seems to adversely affect cardiac autonomic modulation with increased sympathetic and decreased parasympathetic components of HRV.

P563

The impact of infertility on the relationship of people with a Pituitary condition

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Background
Evidence from a Needs Analysis (2006) and Patient Satisfaction Survey (2008) for people with pituitary conditions, suggested infertility was a key yet unexpected problem for the majority of respondents. Being infertile without the co-morbidity of having a pituitary condition confers a huge emotional burden on the couple concerned. The aim of this study was to explore in detail the effects infertility imposed on people with a pituitary condition.
Method
A questionnaire comprising 12 open-ended questions was posted to 100 people with a variety of pituitary conditions (61% response rate; 45 females and 16 males; mean age=49; 61% with a prolactinoma and 39% with hypopitutiarism). The participant responses were analysed independently by two researchers using Inductive Thematic analysis.
Results
Four major themes emerged: 'infertility', 'coping and managing', 'feelings and emotional impact', and 'relationship impact'. The focus here is on the impact on relationships. Developing relationships were affected by the pituitary condition and further compromised by a diagnosis of infertility, often exacerbated by communication difficulties between partners. Some avoided the hurt of rejection

by terminating relationships prematurely or by actively choosing partners who did not want children. Participants described how relationships were strained by the emotional rollercoaster of infertility treatment; and for those conceiving, there was the added burden of managing a pregnancy alongside their daily hormonal regime.
Conclusion
Infertility is often a consequence of a pituitary condition and one which is often overlooked and not communicated by healthcare professionals during the battle to manage the condition. Acknowledging this battle by creating opportunities to listen to patients' and partners experiences coupled with the offer of psychological support could reduce much of the emotional distress they experience.

P564

Carotid intima media thickness and other cardiovascular risk factors in acromegalic patients

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Cardiovascular diasease (CV) is the leading cause of mortality in acromegalic patients. Although acromegalic cardiomyopathy has been extensively investigated, there is a lack of data about atherosclerosis in acromegaly.
We aimed to the evaluate the extent of carotid atherosclerosis with various CV biomarkers in acromegaly. Sixty-one acromegalic patients and 21 age and sex matched healthy controls were included. We measured carotid intima media thickness (CIMT) and performed OGTT and hormonal evaluation. CV risk factors including microalbuminuria (MAU), cystatin C, proBNP, uric acid (UA), CRP and serum lipids were also measured. Cystatin C and total cholestrol levels were higher in acromegalic patients ($P=0.012$ and $P=0.005$, respectively), while LDL levels were lower than controls ($P=0.022$). CRP, UA, proBNP, degree of MAU and CIMT were not different from controls. Patients with normal IGF-1 for age or patients who achieved the nadir GH level of <1 ng/ml after OGTT were found to have lower microalbuminuria when compared to their counterparts ($P<0.028$ and $P<0.028$, respectively). However, other parameters were not statistically different between acromegalic patients achieving normal IGF-1 and/or nadir GH <1 ng/ml. We found no difference between diabetic and nondiabetic acromegalic patients in terms of CV risk parameters. We found a weak but positive correlation between CIMT and cystatin C levels ($R=0.201$, $P<0.001$), CIMT and UA ($R=0.164$, $P<0.005$) and CIMT and proBNP ($R=0.202$, $P<0.001$) in acromegalic patients.
CIMT was not higher than controls despite well documented CV risk factors in acromegalics. Disease activity was not associated with CIMT as well.
Correlation between CIMT and cystatin C, UA and proBNP may suggest a cluster effect of some CV risk factors in acromegalics for developing atherosclerosis. Evaluation of vascular abnormalities in conjuction with other CV risk factors may better predict those at higher risk for atherosclerosis and CV events in acromegalics.

P565

Expression and regulation of HIF-1α and RSUME in murine and human pituitary tumor cells

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Hypoxia inducible factor-1alpha (HIF-1α) is a transcription factor regulating expression of many genes related to oxygen homeostasis in response to hypoxic stress. RSUME is a sumoylation enhancer, which was recently cloned from a pituitary cell line with an increased tumorigenic and angiogenic potential. It has been shown that RSMUE is induced by hypoxia and enhances the sumoylation of HIF-1α. Although many studies demonstrated that the level of HIF-1α is remarkably high in some kinds of tumors to increase oxygen availability by promoting tumor neovascularization, the situation in human pituitary tumors is still unclear. The aim of our work is to investigate the expression of HIF-1α in adenomatous pituitaries and the relationship between HIF-1α and RSUME. In our preliminary study, samples of three corticotrophic adenomas, five somatotrophic adenomas and two prolactinomas were detected by immunohistochemistry, and the results have shown that some of them have significantly higher levels of HIF-1α, compared with normal pituitaries. In order to investigate the regulation of HIF-1α and RSUME under hypoxic condition, we stimulated mouse pituitary tumor cell lines (TtT/GF and AtT20) and human none function pituitary adenoma primary cell cultures with cobalt chloride, which is a well-known hypoxia-mimicking agent. In all of the cell cultures, a time and dose dependent increase of HIF-1α was observed, and this could be suppressed with RSUME siRNA in both TtT/GF and AtT20 cell lines. VEGF was time and dose dependently stimulated in TtT/GF but not in AtT20 cells. By RT-PCR it was shown in both cell lines that cobalt chloride treatment enhanced RSUME mRNA expression, which could be inhibited by siRNA, and 45% human pituitary tumor samples have significantly higher RSUME mRNA expression in 33 samples compared with 4 normal human pituitaries. Our preliminary findings suggest that the level of HIF-1α increases in some pituitary adenomas and RSUME stabilizes HIF-1α in some pituitary cell lines under hypoxia mimicking condition. The co-localization of HIF-1α and RSUME in human pituitary tumors is being studied and more evidences need to be provided to elucidate that RSUME stabilizes HIF-1α in pituitary tumors, which probably gives a new access to control pituitary tumor development.

P566

MicroRNA expression in human sporadic pituitary adenomas

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Introduction
MicroRNAs (miRs) are 16–29 nucleotide long, non-coding RNA molecules that post-transcriptionally regulate gene expression via RNA interference. It has been shown that they participate in control of cell proliferation, cell differentiation, signal transduction, cell death and carcinogenesis.
Aim
To examine the role of the miRs in sporadic pituitary tumourigenesis.
Methods
Twenty-five sporadic pituitary adenoma specimens were analyzed approved by the Ethical Committee of the Hungarian Health Council. Real time quantitative PCR using microRNA low density array (TLDA) with *in silico* target prediction were used for identification of miRs potentially involved in pituitary tumourigenesis.
Results
Expression profile revealed 87 differentially expressed miRs: 17 miRs only in the normal pituitary tissues, 4 only in adenomas and 66 with a difference more than 2.5-fold between normal and adenoma tissues. Examining the miR's chromosomal localizations we found that the 87 differentially expressed miRs were located in 51 chromosomal regions. In the underexpressed group a cluster of 18 miRs was banded to 14q32.31, 4 miRs to Xq37.3, and 8 miRs to Xp11.22 region. Combining these results with the *in silico* target prediction we selected six miRs for further validation using Taqman assays. MiR-17-5p, miR-107, miR-221, miR-98, miR-225 and miR-429 were overexpressed in 88, 80, 68, 76, 56 and 60% of adenoma tissues, respectively. MiR-17-5p potentially targets *PRKAR1A* while miR-107 *AIP*.
Conclusions
The genes of the downregulated miRs map to regions which have already been identified as a hotspot for loss of heterozygosity in several tumours suggesting that in these regions a potential tumour suppressor gene which has not been identified yet is located or, based on our results, miRs located here could function as a tumour suppressor. miR-17-5p and miR-107 through regulation of the expression of the PRKAR1A and AIP may contribute to the pathogenesis of sporadic pituitary tumours.

P567

Progesterone up-regulates transthyretin levels in primary cultures of choroid plexus epithelial cells

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Transthyretin (TTR) is a protein mainly synthesized by the liver and choroid plexus (CP) of the brain. Besides its role as a carrier for thyroid hormones, TTR also sequesters the amyloid beta (Aβ) peptide impairing its deposition in nervous tissues, and its concentrations in the cerebrospinal fluid (CSF) appear to be inversely correlated with Alzheimer's disease (AD) onset and progression. Abundant evidence suggests that the depletion of progesterone (PROG) at menopause is a significant risk factor for the development of AD in women, but the mechanisms involved are poorly understood. So, we examined the effects of PROG on TTR mRNA levels, in primary cultures of rat CP epithelial cells (CPEC) by Real Time PCR. We show that, PROG (100 nM) induced TTR transcription in these cells. The combination of mifepristone (RU486) with PROG in the treatment of CPEC abrogated the induction of TTR expression by PROG. Pre-treatment with ICI 182 780 and flutamide, specific oestrogen and androgen receptor antagonists, respectively, had no effect on TTR levels. These data suggest that the effects observed are due to PROG itself and not from downstream products from its metabolization, such as testosterone and estradiol, and indicates that TTR is up-regulated via a progesterone receptor (PR)-dependent pathway. Our results highlight the importance of PROG on the regulation of TTR, which may be involved in the neuroprotective role of PROG in AD described in the literature.

P568

Long-term follow-up of female patients with prolactinoma: is there a place for surgery in the therapy of prolactinoma?

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Background
Medical therapy with dopamine agonists (DA) is the primary treatment in most patients with prolactinomas. 'Classical' surgical indications are mainly intolerance of DA therapy or non-responders. Focusing on a possible shift of recent indications towards a surgical approach, we retrospectively analyzed the long-term results of surgical treatment and compared them to the medically treated female patients with prolactinomas.
Patients and methods
Between 1977 and 2007, the chards of 105 female patients with prolactinoma were reviewed. Clinical, biochemical characteristics and tumour size were assessed at baseline and at last follow-up in the patients who underwent transsphenoidal surgery (S; $n=71$) and in the medically treated cohort (M; $n=34$). Within the S group a subgroup with intrasellar microadenoma (IS; $n=41$) were analysed separately.
Results
The mean age *at diagnosis*, clinical presentation, prolactin levels and the tumour size was similar in the S and the M group at baseline (age: S: 33.3 (9.9) years, mean (s.d.) versus M: 35.4 (5.0); Prolactin: S: 182.2 (89.7–249), μg/l, median (IQR); M: 110.8 (85.5–679.5); Tumour size: macro-/meso-/microadenoma: S: 17%; 25%; 58% versus M: 23%;9%; 68%; NS). The mean follow-up was similar in both groups (S: 121 (99) months, mean (s.d.) versus M: 112 (93); NS).
At last follow-up, galactorrhea was reported in 9% of the M and in 3% of the S patients ($P=0.17$). Persistent amenorrhoea was documented in 6% and in 5% of the S and M cohort, respectively. Prolactin levels were controlled in 87% (S) vs 72% (M; $P=0.07$) requiring DA therapy in 66% (M) and in 32% (S) of the patients ($P<0.001$). Analysis of the surgical treated group with intrasellar micoradenoma (IS) revealed a control of hyperprolactinaemia in 91% of the patients requiring DA therapy in 26%. Patients with microadenoma medically treated had a control of hyperprolactinaemia in 84% with a persistent need for DA therapy in 52%. Transient complications of transsphenoidal surgery included diabetes insipidus (23%) and liquor fistula (4.2%). Persisting complications consisted of an additional pituitary axis insufficiency in 4.2%, similar to the findings in the M group. There was no mortality associated with the surgical intervention. Transient side effects of DA therapy (nausea, orthostatic problems) were present in 36% of the patients.
Conclusion
(1) Transsphenoidal surgery for prolactinoma in female patients has no mortality in this cohort (2) Transitory side effects of treatment strategy occur in the S and M cohort in a substantial number of cases (3) The long-term control of hyperprolactinaemia in the S and M is similar (4) The present data justify at least the discussion about a neurosurgical approach in selected patients.

P569

Clinical presentation, long-term follow-up and bone morbidity of male patients with prolactinoma

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Background
In contrast to females with prolactinoma, male patients usually present with a history of long-standing hypogonadism and a macroadenoma on MRI-scan. Data is scarce about the effect of hypogonadism on bone health in these patients. We, therefore, investigated retrospectively the cohort of male patients with prolactinoma at our institution.
Patients and methods
Between 1983 and 2007, the chards of 44 male patients with prolactinoma were reviewed. Clinical, biochemical characteristics and tumour size were assessed at baseline and at last follow-up. Qualitatively bone density assessment (i.e. osteopenia, osteoporosis) was registered.
Results
The mean age *at diagnosis* was 47.4 (15.3), years, mean (s.d.), the leading symptoms were loss of libido in 68% and erectile dysfunction in 50% of the patients. Mean BMI at diagnosis was 28.7 (4.5) kg/m^2. Prolactin levels were 1978.5 (779.8–4890.3), μg/l, median (IQR), and MRI scan showed macro- meso- and microadenoma in 77, 5 and 18% respectively. Bone density revealed pathological bone density in 25% of the patients. Nine percent of all patients were diagnosed with osteoporosis. Therapeutical strategy included primary operation in 32% and dopamine agonists in 68% of the patients.
At last follow-up the mean age was 54.0 (15.6), years, loss of libido and erectile dysfunction was reported in 20 and 15% of the patients, respectively. Mean BMI tended to decrease from 28.7 (4.5) to 28.0 (4.4) kg/m^2 ($P=0.08$). Prolactin concentration significantly decreased to 13.8 (7.0–27.1) μg/l, median (IQR; $P<0.001$) and was within normal range in 80% of the patients. The control of hyperprolactinaemia required Dopaminagonist therapy in 75% of the patients (three patients with microadenoma, 2 patients with mesoadenoma, 28 patients with macroadenoma). Fifty five percent of all patients needed Testosterone therapy, 2/3 of them had macroadenomas. Biphosphonate and/or Vitamin D and Calcium was prescribed in 25% of the patients. No significant differences in clinical outcome and need for dopamine agonist or testosterone therapy were observed according to the therapeutical strategy (i.e. primary surgery vs primary medical therapy).
Conclusion
(1) Based on these results assessment of bone densitometry in male patients with prolactinoma can be recommended. (2) The tendency for a decrease of BMI following therapy remains to be confirmed. (3) A surgical procedure besides the classical indication (i.e. intolerance of dopamine agonists and non-responder) cannot be recommended in male patients with macroprolactinoma. (4) A substantial number of patients had testosterone replacement therapy at last follow-up.

P570

Effects of somatostatin analogs on glucose homeostasis: a meta-analysis of acromegaly studies

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Objective
Somatostatin analogues (SSA) are widely used for the treatment of patients with neuroendocrine tumors. These drugs are able to influence glucose metabolism by an inhibitory effect of insulin secretion, but the clinical impact of this effect is uncertain. Most of the data on this topic have been obtained from studies including patients in acromegaly, one the major indication for SSA, which are limited in terms of numerosity and study duration. Therefore, we have carried out a meta-analysis on data from published, long-term trials in acromegaly in order to assess the clinical impact of SSA on glucose metabolism.

Methods
The outcomes analyzed were fasting plasma glucose concentration (FPG), fasting plasma insulin concentration (FPI), haemoglobin A(1c) (HbA1c) and plasma glucose during oral glucose tolerance test (OGTT). Eligibility criteria were: (1) duration of SSA treatment of at least 3 months; (2) available numerical data for at least one of the four biochemical outcomes investigated; (3) measurement of the outcomes before and after SSA treatment; (4) no selection of acromegalic patients for their responsivity to SSA. After revision, only 31 studies fulfilled eligibility criteria.
Results
SSA treatment was found to induce statistically significant decrease in FPI (effect size-0.45, 95% CI: −0.58; −0.32, $P<0.001$), without any significant change of FPG (effect size 0.04, 95% CI: −0.07; 0.15, $P=0.52$) and HbA1c (effect size 0.11, 95% CI: −0.02−0.23, $P=0.09$). Serum glucose values during OGTT were shown to significantly change during SSA treatment (effect size was 0.31, 95% CI: 0.17; 0.45, $P<0.001$) although with high inconsistency among trials.
Conclusions
Our data suggest that modifications of glucose homeostasis induced by SSA have an overall minor clinical impact in acromegaly. Although this does not mean that in selected patients a clinically significant deleterious effect may be observed, our findings suggest that SSA are drugs with a favourable risk/benefit ratio.

P571

Macroprolactinemia in patients with pituitary adenomas

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Elevated macroprolactin level is one of the reason of misdiagnosis and mismanagement of hyperprolactinamia. About 10% of population have incidentalomas and it also can combine with increasing of prolactin (Prl).
Three hundred and thirty patients with hyperprolactinemia (Prl > 600 mU/l) were studied: 192 women and 138 men in age of 30 (25; 39) and 35 (29;46) years respectively. Clinical, biochemical and MRI methods were used. Prl, LH, FSH and Testosterone levels were determined by fluorescence method. Monomeric Prl (monPrl) was determined after polyethylene glycol precipitation (Delfia; Finland): a recovery of <40% was accepted as macroprolactinaemia.
Macroprolactinemia was identified in 64 patients (19.4%): 51 women (26%) and 13 men (9.4%). True hyperprolactinaemia was founded 266 patients (80.6%). Median of Prl level in group with macroprolactinemia was 1080 (922; 1324) mU/l in women and 1004 (698; 1600) mU/l in men; monPrl level was – 271 (222; 331) mU/l and 251 (182; 444) mU/l respectively.
Pituitary adenomas were revealed in 195 (59.1%) patients. Only 17 of them (8.7%) had macroprolactinemia: 12 with microadenomas (7 women and 5 men) and 5 with macroadenomas (1 woman and 4 men). Median of Prl level in this group was 1055 (947; 1509) mU/l in women and 1253 (887; 1770) mU/l in men; monPrl – 248 (193; 302) mU/l and 265 (182; 444) mU/l respectively.
Clinical features of hyperprolactinemia were in 9 men and in 2 women with macroprolactinemia and pituitary adenomas. All of them had increased monPrl level. Cabergoline treatment was prescribed for these patients. There were no clinical symptoms in other patients.
This investigation revealed that macroprolactinaemia in patients with pituitary adenomas is found out rare than in patients with hyperprolactinemia in whole. Reasonability of medical treatment of these patients should be determined on monPrl level, not only on Prl level.

P572

Primary medical treatment of large and giant prolactinomas

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Objective
Prospective study of medical treatment effect in newly diagnosed patients with large and giant prolactinomas.
Patients and methods
The study group included 45 patients with large prolactinomas (tumors more 3,6 cm) and 23 patients with giant prolactinomas (tumors more than 6 cm); from them 8 mainly cystic tumors. The treatment period was 3–24 month (mean 6). Serum prolactin level before treatment ranged between 12 990 and 1 038 000 mU/l (mean 198 000; normal 30–545 mU/l). Fifty-five men and 13 women aged 16–67 years (mean 39) were treated with cabergoline of dose from 0.5 mg to 4.0 mg/week (mean 1.5 mg). Fifty-four patients before treatment had visual impairment. Fourteen patients had optic atrophy. Fourteen patients had epileptic syndrome.
Results
Tumors, including 7 cystic, decreased in size in 55 patients. The size of 13 tumors was without any change. Decrease of prolactin occurred in all patients; serum prolactin level was 350–19 460 mU/l (mean 2500) during treatment. Forty-two patients had improved visual symptoms in the early stage of treatment, other patients showed no visual impairment. In 5 (7%) patients cerebrospinal fluid (CSF) leakage occurred within 14–30 days after initiation of treatment. In 2 patients endoscopic endonasal surgery to repair the fistula was performed.
In other patients the CSF leakage ceased with diuretic therapy and with temporarily decreases of cabergoline dosage. The drug was well tolerated by all patients and no one discontinued the therapy.
Conclusion
Cabergoline should be the first-line therapy for large and giant prolactinomas, even in patients with visual defects. Use of cabergoline results in effective reduction of prolactin, improvement of visual defects and provides tumor shrinkage (including cystic prolactinomas).
Patients with large and giant prolactinomas are at risk of CSF leakage during medical treatment with cabergoline.

P573

Transport activities and plasma membrane localization of MCT8 mutant proteins identified in patients with severe psychomotor retardation depend on cell type. Implications for the interpretation of clinical phenotypes

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Objective
Mutations in the gene encoding the thyroid hormone transport protein, monocarboxylate transporter 8 (MCT8), underlie severe mental retardation. *In vitro* expression of mutant transporters was performed to understand phenotypical differences.
Methods
We established cell lines stably expressing 16 MCT8 variants in JEG1 and MDCK cells. Several of these mutants have never been analysed before. The cell lines were characterized according to MCT8 mRNA and protein expression, T3 transport activity, substrate KM characteristics, surface expression and responsiveness to treatments aiming at rescuing transporter function.
Results
We could clearly demonstrate that functional activities of MCT8 mutants depend on the cell type in which they are expressed (e.g. S194F, V235M, ins235V, ΔF230, R271H, L434W, L512P, L568P). These mutants exhibited considerable transport activity when present at the cell surface as demonstrated by surface biotinylation. All mutations found in patients with milder impairments are partially active in at least one cell type *in vitro*, whereas other mutants are functionally inactive even if present at the cell surface (ins189I, A224V). G418 treatment of the non-sense mutants did not induce read through to yield full-length MCT8 irrespective of dose incubation time.
Conclusions
The finding that the cell type determines surface expression and T3 transport activity of missense mutants in MCT8 is important to understand phenotypic variability among carriers of different mutations. Moreover, the clinical observation that the severity of derangements of thyroid hormone levels does not correlate with mental impairments of the patients, may be based on different residual activity in different cell types e.g. pituitary thyrotrophs and central neurons. Our results indicate that patients may benefit from treatment strategies that enhance surface expression of mutated MCT8.

P574

Is it possible to avoid hypopituitarism after the irradiation of pituitary adenomas by the Leksell gamma-knife?

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Radiation therapy is used in the treatment of pituitary adenomas, especially in failures of neurosurgery and pharmacotherapy to reduce the size of adenomas and normalize their hypersecretion. Conventional fractionated radiotherapy has

achieved good results, but only after a long latency, with considerable postradiation morbidity and with very frequent appearance of hypopituitarism. The focal stereotactic targeting allowed by Leksell gamma-knife (LGK) was supposed to decrease the incidence of hypopituitarism, however our first patients treated by LGK developed hypopituitarism in 38.2%. Consequently, we have analyzed factors leading to this unfavorable outcome and suggested that the mean dose of irradiation on pituitary tissue is the most important cause of hypopituitarism.
Results
Seventy-five patients (47 women and 28 men) with pituitary adenomas (39 with acromegaly, 17 prolactinomas, 8 with Cushing's disease, 1 with Nelson's syndrome and 9 functionless adenomas), where the mean dose of irradiation on pituitary tissue was measured, were followed 60–180 (mean 91.1) months. In 41 patients, the mean dose of irradiation on pituitary was more than 15 Gy. The hypopituitarism at least in one axis developed in 29 (70.7%) patients during 10–126 months after the irradiation. In 34 patients, the mean dose on pituitary was less than 15 Gy. Only one patient (1.2%) developed hypopituitarism 12 months after the irradiation. This patient had undergone two previous pituitary surgeries and had already central hypothyreosis when irradiated.
Conclusion
To avoid hypopituitarism the radiation dose of 15 Gy is the maximum safe limit of the mean dose of radiation to the pituitary tissue surrounding the adenoma. This cut off should become a rule when irradiating pituitary adenomas – just like the dose rules of 7 Gy on the optical tract or 14 Gy on the brainstem.

P575

Effect of prolonged treatment with potassium canrenoate, a MR antagonist, on basal and stimulated hypothalamus–pituitary–adrenal (HPA) axis in humans

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HPA is negatively regulated by glucocorticoid feed-back at hypothalamic, pituitary and hippocampal level by glucocorticoid (GR) and mineralcorticoid (MR) receptors. MR antagonists impair HPA rhythm after acute administration. The aim of this study was to verify HPA activity and glico-insulinemic profile both basally and after acute administration of potassium canrenoate (CAN), before and after chronic treatment. We evaluated ACTH, cortisol (F) and dehydroepiandosterone (DHEA) levels during placebo and CAN infusion (200 mg bolus followed by 200 mg i.v. over 240 min) in six healthy women (27.2 ± 1.5 years, BMI 21.4 ± 1.4 kg/m^2) at baseline and after 21 days CAN treatment (200 mg/die po). Blood samples were taken every 15′ for 240′ starting from 16.00 to 20.00 h, time period in which glucocorticoids preferentially bind MRs. We also evaluated insulin and glucose levels during all the sessions. At baseline, ACTH, F and DHEA showed a progressive decrease, more marked for F. During CAN infusion, ACTH, F and DHEA were significantly higher than during placebo ($P<0.05$ starting from $+15'$). After chronic CAN treatment, basal F and DHEA were higher than at baseline ($P<0.05$ from $+30'$ to $+210'$), while ACTH showed a trend but not significant toward increase. Moreover, acute CAN infusion was unable to further increase all the hormonal levels. No differences in insulin and glucose levels were recorded before and after CAN treatment. These findings demonstrate that acute MR antagonism is able to amplify HPA activity during the quiescent phase of the circadian rhythm, in agreement with previously observed at the nadir of the circadian rhythm. Interestingly, chronic MR antagonism up-regulates the HPA activity and makes it refractory to further increase induced by acute CAN administration. This suggests an alteration in the MR sensitivity after prolonged antagonism leading to a derangement in the MR-mediated glucocorticoid feed-back.

P576

Initial and long-term outcome of surgery in acromegaly: a ten-year, single centre study in 115 patients

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Objective
To analyze characteristics of patients who had surgery for a GH-secreting adenoma in the past decade in our centre, to evaluate their initial outcome and long-term recurrence rate using stringent criteria and identify potential predictive factors of surgical remission.
Methods
This retrospective study included 115 consecutive patients with acromegaly operated at the neurosurgical department of the Timone Hospital Marseille between 1997 and 2007, with a mean follow-up of 3.8 years.
Results
Using stringent criteria (GH nadir at oral glucose tolerance test (OGTT) ≤ 0.4 μg/l and normalisation of IGF-1 level), remission rate at 3 month was 37.4% (65% for microadenomas and 31.6% for macroadenomas). A subset of patients (23.5%) had discordant values in terms of GH/OGTT or IGF-1 levels. Only 7.8% of patients had surgical complications and no mortality was observed. In multivariate analysis, preoperative mean GH level, tumour invasion and surgical observation of total resection were significant predictors of surgical outcome. Beneficial effect of preoperative medical treatment was significant in univariate analysis, independently of confounding factors. We observed only 2% of long-term recurrence. At the end of follow-up, acromegaly was controlled in 90.9% of patients with or without adjunctive treatment (including 49.5% after surgery only).
Conclusion
Using stringent criteria for remission, a very low recurrence rate was observed in this study. The best results of pituitary surgery were obtained in non invasive adenomas with low preoperative GH levels. Presurgical medical treatment was also a significant factor related to remission. Nevertheless improvement of long-term control of acromegaly in the context of newly available therapeutic options is likely to modify the management of such patients.

P577

Two repeated restraint stress paradigms differing in duration and frequency result in similar levels of HPA habituation, but differences in neuropeptide expression and testosterone levels

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The dampening of hypothalamic–pituitary–adrenal (HPA) axis responses to a repeated stress is termed HPA habituation. We investigated here the effects of two commonly used repeated restraint stress paradigms, in which rats are exposed either to 10 episodes of 0.5 h restraint or 5 episodes of 3 h restraint. We compared ACTH, corticosterone, and testosterone responses, as well as corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) mRNA levels within brain regions involved in HPA regulation and ACTH secretion. Relative to initial exposures, declines in HPA activation measured by endocrine ACTH and corticosterone responses were remarkably similar among paradigms following repeated exposures. Compared to nonstressed controls, CRH mRNA in the paraventricular nucleus of the hypothalamus remained unchanged, while AVP mRNA was increased following 3 h, but not 0.5 h repeated restraint. 3 h repeated restraint increased AVP mRNA in the bed nucleus of the stria terminalis (BST), and CRH mRNA was increased in the BST and central amygdala. 0.5 h repeated restraint increased AVP mRNA levels in the medial amygdala, with no changes in the BST and central amygdala. In summary, despite comparable reductions in HPA activation, CRH and AVP expression within limbic and hypothalamic HPA neurons were differentially affected by each paradigm. Thus, the pathways recruited for HPA habituation appear to be distinct between these paradigms. Testosterone could be key to the differential peptide response, at least with respect to AVP expression, considering the extreme sensitivity of this peptide to changes in gonadal status.

P578

Association of Interleukin-6 with hot flashes in perimenopausal women

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Introduction
Hot flashes and night sweating are the most common medical complaints in women during the menopausal transition and they are strongly associated with the presence of perimenopausal depression. The pathophysiology of hot flashes has not been elucidated. Peripheral vasodilation and increase in skin temperature observed in women with hot flashes might be induced by changes in brain neurotransmitters and instability in the hypothalamic thermoregulatory center due

to estrogen withdrawal. Cytokines such as IL-6 and TNF-α have been reported to be potent vasodilators. We investigated if there is any association between IL-6, TNF-α and hot flashes in perimenopausal women.
Material and methods
We examined 65 perimenopausal women. All women were between the ages 40 and 55 and presented with a history of menstrual cycle irregularity of at least 6 months duration but not longer than 1 year of amenorrhea. Menopause rating scale (MRS) was given to women in order to examine the presence and severity of hot flashes. IL-6 and TNF-α were analysed with standard laboratory methods. Pearson's correlations were applied to evaluate the relationship between cytokines and vasomotor symptoms.
Results
Serum IL-6 concentrations in perimenopausal women with severe hot flashes were significantly higher than the concentrations in women without hot flashes or with mild and moderate hot flashes. In the contrary, there is no difference in serum TNF-α concentrations in the population we examined.
Conclusions
IL-6 may be associated with peripheral vasodilation in women with hot flashes.

P579

Empty sella and primary autoimmune hypothyroidism

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Objective
To assess the association between empty sella (ES) and primary autoimmune hypothyroidism, and the possibility of a common pathogenesis.
Patients and methods
We retrospectively studied all patients with presumed ES diagnosed in the last 20 years, most of whom were treated and followed up by our Endocrinology Department. Subjects with a known aetiology were excluded. Incomplete records or those with a doubtful diagnosis were also excluded. A total of 56 subjects were included in the study. ES was diagnosed by pituitary MRI. The measurement of free T4, TSH, and antithyroid antibodies (TPOAb and TgAb) was assayed using commercial kits. Hypothyroidism was defined as a serum TSH titer higher than 10 μU/ml and/or FT4 less than 0.6 ng/dl. Subclinical hypothyroidism was defined as a serum TSH titer of 4.5 to 10 μU/ml and normal FT4.
Results
Fifteen (26.78%) patients of 56 with ES had autoimmune thyroid disease (6 with primary hypothyroidism, 6 with subclinical hypothyroidism, and 3 with normal FT4 and TSH values). Primary hypothyroidism with negative antithyroid autoantibodies was found in a further 13 patients (23.21%).
Conclusions
There is an important association between ES and autoimmune thyroid disease, which reached 26.78% in our series. The percentage would probably be higher if the antithyroid antibody test had been performed in all cases of hypothyroidism. We suggest the possibility of a common pathogenesis for certain cases of ES and autoimmune thyroid disease, in which ES may occur as the natural progression of autoimmune hypophysitis more or less simultaneously with autoimmune thyroid disease, with the end point of ES in the pituitary and atrophy in the thyroid gland. Advances in laboratory methods for antipituitary autoantibody determination would help to resolve this question.

P580

Human corticotropin releasing hormone (hCRH) test performance in the differential diagnosis between Cushing's disease and pseudo-Cushing state is enhanced by combined ACTH and cortisol analysis

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Objective
The corticotropin releasing hormone (CRH) test does not reliably distinguish between Cushing's disease (CD) and normality or pseudo-Cushing state (PC). In this study we assessed whether the application of novel criteria could enhance its diagnostic performance.
Design
Retrospective study.
Patients
Fifty-one subjects with CD, 26 with PC and 31 control subjects (CT).
Measurements
All subjects underwent human CRH (hCRH) test and standard diagnostic procedures for the diagnosis of Cushing's syndrome (CS).
Results
The area under the curve (AUC)-ACTH exhibited a significant negative correlation with baseline serum cortisol in CT and PC subjects, but not in CD patients. The ACTH response to hCRH was blunted in PC compared with CT subjects. These findings suggested that CD can be diagnosed by the simultaneous presence of two hCRH test parameters and excluded in the absence of either or both.
The two-parameter combinations proposed are (1) basal serum cortisol >331 nmol/l and absolute peak plasma ACTH >12 pmol/l, or (2) absolute peak serum cortisol >580 nmol/l and absolute peak plasma ACTH >10 pmol/l. The combined criteria had a sensitivity (SE) of 90.1 and 94.1% and a specificity (SP) of 98.2% and 91.2%, respectively.
Conclusions
The proposed combinations enabled the hCRH test to distinguish CD patients from PC and CT subjects.

P581

Hemostatic and fibrinolytic changes in patients with Cushing's disease

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Introduction
Chronic endogenous hypercortisolism is characterized by abdominal obesity, systemic arterial hypertension, glucose and lipid abnormalities, insulin resistance, osteoporosis. This syndrome also has features of hypercoagulation. The mortality rate in patients with active Cushing's disease (CD) is four times higher than in age- and sex- matched population. The main cause is cardiovascular disease with thrombotic complications.
Objective
To study hemostatic and fibrinolytic state in patients with CD.
Materials and methods
We studied 31 patients with active CD (group 1), 21 patients during one year CD remission after successful surgical treatment (group 2) and 16 patients were the control (group 3). Prothrombin time (PT), thrombin time (TT), activated partial thromboplastine time (APTT), fibrinogen, plasminogen, tissue plasminogen activator (tPA) and inhibitor (PAI-1) were investigated. Statistical analysis was performed using Crasckell-Walles criteria. Results are presented as median and 25; 75 percentiles.
Results
Results are given for the groups 1, 2 and 3, respectively. Fibrinogen level, mg/dl: 341 (306; 370), 329 (316; 391), 285 (226; 339) ($P=0.0333$); PTI, %: 96 (89.5; 101), 85 (78; 93), 85 (75; 93) ($P=0.0074$, $P1.2=0.0055$, $P1.3=0.0351$, $P2.3=0.683$); TT, s: 18.1 (16.9; 19.7), 17.1 (16.6; 22.3), 17.2 (16.7; 18.5) ($P=0.6770$); APTT, s: 29.2 (36; 27.45), 33.6 (32.9; 35.4), 34.8 (33; 39) ($P=0.0001$, $P1.2=0.001$, $P1.3=0.0009$, $P2.3=0.276$); plasminogen, %: 96 (87; 102), 86 (73; 92), 79.5 (70; 85) ($P=0.0002$, $P1.2=0.001$, $P1.3=0.001$, $P2.3=0.331$); tPA, ng/dl: 3.24 (1.90; 5.51), 2.026 (1.67; 2.67), 1.61 (1.49; 2.06) ($P=0.002$, $P1.2=0.021$, $P1.3=0.001$, $P2.3=0.09$); PAI-1, ng/ml: 69.63 (39.33; 90.46), 41.80 (16.85; 86.80), 37.92 (28.16; 42.35) ($P=0.0163$, $P1.2=0.02$, $P1.3=0.007$). Thus, patients of group 1 had significantly higher fibrinogen level and lower APTT than patients of groups 2 and 3. tPA, PAI-1 levels were significantly increased before treatment and decreased slowly after treatment to become normal in 6–12 month. The haemostatic parameters of patients in group 2 and group 3 did not differ significantly.
Conclusion
Our results suggest that hypercoagulation found in patients with active CD is predominantly associated with fibrinolytic system alterations.

P582

Immunoassay determination of macroprolactin in hyperprolactinemic patients: an interassay comparative study

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Prolactin is mainly found in the monomeric form although it can also occur in the big-PRL and bigbigPRL (bbPRL) form: a complex of prolactin and

immunoglobulin G. The latter has reduced bioactivity despite maintaining its imunorreactivity. This can cause false positive results representing the main cause of interassay variability in prolactin measurement. It should therefore be considered in every study concerning hyperprolactinemia. We aimed to evaluate the prevalence of bbPRL in a sample of patients with hyperprolactinemia and to investigate its interference in two immunoassay systems: IMMULITE2000 and KRYPTOR.
Retrospective analysis of seventy-four samples of patients with hyperprolactnemia was undertaken. The mean age was 44 ± 12.6 years; seven patients were males. Prolactin was measured with an immunometric chemiluminescent (IMMULITE2000) and an immunofluorescent (KRYPTOR) method; additionally, bbPRL was precipitated using polyethyleneglycol (PEG) followed by prolactin measurement in the supernatant with IMM2000. We considered bbPRL to cause interference when the percentage of recuperated prolactin was fewer than 40%.
In nineteen patients the hyperprolactinemia was due to bbPRL; in one case there was an excess of prolactin monomers simultaneously. In those patients with macroprolactinemia, the mean values found by IMM2000, KRYPTOR and PEG where, respectively, 47.6 ± 21.9; 17.5 ± 10.9 and 10.1 ± 8.2 ng/ml ($P < 0{,}05$).
In conclusion, KRYPTOR has less immunorreactivity than IMM2000. Measurements after PEG precipitation were useful in the diagnosis of macroprolactinemia allowing, in this sample, to alter the management of 26% of patients.

P583

Adiponectin and visfatin: a link with bone mineral density in acromegaly

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Two adipokines highly expressed in fat mass, adiponectin with antiinflammatory and antiatherogenic properties, and visfatin with an insulin-mimetic effect, are potential contributors to bone metabolism. In acromegaly data on adiponectin are contradictory and there are no data on visfatin.
Objectives
To evaluate adiponectin and visfatin in acromegaly, compared to control subjects and to analyze their relationship with body composition and bone mineral markers.
Methods
Bone markers (osteocalcin, amino-terminal propeptide of type 1 procollagen (P1NP), cross-laps), body composition (by DEXA), adiponectin (ELISA) and visfatin (immunoanalysis) were evaluated in 60 acromegalic patients (24 males and 36 females) and in 105 age- and gender matched healthy controls (33 males and 72 females). Acromegalic patients were classified as controlled, with normal IGF-I and GH ≤ 1 μg/l ($n = 41$) or active ($n = 19$).
Results
Acromegalic patients had lower adiponectin ($P < 0.01$), more lean body mass ($P < 0.01$) and total body mass ($P < 0.01$), higher bone formation markers (osteocalcin and P1NP, $P < 0.05$ and $P < 0.01$ respectively), but lower bone resorption markers (cross-laps) and fat mass (both total and trunk, $P < 0.05$) than controls ($P < 0.001$). No differences in visfatin and bone mineral density (BMD) were found between patients and controls. Adiponectin correlated negatively with BMD ($r = -0.374$; $P < 0.05$) and lean mass ($r = -0.301$; $P < 0.05$) and positively with age ($r = 0.347$; $P < 0.001$). Visfatin correlated negatively with BMD ($r = -0.359$; $P < 0.05$).
Conclusions
Acromegalic patients present hypoadiponectinemia. BMD is predictor for adiponectin and visfatin in patients with acromegaly. No correlations were found between individual bone markers and both cytokines. Adiponectin and visfatin could be a link between fat mass and bone in acromegalic patients.
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P584

Feedback inhibition of prednisolone on vasopressin (AVP) secretion but preserved renal water reabsorption after thirsting point to an AVP independent antidiuretic action

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Adrenal insufficiency can result in severe hyponatremia due to inappropriate high plasma vasopressin (pAVP). To elucidate the glucocorticoid AVP feedback we monitored thirsting of 12 male volunteers without and after on or five days of prednisolone (30 mg/d). Although prednisolone suppressed pAVP below 0.2 pg/ml the rise in plasma osmolality during thirsting was not influenced by prednisolone. Independent of exogenous glucocorticoid thirsting resulted in higher urine osmolality and decreased urine volume. Although pAVP was extremely low there was no difference in urinary secretion of aquaporin-2 (AQP2). Exogenous stimulation of the AVP V2 receptor by 4 μg desmopressin resulted in normal renal response with increased urine osmolality and decreased urine volume. The V2 agonist induced a significant increase of urinary AQP2 secretion. This increase is independent of prednisolone intake and suggests that AVP is able to act normally on the translocation of the water channel AQP2 in the principal cells of the collecting duct, compatible with our data of a prednisolone unrestricted rise in urinary cAMP excretion after desmopressin injection. Evidence has been reported that both secretion of atrial natriuretic peptide (ANP) and synthesis of prostaglandin E2 (PGE2) are influenced by the action of glucocorticoids and that they may modulate renal AVP action. In this study urinary excretion of PGE2 was not influenced by prednisolone intake. Plasma ANP concentration were higher during prednisolone treatment. An AVP independent effect of elevated ANP on AQP2 translocation would be compatible with the reported phosphorylation of AQP2 at Ser256 by protein kinase G (PKG) and subsequent AQP2 membrane translocation.
The experiments show a strong feedback inhibition of the glucocorticoid prednisolone on AVP secretion. Preserved renal water reabsorption after thirsting in the presence of prednisolone suggests an AVP independent mechanism that may be influenced by higher ANP plasma concentrations.

P585

Genetic alterations of phosphodiesterase 11A (PDE11A) in acromegalic patients

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Background
Aberrant cAMP signalling is involved in the pathogenesis of somatotropinomas. Recently, variants of phosphodiesterase type 11A (PDE11A) gene have been described in patients with adrenocortical tumors. Aim of the study was to investigate the presence of these variants in patients with somatotropinomas.
Subjects and methods
Germ-line DNA of 78 acromegalic patients was screened for known genetic variants of PDE11A by direct sequencing or restriction analysis. Immunohistochemistry for PDE11A was performed in a subgroup of adenomas and in normal pituitary samples.
Results
We found non-synonymous germ-line substitutions in 17% of acromegalic patients, i.e. 14 missense variants (6 Y727C, 1 R804H, 4 R867G and 3M878V) and 1 truncating mutation in 1 patient who also presented R867G variant. Tumor DNA from these patients showed both the variant and wild-type PDE11A sequences, with the expected percentage of gsp mutations (38%). Immunohistochemistry revealed variable PDE11A expression, absence of PDE11A staining being limited to the tumor with truncating mutation. No significant differences in hormonal and clinical parameters between patients with or without PDE11A variants were observed, although patients with PDE11A variants showed a tendency to have a more aggressive tumor compared to patients with wild-type sequence (extrasellar extension in 69% *vs* 45%).
Conclusions
This study first demonstrated the presence of nonsense/missense PDE11A variants in a subset of acromegalic patients. The retaining of the wild type allele resulting in a normal expression of the enzyme in the tumor tissues, together with the lack of significant clinical phenotype suggest that these variants might only marginally contribute to the development of somatotropinomas.

P586

Neuroendocrine effects of stress in hostile heavy social drinkers: exploring the involvement of serotonin using dietary tryptophan manipulation

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Previous studies suggest that individuals characterized by high trait hostility may be more sensitive to stress and that this may contribute to their greater vulnerability to alcohol abuse. To characterize the stress reactivity of hostile individuals and to evaluate the possibility that serotonergic dysfunction may underlie their susceptibility to stress, we examined the effects of acute dietary tryptophan enhancement and stress on mood and physiological reactivity in low (LoH) and high hostile (HiH) heavy social drinkers.
Thirty-four LoH and thirty-three HiH heavy social drinkers received either tryptophan-enriched or control diet and underwent a stress-induction procedure. Trait differences between the two hostile groups were explored using personality, anxiety and depression questionnaires. Mood and salivary cortisol levels (CORT) were measured before and after tryptophan diet and after stress-induction. Heart rate (HR) was measured during stress-induction.
HiHs compared to LoHs were characterized by greater trait anxiety and depression and reported more stress exposure over the past month. They also experienced more negative mood throughout the testing session. Stress increased CORT, HR and negative mood in most participants. Compared to LoHs, HiH individuals displayed a higher CORT increase and lower HR as well as a tendency to report more anger in response to stress. Tryptophan manipulation did not modulate any of the subjective and physiological effects of stress.
Among heavy drinkers HiHs show greater reactivity to stress as measured by CORT and negative mood but lower heart rate response. Dissociation between cardiovascular and hypothalamo–pituitary–adrenocrotical axis (HPA) response to stress suggests that HiH heavy drinkers may indeed be at a greater risk of HPA-related disorders such as depression and alcohol abuse but not at a greater risk of cardiovascular problems. The present data do not support the hypothesis that the greater sensitivity of HiH individuals to stress may be due to a serotonergic dysfunction.

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GH response to oral glucose tolerance test: a comparison between patients with pituitary disease and healthy subjects

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Background
GH response to oral glucose tolerance test (OGTT) is currently used for the definition of disease remission in acromegaly. This test has been poorly investigated in other pituitary diseases.
Aim
To evaluate the impact of a pituitary disease other than acromegaly on GH response to OGTT.
Patients and methods
Eighteen patients (13 F & 5 M, age: 50.7 ± 13.1 years) with different pituitary diseases (i.e. non-functioning pituitary adenomas, $n=14$, empty sella, $n=1$, meningiomas: $n=3$) were evaluated. Eight of these patients underwent neurosurgery and 2 underwent radiotherapy. None of them had history of diabetes mellitus, GH deficiency or acromegaly, renal/liver failure or nutritional disorders and none was treated with drugs interfering with GH secretion. 45 sex-, age- and BMI-matched healthy subjects were investigated as controls. All patients and subjects were studied for IGF-I and GH levels before and during OGTT.
Results
IGF-I levels, evaluated as standard deviation score, were similar between the 2 groups (-1.00 ± 0.58 vs -0.38 ± 1.14, $P=$NS). All patients and controls had post-glucose GH nadir levels <1 ng/ml. Mean GH nadir was slightly higher in patients with pituitary disease than in controls (0.13 ± 0.10 vs 0.08 ± 0.09 ng/ml, $P=0.08$), being higher in females than in males only in the control group (0.10 ± 0.09 vs 0.075 ± 0.08 ng/ml, $P<0.05$). However, the time course of GH response was different between the two groups, GH levels being significantly higher in patients than in controls at time 90 and 120 min ($P<0.01$).
Conclusion
GH response to OGTT appears to be dysregulated in patients with pituitary tumors, possibly as a consequence of hypothalamic alterations.

P588

Quality of life (QoL) in patients with Cushing's syndrome in a Spanish population: new experience with the CushingQoL questionnaire

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Quality of life is impaired in patients who have suffered Cushing's syndrome (CS).
Aim
Evaluate QoL with the new questionnaire CushingQoL in Spanish patients in a clinical practice setting and compare it with a generic QoL questionnaire, EuroQoL-5 Dimensions (5D) and its Visual Analogue Scale (VAS).
Patients and methods
Forty-three patients with CS (38 pituitary-dependent, 28 cured) were approached during their regular endocrine follow-up and asked to complete the EuroQoL and CushingQoL questionnaires.
Results
Mean EuroQoL-VAS in patients with cured CS ($69.7 + 19.4$) did not differ from that of active CS patients ($63.9 + 22.3$), while the CushingQoL score was signifficantly worse in active patients ($62.8 + 20.7$ vs $45 + 17.6$, $P<0.01$). A positive correlation was observed between both questionnaires, both for the whole group of patients ($r=0.635$), cured ($r=0.643$) and active CS patients ($r=0.648$). The only dimension of the EuroQoL-5D questionnaire which was significantly less affected in cured than in active patients was that which referred to Usual Activities ($P<0.035$).
Conclusions
With the disease-generated CushingQoL questionnaire, patients with active CS have a greater impairment of QoL than patients in whom hypercortisolemia has been controlled. No difference was seen when the generic EuroQoL-VAS was used. Therefore, the CushingQoL questionnaire is more sensitive than the generic tool used to identify dimensions important for QoL impairment in patients with CS in a clinical practice setting. Study supported in part by the ERCUSYN PHP 800200 project.

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Five year experience with GH replacement therapy on tumor occurrence or recurrence: one center study

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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 IGF-1 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 1995–2002 with the following diagnoses: 48 adenomas (34 clinically functionless pituitary tumors, 3 corticotropinomas, 9 prolactinomas, 2 somatotropinomas), 16 craniopharingiomas, 1 dysgerminoma, 1 arachnoid cyst, 1 tumor gigantocellular, 17 idiopathic GHD. Diagnosis of GHD was performed after 6–12 month 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.

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Androgen deficiency is additional risk factor for bone health in growth hormone deficient men

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Growth hormone deficiency (GHD) impairs bone mass and strength. Androgen deficient men exhibit impaired bone mass, but the extent to which testosterone replacement can prevent this remains disputable. The aim of this study was to assess whether androgen deficiency presents an additional risk factor for bone health in male patients with GHD and whether this is correctable by testosterone substitution.
A group of 81 male patients with GHD, not receiving GH replacement, aged 49.6 ± 1.7 years, with BMI of 28.23 ± 0.61 kg/m^2, was divided in 3 groups according to androgen deficiency status: (1) androgen sufficient men with isolated GHD ($n=16$) (2) hypogonadal men not receiving any androgen replacement ($n=15$) and (3) hypogonadal men on androgen replacement ($n=50$). Lumbar spine BMD and Lumbar spine *Z*-score (Hologic DXA) were analyzed as well as markers of bone turnover – serum osteocalcin (OC) and beta-cross-laps (BCL).
Lumbar spine BMD was greater in the androgen replaced than in unreplaced hypogonadal GHD men (1.083 ± 0.201 vs 0.950 ± 0.136 g/cm^2; $P>0.05$) but both groups had lower BMD than androgen sufficient (isolated GHD) group (1.120 ± 0.218 g/cm^2). Lumbar spine *Z*-score in unreplaced hypogonadal GHD men was significantly lower than in androgen sufficient men (isolated GHD) (-1.88 vs 0.4; $P<0.05$) but *Z*-score was also lower in the androgen replaced hypogonadal men than in androgen sufficient men (isolated GHD) (-0.32 vs 0.4; $P>0.05$). Both OC (17.47 ± 10.6 vs 18.21 ± 9.64 ng/ml; $P>0.05$) and BCL (275 ± 233 vs 282 ± 221 pg/ml; $P>0.05$) were lower in the androgen replaced than in unreplaced hypogonadal GHD men.
In conclusion, the observed trends in bone density values point to androgen deficiency as probably presenting an additional risk factor for bone health in male patients with growth hormone deficiency, which can only be partially corrected by testosterone replacement.

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The correlations of ghrelin concentrations and lipid profile in patients with acromegaly

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The role of ghrelin in the pathogenesis of acromegaly is doubtful. Ghrelin also regulates glucose and adipose tissue metabolism. It has not been demonstrated whether it contributes to the development of metabolic complications of acromegaly.
Aim
The aim of the study was to assess: (1) whether serum concentrations of total and acyl ghrelin in patients with acromegaly differ in relation to coexisting metabolic complications (hypercholesterolemia, hyperinsulinemia, hyperglicemia). (2) correlations between concentrations of ghrelin and concentrations of GH, IGF-1, cholesterol, insulin and glucose in patients with acromegaly.
Materials
Twenty-four patients with previously diagnosed acromegaly (16 women and 8 men, 27–71 years old) (11 subjects with active and 13 subjects with inactive disease) and 12 healthy subjects. Twenty-three subjects were treated in the past with neurosurgery, 3 subjects with radiotherapy. Seven patients were receiving octreotide LAR at the time of the study. Methods: In all studied subjects the concentrations of total ghrelin, acyl ghrelin, GH, IGF-1, insulin, glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglicerydes, were measured.
Results
(1) Mean concentrations of total and acyl ghrelin were significantly higher in patients with acromegaly and hypercholesterolemia compared with patients with normocholesterolemia ($P=0.01$ total ghrelin; $P=0.05$ acyl ghrelin). (2) In patients with hypercholesterolemia the ratio of acyl/total ghrelin was 16%. (3) In patients with active acromegaly there was a statistically significant positive correlation between the concentration of total ghrelin and the concentration of total cholesterol ($P=0.03$, $r=0.63$) and LDL cholesterol ($P=0.03$, $r=0.64$). There was also a positive correlation between the concentration of acyl ghrelin and LDL cholesterol (without statistical significance, $P=0.07$). (4) In patients with inactive acromegaly there was a statistically significant positive correlation between the concentration of acyl ghrelin and the concentration of triglycerides ($P=0.03$, $r=0.6$) and a positive correlation between total ghrelin and triglycerides, but statistically insignificant ($P=0.08$).
Conclusions
Ghrelin might be the factor contributing to development of hypercholesterolemia in patients with acromegaly. Presumably, some metabolic complications of the disease result not only from GH hypersecretion but also from altered ghrelin secretion.

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Glucose metabolism alterations in acromegaly

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Glucose metabolism alterations are frequently observed in acromegalic patients. Somatostatin analogues (SSTA) are the most widely used drugs to treat acromegaly, since they inhibit GH and IGF-1 levels, reduce pituitary mass, but can affect glucose metabolism. Aim of our study was to evaluate glucose metabolism alterations in acromegalic patients cured after surgery and in patients with active disease during treatment with SSTA. We studied 10 patients (group A, 5F, 55.66 ± 10.83 years, BMI$=27.53 \pm 5.11$ kg/m^2) who undergone transphenoidal surgery with disease remission (GH <1 µg/l after OGTT, normal sex and age matched IGF-1 levels) and 10 patients (group B, 6F, 44.88 ± 14.69 years, BMI$=27.16 \pm 4.43$ kg/m^2) with active disease, treated with SSTA. We measured at baseline and 12 months after medical o surgical therapy, GH and IGF-1 levels, fasting and post-load (OGTT) glucose and insulin levels, also evaluating the area under the curve (AUC). At baseline, 44.4% of patients had impaired glucose tolerance and 1′11.1% diabetes mellitus. No significant differences were observed in GH and IGF-1 levels between the two groups. At 12 months group A patients had normal GH and sex and age matched IGF-1 levels. Fasting and post-load glucose levels, fasting insulin levels and glucose AUC values were significantly reduced ($P<0.05$) compared to baseline. On the contrary, post OGTT insulin secretion and insulin AUC did not change. In group B, GH and IGF-1 levels, insulin peak during OGTT, and insulin AUC were significantly reduced ($P<0.05$) compared to baseline. Fasting and post-OGTT glucose levels did not change. Our results confirm that glucose metabolism alterations are frequently observed in active acromegaly. In acromegalic patients with disease remission, an improvement of fasting glucose and insulin levels is observed, likely due to reduction in GH and IGF-1 levels. Therapy with SSTA controls GH and IGF-1 excess, but impairs insulin secretion.

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ACTH-secreting bronchial carcinoid: a role of somatostatin analogues in diagnosis and treatment

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Objective
To report a case of Cushing's syndrome due to ectopic ACTH secretion and to evaluate role of somatostatin analogues for the localization and postoperative treatment of adrenocorticotropin-secreting bronchial carcinoid tumor.
Methods
We describe the clinical presentation of our case and discuss its management.
Results
A 57-year-old woman presented with symptoms and physical findings strongly suggestive of Cushing syndrome. Findings on biochemical evaluation were consistent with ectopic ACTH syndrome. Conventional radiographic imaging has revealed primary tumor in bronchopulmonary segments C9–C10 of left lung (about 12 mm) and several small focuses. Surgical exploration was undertaken – both a primary tumor and metastatic disease were identified, and the patient underwent resection of the lower portion of left lung. Histological examination of the resected specimen confirmed bronchial carcinoid staining positive for ACTH. Eventual resection of the lung nodule resulted in cure of hypercortisolism and normalization of 24-hour urinary free cortisol and ACTH levels in first 2 months after operation. Then the state of patient began to progressively deteriorate, and the patient underwent 6 courses of chemotherapy (etoposide, carboplatin, bleocin) without effect. The scintigraphy with radiolabelled octreotide has found multiple focuses of the accumulation of radiolabelled preparation in the left lung.

Subsequent therapeutic administration of long-acting octreotide (30 mg every 4 weeks) normalised urine free cortisol level, with symptomatic improvement. After 12 months of long-acting octreotide treatment urine free cortisol level has increased and ketoconazole was added. The duration of follow-up of this patient consists now 36 months.
Conclusion
This case illustrates the difficulties encountered in the assessment and management of a patient with ectopic ACTH syndrome. This is one of the few cases described where ectopic ACTH secretion secondary to bronchial carcinoid responded to somatostatin analogue therapy.

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Dose interval comparison of Lanreotide Autogel 120 mg in acromegalic patients previously treated with Octreotide LAR

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Acromegalic patients under treatment with Octreotide LAR (Oct), 10, 20 or 30 mg were switched to Lanreotide Autogel 120 mg (Lan) at different dose intervals: 56, 42 and 28 days respectively. Just before the fourth Lan injection, IGF-I values were measured and the dose interval for the final three injections adjusted accordingly: if IGF-I values were between 1 and 2 standard deviations (S.D.) above the age and sex related mean value, no change in dose-interval was made; if IGF-I was above 2 S.D., the dose interval was reduced (from 56 to 42 or 42 to 28 days); if the IGF-I value was below 1 S.D., the dose interval was increased (from 28 to 42 or 42 to 56 days).
The ITT population comprised 37 patients who were treated at least once; 33 were titrated and 18, 7 and 8 patients finished the study with 28, 42 and 56 day injection intervals respectively.
Median IGF-I, GH and quality of life (QUOL) values were similar at study start and end: IGF-I 89.6 and 87.9% of upper limit of normal range; GH 1.02 and 1.64 ng/ml; QUOL 66 and 66%.
Fifty-one percent of investigators preferred the Lan Injections and 26% Oct. Patients on the longer injection intervals seemed to prefer the Lan treatment more: 41, 54 and 71% of the patients in the 28, 42 and 56 day groups expressed a preference for treatment with Lan (Oct: 35, 9 and 29% respectively).
No treatment-related serious or unexpected adverse events occurred.
Conclusions
Lanreotide Autogel 120 mg injected at intervals of 28, 42 and 56 days provided equivalent control of IGF-I, GH and QUOL when compared to treatment with 30, 20 and 10 mg Octreotide LAR respectively. For patients requiring lower doses, a longer injection interval leads to a higher preference for the treatment.

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Growth hormone replacement therapy in adult onset growth hormone deficiency induces favorable long-term effects on quality of life, bone, body composition and lipids: a 55 month prospective study

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Objective
To investigate long-term effects of GH replacement therapy.
Material and methods
Thirty-nine patients (mean age 52.5 years, 14 women) with adult-onset growth hormone deficiency (AOGHD), recruited from a randomized placebo-controlled crossover study of treatment with growth hormone (GH) and placebo for 9months each, were enrolled in an open prospective follow up study. GH replacement was given for additional thirty-three months and was individually dosed to obtain an IGF-1 concentration within the upper part of the normal range for age and sex.
Results
During treatment, IGF-1 increased significantly and reached target levels, $P<0.001$. The final mean dose was 0.88 (S.D. = 0.60) mg/d for women and 0.56 (0.22) mg/d for men, a significant difference between genders, $P=0.03$. QoL was improved as assessed by HSCL-58 sum score – 7.4 (22.4), $P=0.03$ and AGDHA sum score −2.2 (6.0), $P=0.03$, increase in physical activity, $P=0.05$ and improvement in SF-36 dimension vitality, $P=0.006$. Bone mineral content and bone mineral density increased significantly, both in lumbar (L2–L4) spine, $P=0.001$ and $P=0.007$ respectively, and in total body, $P<0.001$ and $P=0.01$ respectively. Changes in body fat mass (BFM) and lean body mass (LBM) observed in the controlled part of the study was sustained with a reduction in BFM by −2.18 (4.87) kg, $P=0.01$ and an increase in LBM by 2.01 (3.25) kg, $P=0,007$. LDL-cholesterol was reduced – 0.6 (1.1) mmol/l, $P=0.002$, and HDL-cholesterol increased 0.2 (0.3) mmol/l, $P<0.001$. No changes were observed in total cholesterol, fasting triglycerides, HbA1c %, fasting plasma insulin and fasting plasma glucose.
Conclusions
Long-term replacement of low dose growth hormone in AOGHD induces favourable effects on QoL, bone and several metabolic parameters.

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GH deficiency in HIV-infected patients with lipodystrophy: preliminary data on the effects of r-hGH treatment on body composition

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Introduction
HIV-infected patients often display a moderate to severe GH deficiency (GHD). To investigate the effects of r-hGH on hormonal parameters and body composition in patients with HIV-related lipodystrophy (HIV-r-L) and concomitant GHD, we studied 60 patients with HIV-r-L, 28 male and 32 females aged 18–65 years. According to their response to GHRH + Arginine (GH peak assumed to be normal when >7.5 ng/ml) patients were assigned to the following 3 groups: Group 1, 35 subjects with a normal GH peak after GHRH + Arg (>7.5); Group 2, 10 subjects with GHD (peak <7.5) treated with a low dose of r-hGH (0.018 mg/kg/die), Group 3, 15 subjects with GHD (peak <7.5) not treated with r-hGH.
Methods
Hormones: GH, IGF-1, IGFBP-3; abdominal CT scan for VAT and SAT measurement; DEXA for the measurement of lean and fat body mass. Measurements were performed at baseline and after 24 months.
Results
Serum IGF-1 (mean ± S.D.: 130.2 ± 48.2 at baseline, 175.1 ± 55.4 ng/ml at 24 months; $P<0.01$) and IGFBP3 (mean ± S.D.: 2901.16 ± 1328.4 at baseline 4315.6 ± 1150.3 ng/ml at 24 months; $P<0.0001$) increased significantly only in Group 2, as expected. VAT decreased in Group 2 (from 195.3 ± 75.3 to 158.3 ± 46.9 cm^2), but the difference was not significant, while it increased significantly in Group 3 (from 158.3 ± 79.6 to 201.7 ± 88.9 cm^2; $P=0.02$). The same trend was evident for SAT that increased also in Group 1.
Conclusions
The replacement treatment with r-hGH of patients with HIV-r-L and concomitant GHD may be effective in controlling body composition and to prevent fat accumulation, an event that usually occur in patients with HIV-r-L. In particular, the reduction of VAT seems to be higher in patients with higher fat accumulation at baseline.

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Can the pineal gland modulate the effects of kisspeptin on the puberty onset in female rats?

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Chronic central administration of KiSS-1 peptide to immature female rats induces pubertal maturation, characterized by advanced vaginal opening, increased uterine and ovarian weights, elevated levels of luteinizing hormone (LH) and estrogen. There is evidence that the pineal gland may be also involved in puberty onset because melatonin secretion declines near puberty. We have investigated whether there is an interaction between kisspeptin and melatonin in timing of puberty onset. Wistar female rats ($n=24$) were weaned on day 21 and used. Twelve animals were pinealectomized (PNX), and the other half was exposed to sham operation (SHAM). Both SHAM and PNX rats received intraperitoneally

either 100 nmol KiSS-1 per day or vehicle only. The animals were individually caged with free access to food and water. Vaginal opening was daily monitored starting from day 25, and the animals were decapitated when the first diestrus observed. Upon decapitation, trunk blood was collected for LH and estrogen, and the hypothalami were obtained for kisspeptin expression. Uterus and ovaries were dissected and weighed out. All animals presented vaginal opening at the age of 38 days except one rat from SHAM plus KiSS-1 and one from PNX plus vehicle groups. Peripheral administration of KiSS-1 increased uterine weight in both SHAM and PNX groups. PNX rats receiving KiSS-1 had higher ($P<0.05$) ovarian weight (44.84 ± 3.6 mg) compared to PNX rats injected with vehicle (36.85 ± 07.06 mg). The present results show that peripheral administration of KiSS-1 does not induce vaginal opening at least in the dose of 100 nmol, which was used in our study, unlike the effect of central administration. However, KiSS-1 caused an increase in ovarian weight in PNX group, which is another sign of pubertal maturation. The present findings suggest that the pineal gland may modulate the effect of kisspeptin on reproductive functions.

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Central diabetes insipidus after transsphenoidal treatment for tumors of the sellar region: prognostic factors for transient course of the disease

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Central diabetes insipidus (CDI) is a common complication after transsphenoidal treatment for tumors of the sellar region and may exhibit different patterns: transient, permanent or triphasic.
The aim of our study was to determine the incidence and course of the CDI and to characterize the factors associated with resolution of this disease.
The incidence of CDI was based on records of 318 patients who underwent transsphenoidal surgery at our institution between 2004 and 2008 by a single surgeon. The main study included 48 patients (32 with transient and 16 with permanent) with postoperative CDI, operated in part in other neurosurgical institutions, and were available for follow-up.
CDI was diagnosed in 12.9% of our patients (41/319). There were no differences between groups of transient and permanent CDI in patient's age (36.2 vs 32.1 years), body mass index (27.4 vs 26.7 kg/m^2), duration of preoperational anamnesis of main disease (5.6 vs 6.2 years), tumor volume (1.7 vs 3.1 cm^3; $P=0.29$), cavernous sinus involvement ($P=0.44$) or pituitary stalk deviation ($P=0.48$). Median time to resolution of CDI was 9 weeks ranging from 2 days to 1.5 years. We were not able to show that narcosis duration, amount of haemorrhage, liquorrhea, traumatization of neurohypophysis, intraoperational use of H_2O_2 or formic acid as well as number of mitoses, stromal edema or degenerative changes at histological appearance of removed tumor could influence the resolution of postoperative CDI (for all $P>0.05$). Transient CDI was associated with previous therapeutic treatment of the tumor ($P=0.04$), hormone hypersecretion ($P=0.007$) and tumor size less than 1 cm in diameter ($P=0.02$).
Thus, herein we report an incidence of CDI within our postoperative population and describe factors that favor transient course of the disease.

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Food deprivation affects arcuate neurons in the hypothalamus of young rats

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The arcuate nucleus of the hypothalamus (ARH) is localized within lateral walls of the third ventricle above the median eminence. From the functional point of view ARH takes part in the regulation of food intake, energy expenditure and body weight. The aim of this study was to investigate the influence of food deprivation on ultrastructural alterations of the rough endoplasmic reticulum (RER)/Golgi network in the arcuate neurons of young rats. There have been no reports describing the effect of food deprivation on the formation of membranous whorls, the function of which is still unclear. Otherwise, under different stress conditions like morphine, colchicine or mercury treatment, the arcuate neurons exhibited those structures. Inbred male rats aged 5 months were divided into three groups (4 in each group): control (normally fed), and deprived of food for 48 hrs and 96 h. Simultaneously, total 8-isoprostane serum level was assayed as a marker of oxidative stress inducing lipid peroxidation *in vivo*. In both groups of food deprived animals we observed rearrangements of the RER in the form of lamellar bodies and membranous whorls. The lamellar bodies in controls were rather short and dispersed in the neuronal cytoplasm. Whereas, in food deprived animals they became longer and participated in the formation of membranous whorls composed of concentric layers of endoplasmic reticulum. The membranous whorls were often placed in the vicinity of very well developed Golgi complexes. Some Golgi complexes displayed an early stage of whorls formation. This observation correlates with a significant increase in serum 8-isoprostane levels in food deprived animals as compared to the fed control.

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Plasma levels of neuropeptide Y and peptide YY in patients affected by Anorexia nervosa and severe obesity

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Anorexia nervosa (AN) and obesity are prevalent in modern societies.
This study evaluate circulating levels of Neuropeptide Y (NPY) and of Peptide Tyrosine-Tyrosine (PYY) in 60 women: 20 affected by AN (Body Mass Index = BMI 15.74 ± 2.09 kg/m^2, age 30.19 ± 10.52 years), 10 *restricter* (BMI 14.89 ± 1.64 kg/m^2) and 10 *binge-purge* subtype (BMI 18.27 ± 0.81 kg/m^2); 20 affected by severe Obesity (BMI >40 kg/m^2, age 33.56 ± 5.2 years) with type II diabetes with no therapy, and 20 healthy controls (BMI 22.06 ± 0.93 kg/m^2, age 32.44 ± 4.35 years).
NPY is higher in AN than obese and controls (70.17 ± 20.84 vs 25.12 ± 7.26 and 52.20 ± 10.88 pmol/l; $P<0.001$) and lower in obese than controls ($P<0.001$). PYY is higher in AN than obese and controls (219.77 ± 83.51 vs 116.42 ± 41.42 and 94.97 ± 12.74 pg/ml; $P<0.001$), with no differences between obese and controls.
In AN, NPY and PYY are quite higher ($P=0.059$; $P=0.06$) in *restricter* (75.77 ± 20.86 pmol/l; 241.78 ± 84.6 pg/ml) than in *binge-purge* subtype (53.39 ± 8.72 pmol/l; 153.73 ± 29.45 pg/ml). Increase of NPY despite simultaneous PYY increase in AN might be related to reduced sensitivity to PYY inhibitory effect on NPY production or increased production of NPY from simpathetic peripheral nervous system, a finding evident mainly in *restricter* AN. In obese PYY is close to controls suggesting a reduced intestinal production of this peptide because of the stimulus of continous overfeeding, whereas the reduced NPY production could be explained by increased levels of insulin and leptin. Reduced NPY levels suggest that in these obese the overfeeding is not dependent on increased hungry signal, but on inadequate satiety signal.

P601

From the horse's mouth: recommendations to improve care for pituitary patients. Results from a survey on pituitary patients' satisfaction with information and support from healthcare professionals

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Introduction
Pituitary conditions are rare and diagnosis may be slow because symptoms are ambiguous. The treatment may be a combination of surgery, radiotherapy and medication so patients see many healthcare professionals (HCPs). This survey sought to assess patient satisfaction with the information and support they receive from GPs, endocrinologists, neurosurgeons, radiotherapists, specialist nurses, and other agencies (e.g. the pituitary foundation).
Method
A questionnaire based upon the 2006 cancer backup survey was sent to 1000 members of the pituitary foundation. Of 488 questionnaires were returned with 429 containing enough responses to be included in the subsequent analyses. Of these 40% were from male and 60% from female respondents aged between 10 and 85 years (average 56 years).
Results
Overall the picture was reasonably good, but the areas of concern related to the process of diagnosis, and issues related to ongoing medication. There were also some concerns in relation to provision of information to individuals with a pituitary condition and the extent to which they are involved in regular treatment reviews; and the fact that a significant number (53%) did not know where to get information on possible treatments for their condition. Most individuals surveyed

(96%) liked to take and maintain control of managing their condition and enthusiastically sought information to do so, preferably in a face-to-face interaction.
Conclusions
Individuals with a pituitary condition need support to learn the skills to help them manage their condition to the best of their ability, enabling them to enjoy an improved quality of life. To this end, mechanisms should be in place to enable individuals to have regular treatment reviews in partnership with their HCPs. HCPs should be encouraged and enabled to offer more information to patients in a 1:1 setting.

P602

Pituitary insufficiency with a HESX1 mutation: a new case

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A 14 year-old Turkish boy sought advice for growth retardation. Pituitary insufficiency with GH, TSH, ACTH and gonadotrophin defect was diagnosed and treated. He was born from a consanguineous family and was married at 24. Three years later he consulted wishing to father a child. He was treated with levothyroxine 150 µg, hGH 0,5 mg/day, hydrocortisone 20 mg/day and was switched from testosterone enantate to hCG + FSH. Azoospermia was initially found and oligospermia after treatment. Pituitary MRI noticed a very hypoplastic anterior pituitary gland without abnormality either of the pituitary stalk or of the neuro-hypohysis. No septo-optic dysplasia or cerebral midline defects were visualized.
Nevertheless, taking into account multiple pituitary secretion defects and consanguinity, a mutation was looked for on HESX1. A non-sens mutation was discovered with a stop codon in exon 2 (R 109 X) leading to a truncated protein. Familial investigation found the same mutation in his father and mother with a normal phenotype and the propositus is therefore homozygote for the mutation. One of his two sisters is heterozygote.
Mutations of HESX1 are rare and more often associated with brain abnormalities. HESX1 is a member of the paired-like class of homeobox genes which functions as a transcriptional repressor and is one of the earliest markers of pituitary development. The repression of HESX1 allows the expression of PROP1. Transmission is recessive or dominant and phenotype highly variable with sometimes minor abnormalities as observed in our case, ranging from isolated GH deficiency to panhypopituitarism with diabetes insispidus.

P603

Prevalence of colonic polyps in acromegalic patients and relationship between glycemic status

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Objective
Acromegaly is associated with an increased prevalence of colonic polyps. The aim of this study was to evaluate the prevalence of colonic polyps in acromegalic subjects, and also whether there is a relationship between insulin-like growth factor 1 (IGF-1), growth hormon (GH), fasting insulin plasma levels and the presence of polyps.
Material and methods
Fifty-four consecutive acromegalic patients and 45 IBS patients were enrolled to study between 2004 and 2008. Groups including acromegalic patients and controls were age and sex matched. All patients underwent colonoscopy and received a histological diagnosis of colorectal lesions. Serum GH, IGF-1, insulin levels were compared between acromegalic patients with and without colorectal lesion.
Results
Acromegalic patient's mean age was 44 ± 10.9 years (20 males and 34 females, mean duration of disease 53.5 ± 47.6 months) and IBS patient's mean age was 47 ± 11.1 years (15 males and 30 females). 14 of 54 cases (25.9%) had colonic polyps. Eight (57.1%) had hyperplastic polyps, 5 (35.7%) had adenomatous polyps, and 1 (7.1%) had leomyoma. In the IBS group, 4 (8.8%) had colonic polyps; all polyps were hyperplastic. The prevalence of hyperplastic and adenomatous polyps were significantly higher in acromegalic patients ($P = 0.02$). The group of acromegalic patients with and without polyps did not differ significantly in duration of disease, body mass index, plasma GH, IGF-1, fasting insulin levels and glycemic status (Impaired glucose tolerance, impaired fasting glucose and diabetes mellitus). The presence of colonic polyps was correlated with patients age ($P = 0.009$) and male gender ($P = 0.01$).
Conclusion
Acromegalic patients have a higher prevalence of colonic polyps than IBS subjects. There was no correlation between IGF-1, GH, fasting insulin plasma levels, glycemic status, HOMA-IR and colonic polyps in acromegalic patients.

P604

Results of acromegaly patients treated with surgery and/or somatostatin analog and/or radiotherapy in a University Hospital in Turkey

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Background
Transsphenoidal adenomectomy is the first choice of therapy, but about 50% of patients are not cured and require medical treatment and/or radiotherapy. We retrospectively analyzed the data on surgery, sandostatin analog therapy, radiotherapy and the combination of them.
Materials-methods
The records of the 96 female and 70 male, totally 166 acromegalic patients who had been followed in Ankara University, Endocrinology and Metabolic Diseases Department from 1985 till now were documented. Collected data include estimated data of initial symptoms, date of diagnosis, results of pituitary imaging, the treatment modalities and the remission rates. Remission criteria was GH < 1 with oral glucose tolerance test (OGTT) and IGF-1 normal with respect to age and gender.
Results
Macroadenomas were detected in 74% and microadenomas in 26% of the patients. In patients with macroadenoma median growth hormone (GH) was 19.4 ng/ml (1.6–236.8) and median IGF-1 was 1000 ng/ml (323–4500) while in patients with microadenoma median GH was 9.2 ng/ml (2.1–56) and median IGF-1 was 668.5 ng/ml (150–1801). The remission rate was 46% in microadenomas and 31% in macroadenomas giving a total rate of 35% after the first operation. When GH cut off was reduced to < 0.4 the remission rate decreased to 23% in microadenomas, 17% in makroadenomas and 18% totally. Somatostatin analog (SSA) treatment was given to 46 patients after the surgery and remission was detected in 46% of the patients. Radiotherapy (RT) was applied to 31 patients (19 conventional RT, 12 gama-knife). Of 24 of them had also SSA treatment. When RT was applied alone after surgery remission rate was 11%, but if RT and SSA combination was given following surgery the remission rate increased to 50%.
Conclusion
Pituitary surgery is an effective treatment for lowering GH and IGF-1, but adjuvant therapy as SSA and/or RT are required to reach higher remission rates.

P605

Hypothalamic–pituitary–adrenal axis in chronic fatigue syndrome

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Background/objectives
The aim of the study was to assess hypothalamic–pituitary–adrenal (HPA) axis in patients with chronic fatigue syndrome (CFS).
Design and methods
Twenty-three consecutive patients who fulfilled centers for disease control (CDC) criteria for CFS and twenty healthy controls were included in the study. Low dose ACTH test (1mcg of synthetic adrenocorticotropic hormone Synacthen) and insulin tolerance test (ITT) were done and blood cortisol was measured. Data are presented as median with 95 percentile range (2.5–97.5 percentile). Maximum cortisol level was 640.1 mmol/l (338.9–920.1 mmol/l) in CFS patients and 701.2 mmol/l (469.8–1233 mmol/l) in healthy control group.

Results
Dose of ACTH did not differ between patients and controls given in ng/kg of body weight or ng/m^2 of body area. Basal cortisol concentrations did not differ between CFS patients and controls. Maximum cortisol response in ACTH test was significantly lower in CFS versus control group. Maximum cortisol response and area under curve in ITT was significantly lower in CFS patients versus control group.
Conclusions
These results indicate disturbance in cortex of adrenal gland in patients with chronic fatigue syndrome. From our data we cannot conclude whether there are some other disturbances in HPA axis.

P606

Normalization of ACTH and cortisol responses to ghrelin in patients cured from Cushing's disease

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Previous studies have shown exaggerated ACTH and cortisol responses to ghrelin. Seven patients who were cured by transsphenoidal operation from CD were included in the study and tested with ghrelin (1 mcg/kg i.v.) before and six months after the operation. Their results were compared with eight healthy control subjects. Results are presented as mean$\pm$S.D. Mann–Whitney and Wilcoxon signed rank test were used for statistical analysis.
Before the operation, baseline ACTH (86.7$\pm$58.9 vs 24.2$\pm$16.2 ng/ml, $P<0.05$) and cortisol concentrations (671.1$\pm$224.3 vs 338.6$\pm$135.2 nmol/l, $P<0.01$) were significantly higher in patients with CD compared to control subjects. Peak ACTH (185.44$\pm$99.3 vs 59.5$\pm$41.2 ng/ml, $P<0.01$) and cortisol responses (1021$\pm$201 vs 610.8$\pm$170.6, $P<$ 0.01) were also significantly higher in patients with CD, before operation, compared to controls.
After operation, baseline ACTH (from 86.7$\pm$58.9 to 21.7$\pm$19.9 ng/ml, $P<0.05$) and cortisol concentrations (from 671.2$\pm$224.3 to 393.7$\pm$99.5 nmol/l, $P<0.05$) significantly decreased as well as peak ACTH (from 185.4$\pm$99.3 to 40.5$\pm$34.7 ng/ml, $P<$ 0.05) and cortisol responses (from 1024$\pm$201 to 603.8$\pm$147.3 nmol/l, $P<0.05$) to ghrelin. After the operation, baseline and peak ACTH and cortisol values in patients cured from CD were not significantly different from those observed in control subjects ($P>0.05$).
Normalization of ACTH and cortisol responses to ghrelin was observed, six months after the transsphenoidal adenectomy, in our patients treated for CD.

P607

The role of androgen receptors in the medial amygdala on biosynthesis and stress-induced cellular activation of the paraventricular nucleus of the hypothalamus

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Although it is becoming increasingly clear that testosterone exerts an inhibitory influence on stress-induced adrenocorticotropin (ACTH) and corticosterone release, where and how this occurs in the brain remains poorly understood. We previously determined that androgen receptors are not distributed within anterior pituitary communicating neurons of the paraventricular nucleus (PVN) of the hypothalamus (Bingham *et al.* J Comp Neurol 2006). However, they are contained within several upstream brain regions involved in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis and (ACTH) secretion. The medial amygdala (MeA) concentrates androgen receptors and regulates the HPA response to acute restraint stress (Dayas *et al.* Eur J Neurosci, 2002). Furthermore, animals that are gonadectomized as neonates have higher levels of plasma corticosterone and Fos in the PVN under basal conditions and in response to restraint stress, despite equivalent adult testosterone replacement (Bingham & Viau, Endocrinology, 2008). Interestingly, these same animals showed a decrease in the number of androgen receptors in the MeA. Based on these findings, we are examining the role of androgen receptors contained within the MeA on the HPA response to a single episode of restraint. Adult male rats received bilateral implants of the androgen receptor antagonist hydroxyflutamide, or testosterone suspended in beeswax, aimed towards the MeA. Two weeks after surgery, animals were subjected to restraint stress for 30 min and anesthetized for perfusion 2 h following the onset of restraint. We are currently investigating the effects of these implants on PVN biosynthesis and cellular activation and hormonal output.

P608

GH resistance in a group of chronic fatigue syndrome patients

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Objective
The rule of GH and IGF1 in the pathogenic of CFS is unclear. One study (related articles number 2) found no difference in IFG1 levels between controls and patients. However in fibromyalgia there are reports of subgroups of patients with a low IGF1 and normal to high GH. This phenomenon is also known with anorexia nervosa patients due to the malnutrition.
Method
We selected a group of ambulatory CFS patients fulfilling the Fukuda criteria with a low IGF1. An ITT was performed with those patients to explore the GH secretion in response hypoglycaemia (glycaemia: $<$40 mg/dl).
Results
We selected 13 patients with a mean age of 44 years (11 females and 2 males) without malnutrition. These patients have a BMI higher than 18 and lower than 30. The mean IFG1 of the patient group is (99.5$\pm$24.5 ng/ml). The normal value of IGF1 in a control group is (200$\pm$48 ng/ml). The peak GH after ITT in the group is (56.4$\pm$36.6 mIU/l).
Discussion
In this group of 13 CFS patients without known endocrine abnormalities we find a significant low IFG1 of 99.5 ng/ml in comparison to a control group (normal value: 200 ng/ml). These patients show a normal to high GH response after hypoglycaemia. Low IGF1 and high GH response is characteristic for growth hormone resistance. This phenomenon is known with chronic inflammation and anorexia nervosa. There are no reports of GH resistance in CFS. Further studies are needed to discover the pathogenisis and mechanisms in CFS.

P609

Presenting pattern and etiologies of hyperprolactinemia in north west of Iran

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Background
Hyperprolactinemia is the most common endocrine disorder of the hypothalamic–pituitary axis. We have not any documented data about frequency of various etiologies of hyperprolactinemia in our region. For determination of the pattern of presentation and distribution of causes of hyperprolactinemia the presenting study were designed and conducted.
Methods and materials
In a descriptive study, we extract medical records of patient presented to our clinic with diagnosis of hyperprolactinemia between 1 day of 2001 until last day of 2006. At this time interval we found 127 patients with diagnosis of hyperprolactinemia, of which 14 excluded due to absence of sufficient data for determination of etiology. The remaining 113 subjects' data were collected and analyzed.
Results
In our study 27 (23%) of patients were male and 86 (76%) were female. Grater than 63% of subjects had 20–40 years old. The presenting sings and symptoms of patients were in order of frequency: Menstrual abnormalities, galactorrhea, infertility, headache, visual disturbances, and sexual dysfunction. Pituitary adenoma was the most frequent etiology of hyperprolactinemia in our subjects. Other causes of hyperprolactinemia were: idiopathic hyperprolactinemia, hypothyroidism, medications, and polycystic ovary syndrome.
Conclusion
We found that in our series the most frequent presenting problem of patients with hyperprolactinemia was menstrual disturbances in females and sexual dysfunction in males. The most frequent cause of hyperprolactinemia was pituitary prolactin secreting adenomas.

P610

An exceptional image: multiple sclerosis and hypogonadism

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We present a 27 year-old female patient suffering from multiple sclerosis (MS) since 3 years ago. She currently consults for menstrual disturbances. She has not any neurological secuelae of MS now and she is not on medical treatment. She is with amenorrhea since 10 months ago, but she had been oligomenorrhea just before for 24 months. She doesn't have any symptoms suspicious for other hormonal impairment. Physical examination is normal.
The laboratory test reveals a hypogonadotropic hypogonadism with a normal thyrotropin, prolactin, cortisol, corticotrophin hormone, androgens and 17 OH progesterone levels. The Magnetic Resonance of the brain shows multiple signal alterations of white matter, hyperintense in T2 and without enhancing.
She has a demyelinization plaque in the right side of the hypothalamus.
There are some clinical cases in the literature about MS and hypogonadism. One of them describes also growth hormone deficit. Another one shows subclinical pituitary deficits in these patients. But the authors doesn't find any etiologic lesion on imaging tests.
In our case we can show the hypothalamic lesion causing hypogonadotropic hypogonadism. This should make us remember about the possibility of pituitary deficits in patients with multiple sclerosis.

P611

Health related quality of life in patients with diabetes mellitus type 2 in comparison to patients with other endocrine diseases

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Pathophysiological mechanisms of endocrine diseases (and diabetes mellitus type 2 (DM2) among them) impacts person's health perception and health related behavior as measured by the health related quality of life.
Aim
To compare peculiarities of health related quality of life in patients with DM2 and other endocrine diseases under hospital treatment.
Methods
Of 60 patients with DM2 and 71 patients with other endocrine diseases from the Department of Endocrinology of University Hospital were investigated by using WHOQoL-100 questionnaire.
Results
Physical domain of quality of life in patients with DM2 (11.4 ± 2.4 vs 12.5 ± 2.8, $P = 0.022$), individuality domain (11.5 ± 2.5 vs 13.2 ± 2.6, $P < 0.001$) and spirituality domain (11.3 ± 3.3 vs 12.7 ± 3.3, $P = 0.022$) were significantly lower than in patients with other endocrine diseases, showing worse quality of life. Global score of health related quality of life showed tendency to be lower in DM2 patients than in patients with other endocrine diseases (11.6 ± 2.7 vs 12.3 ± 2.6, $P = 0.097$).
In patients with DM2 significant correlations between BMI and individuality domain ($r = -0.307$, $P = 0.020$) and between duration of illness and individuality domain ($r = -0.264$, $P = 0.047$) were detected.
In conclusion
The impact of diabetes mellitus type 2 on psychological well – being as measured by health related quality of life questionnaire is more expressed than this of other endocrine diseases. The pattern of impairment may be helpful in planning psychological rehabilitation in DM2.

P612

Pregnancy occurs rarely in acromegalic patients: although octreotide therapy in pregnancy seems to be feasible and safe, enough information regarding the use of OCT in pregnancy is not available yet

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The patient who was operated because of pituitary macroadenoma causing acromegaly disease had insufficient suppression respond of growth hormone to postoperative oral glucose tolerance test (OGTT). OCT LAR treatment had been started. On the 15.month of the therapy, the patient presented with failure of menstruation since four months, so pregnancy test was performed and pregnancy was diagnosed. The patient had used OCT LAR during the period without knowing that she was pregnant. After it has been shown with magnetic rezonans imaging (MRI) that the pituitary adenoma did not grow, we stopped the OCT LAR treatment. The patient delivered a healthy newborn girl at the 37. gestational week newborn of 2650 g in weight and 50 cm in length. MRI was used for postpartum macroadenoma assessment and it had not grow during this period. Two months after the delivery, because of insufficient suppression respond of growth hormone to OGTT, OCT LAR treatment was restarted.
We believe that the size of the adenoma must be checked to make a decision in discontinuing or continuing treatment when pregnancy developed in acromegalic patients is the most suitable approach. Furthermore, we advocate that OCT should be discontinued during pregnancy until more safety data are obtained.

P613

Observational outcome of Korean acromegalics based on OASIS registry

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We enrolled 30 acromegalics from 2005.4 to 2007.7 at endocrine outpatient clinic of four academic hospitals. Male to female ratio was equal (15/15). Unsuppressed GH confirmed 28 out of 30 patients with oral glucose tolerance test. Average serum GH level was 28.7 ng/ml, and average IGF-I, 985 ng/ml. Micro- to macroadenoma was 8/22 (26%). Before treatment, 28 out of 30 acromegalics were taken 100 μg of somatostatin suppression test, complete responder (GH was suppressed below 1 ng/ml) was 39% (11/28), lower IGF-I (879 ± 322 vs 1035 ± 403 ng/ml) and most of them (6/7, 86%) were microadenoma. Biochemical cure rate after surgery was assessed with suppressed GH after oGTT below 1 n/ml, macroadenoma revealed 67% (8/12) and 100% (5/5) in microadenoma. Paradoxical response of GH to TRH (18/30) was observed in 78% (14/18) of acromegalics. Overall cure rate with trans-sphenoidal adenoidectomy (TSA) was 80% (16/19). All 3 microadenomas were removed completely.
On focusing to 12 acromegalics, preoperative use of octreotide-LAR (median duration, 12 weeks) showed complete removal of tumors regardless of tumor size (macro:micro = 9:3). Tumor volume, hormone profiles before and after use of octreotide-LAR showed in Table 1. The preoperative use of Octreotide-LAR could achieve complete removal of pituitary tumors and biochemical cure of acromegaly. But, in a surgical point of view, sergeons encountered many problems during operation. Because newly developed severe fibrosis between normal and shrunken tumor tissue due to preoperative use of octreotide-LAR regardless of its duration should make operation too hard to more shrink arachnoid space, more chance to CSF leak. These findings suggested that how surgeon's skill was important. In conclusion, preoperative use of octreotide-LAR might be predicted improvement of surgical outcome via reduction of tumor volume, but severe fibrosis should be a critical obstacle to easy removal of tumors and more chance to get operative complication as CSF leakage.

P614

Plasma thrombin-activatable fibrinolysis inhibitor antigen levels in acromegalic patients

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Objective
Acromegaly is associated with increased morbidity and mortality, mostly due to cardiovascular complications. Thrombin-activatable fibrinolysis inhibitor (TAFI) is associated with coagulation/fibrinolysis and inflammation. Plasma TAFI may participate in arterial thrombosis in cardiovascular diseases (CVD). In this study, we aimed to evaluate the levels of TAFI antigen and also its relationship with other markers in a group of patients with acromegaly in compoarison with healthy controls.

Research design and methods
We studied 29 acromegaly patients and 26 healthy controls. We measured TAFIa/ai antigen in plasma samples with a commercially available ELISA kit.
Results
Routine biochemical parameters, fasting and postprandial glucose levels, BUN, creatinine, CBC, prolactin, TSH, total-cholesterol, LDL cholesterol, triglyceride, homocysteine, and hs-CRP were similar in the two groups ($P>0.05$), whereas, plasma TAFI antigen levels were significantly elevated in acromegalic patients ($154.7\pm94.0\%$) when compared with control subjects ($107.2\pm61.6\%$) ($P<0.03$). A positive corralation was found by Pearson corralation test between plasma TAFI antigen levels and hs-CRP. ($r=0.364$, $P<0.01$).
Conclusions
In our study, significant alteration in plasma TAFI antigen levels were detected in patients with acromegaly during treatment. Increased TAFI antigen levels in acromegaly should be considered as an important finding in explaining CVD in this disease.

P615

Prolactinoma in east-black sea Turkey: a description of 51 cases

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PRL hypersecretion is the most common endocrine abnormality due to hypothalamic–pituitary disorders. PRL is the hormone most commonly secreted in excess by pituitary adenomas. Patients usually present with oligo-amenorrhea, galactorrhea, loss of libido, impotence and infertility. If the tumor extends outside of the sella, visual deficit or other mass effects may be seen.
A retrospective study was performed during the period 1999–2008. The diagnosis of prolactinoma was based on objective examination, hormonal levels and MRI. During these time 51 cases were diagnosed as prolactinoma; 68.62% were females and 31.38% were males. Female/male ratio was 2.18:1. The mean age of diagnosis was 36.31 ± 13.58 (range 18–77 years) years old. Oligomenorrhea or amenorrhea were the major symptoms in 21 patients (41.1%) and followed by headache in 11 patients (21.5%), galactorrhea in 9 patients (17.6%), decreased libido in 6 patients (11.7%) and visual deficit in 1 patient. Of 3 patients (5.88%) was asymptomatic. Hypopituitarism was found 18 patients (35.3%). The diagnosis of prolactinoma was confirmed by MRI. The 56.8% of cases was macroadenoma and 41.2% of cases was microadenoma.
The mean value of PRL at the moment of diagnosis was 720.64 ± 1086.2 (range 61.5–3919) ng/ml. PRL normal range is 3.4–24.1 ng/ml.

P616

Acromegaly in the eastern black sea region of Turkey: a description of 42 cases

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Background
Acromegaly is a relatively uncommon disorder (40–68 cases per million population) caused by oversecretion of growth hormone by a tumor of the pituitary gland. Excessive growth hormone (GH) and insulin-like growth factor (IGF) I concentrations cause gradual changes in facial and acral appearances as well as in many internal tissues.
Medhods
Medical records of patients with acromegaly seen between 2000 and 2008 at our hospital were reviewed and epidemiological data regarding the demographic characteristics has been analyzed.
Results
Information was available on 42 patients, of whom 23 (55%) were women and 19 (45%) were men. The mean age at diagnosis was 43 ± 13 years. Body mass indexes were between 22.5 and 43 kg/m^2, mean 29.3 ± 5.13 kg/m^2. GH levels were between 3 and 124 ng/ml, mean 32.6 ± 28.3 ng/ml. IGF-I levels were between 310 and 2620 ng/ml, mean 927 ± 616.14 ng/ml. In nine patients (21%) there were microadenomas while 33 patients (79%) had macroadenomas at the diagnosis. Diameters of adenomas were between 6 and 70 mm, mean value 22.6 ± 13 mm. Most prominent symptoms were acral growth (83%), headache (26.2%) and visual defects (23.8%). Diabetes mellitus have found in 13 patients (31%) while two patients (5%) have had impaired glucose tolerance test. Patients who had neurosurgical procedures for adenomas were 82%. Postoperative hipopitiutarism have arised in 12% of patients.
Conclusion
Acromegaly is a rare disorder that progress slowly for years before become clinically apparent. Suspection from symptoms is important for diagnosis of acromegaly in early period.

P617

Long-term treatment of a misdiagnosed TSH-oma patient with antidepressants and antithyroid drugs

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Background
Among the disorders causing hyperthyroidism thyrotropin-secreting pituitary adenomas (TSH-omas) are extremely rare and account for $<2\%$ of all pituitary adenomas. Failure to recognize a TSH-oma may lead to improper therapy attempts and dramatic consequences. We have reported a patient that wrongly had diagnosed and treated as primary hyperthyroidism for fifteen years.
Case
A 50 years old woman who was on the treatment of propilhyourasil (150 mg/day) referred our endocrinology clinic from physichiatry clinic due to high serum thyroid-stimulating hormone (TSH), free thyroxine (fT_4) and free triiodothyronine (fT_3) levels. She was complaining of sweating and shortness of breathing. Physical examination revealed slightly enlarged thyroid gland but were otherwise unremarkable. Laboratory investigations revealed TSH level of 5.15 (n:0.27–4.2) mU/l, fT_4 level of 2.78 (n:0.9–1.7) ng/dl and free T_3 level of 6.2 (n:1.8–4.6) pg/ml. The serum alfa-subunit level was 0.84 ($n<0.90$) IU/l and alfa-subunit: TSH molar ratio was 1.63 ($n<1$). There was impaired TSH response to TRH stimulation and no supression of TSH with T_3 supression test. An MRI scan revealed 20 mm adenoma in the right side of the pituitary gland. These biochemical and radiological investigations were consistent with the diagnosis of TSH-oma. We have planed to attempt neurosurgical removal after a course of medical treatment with octreotide LAR 20 mg/month for a period of 3–6 months. After 3 months on the octreotide treatment all of TSH, fT_4 and fT_3 levels have decreased (3.09 mU/l, 2.20 ng/dl, 4.78 pg/ml respectively). On MRI scan adenom has regressed to 17 mm in diameter.
Conclusion
In a patient with high levels of tyroid hormones if TSH level is high or normal TSH-oma must be suspected. Misdiagnosed TSH-oma patients undergo wrong treatments and due to persistant hyperthyroidism physichosomatic symptoms may get them to physiciatry clinics. Neurosurgical treatment may be first choice after restoration of thyroid state with octreotide treatment.

Reproduction

P618

The effect of nutritional supplement (Menopace®) on the frequency of hot flushes, night sweats, mood and quality of life in post-menopausal women: a placebo-controlled double blind study

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Objective
Hot flushes and night sweats experienced by 60–70% of postmenopausal women are considered as classical signs of menopause. Estrogens is the gold standard treatment, but in view of its potential risks, various herbal preparations and vitamin supplements have a great appeal to women. Aim of this study was to assess the effect of a nutritional supplement (Menopace®) on the frequency and severity of hot flushes and the quality of life in postmenopausal women in a randomized, placebo-controlled, double blind study over three months.
Subjects and methods
Ninety-one post-menopausal women aged 53.73 (±0.74) years (range 41–71 years) were randomized to receive either placebo ($n=45$) or Menopace® ($n=46$). Sixty-eight women completed diaries (35 from Menopace® group and 33 from placebo) and came for a second assessment after 14 weeks. During the study, women also completed self-report questionnaires on the frequency and severity of hot flushes and night sweats, the Profile of Mood State (POMS) questionnaire, the World Health Organisation Quality of Life Questionnaire (WHOQOL-BREF-UK version), the National Adult Reading Test and the Rey Auditory-Verbal Learning Test.

Results
There was a significant decrease ($P<0.01$) in the number of hot flushes experienced per week in both the Menopace® (pre 31.3±4.7; post 23.1±4.8) and the placebo group (pre 28.1±4.7; post 17.3±4.0), and also a significant decrease ($P<0.001$) in the number of night sweats experienced per week in both the Menopace® (pre 6.1±1.0, post 4.2±0.7) and the placebo group (pre 5.9±0.7, post 3.7±0.7).
Discussion
Our study showed a significant placebo effect on hot flushes and night sweats which is consistent with other studies. There was a significant decrease in the number of hot flushes and night sweats in both the Menopace® and the placebo group. The level of education appeared as the main determining factor of the way that women cope with hot flushes.

P619

Clinical characteristics of Algerian PCOS women

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Polycystic ovary syndrome (PCOS) is the most frequent endocrinopathy in premenopausal women, and has many clinical features in common with the metabolic syndrome.
Objective
The aim of our study was to evaluate the clinical characteristics of our PCOS women.
Methods
Of 181 PCOS defined by the Rotterdam criteria and 90 controls were evaluated for anthropometric parameters by physical examination.
Results
The PCOS and controls clinical parameters were compared. The mean age of PCOS was 27.52±7.23 vs 28.02±8.45 years $P=0.18$; the BMI was 28.67±7.72 vs 27.3±6.37 kg/m^2 $P=0.09$; the waist circumference was 99.51±18.14 vs 92.23±12.01 cm $P=0.001$; the waist to hip ratio was 0.87±0.1 vs 0.82±0.1 $P=0.001$; the systolic blood pressure was 136.14±12.89 vs 112.2±11.4 mmHg $P=0.001$ and the diastolic blood pressure was 81.25±9.59 vs 74.3±7.1 mmHg $P=0.01$.
Conclusion
PCOS women have greater waist circumference, W/H ratio, systolic and diastolic blood pressure.

P620

Influence of hyperprolactinemia on prolactin receptor manifestation and hepatic bilirubin excretory activity under condition of female rat obstructive cholestasis

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Ordinary complication of pregnancy is shift of hepatic excretory activity leading to cholestasis. Gallstone disease is also predominated in women. Both high prolactin (Prl) concentration and high level of liver prolactin receptors (PrlR) in normal and pregnant women were assumed to participate in obstructive cholestasis development.
Using female rat model of hyperprolactinemia combined with obstructive cholestasis we aimed to investigate Prl influence on female rat liver PrlR expression, alterations of hepatic structure and liver bilirubin excretory activity. Obstructive cholestasis was induced by common bile duct ligation and hyperprolactinemia by female donor pituitary transplantation under kidney capsule of female recipient. Intensity of PrlR manifestation was analyzed with indirect immunohistochemical technique with quantitative computer analysis of imaging. Bilirubin concentration in bile, blood, and urine, bile flow and bilirubin excretion rates were tested.
Hyperprolactinemia induced elevation of PrlR manifestation in hepatocytes under normal and obstructive cholestasis conditions. PrlR expression in cholangiocytes was sharply increased in obstructive cholestasis with no additional influence of hyperprolactinemia and decreased after bile duct decompression.
Hyperprolactinemia in conjunction with elevated hepatocyte and cholangiocyte PrlR manifestation caused additional alterations in hepatic structure and functions as compared to obstructive cholestasis influence: 1) amount and size of bile ducts were additionally increased with occasionally observed ducts with elements of intestinal metaplasia; 2) fibrosis and inflammation of periportal areas were more prominent; 3) depending on hyperprolactinemia duration restoration of bile flow after bile duct decompression was firstly suppressed and then not restored; and 4) bilirubin concentration was firstly elevated in blood and urine and then decreased in bile.
Thus, hyperprolactinemia under obstructive cholestasis condition is accompanied by elevation of hepatocyte PrlR manifestation and may further damage liver structure, aggravate liver functions and participate in redirection of bile flux from liver to blood and urine.

P621

The prevalence of subclinical late – onset hypogonadism in men with diabetes type 2

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The aim
To investigate the prevalence of subclinical late-onset hypogonadism (SLOH) in men with diabetes type 2 and relationship between testosterone concentrations and duration of diabetes.
Material and methods
We investigated 114 men with DT 2 aged from 45–60 years (M±SD 54.0±4.63). Luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (T), calculated free testosterone (cFT) and bioavailable testosterone (bT), sex hormone binding globulin (SHBG), the T/LH ratio, HbA1C, BMI were all determined. Control group included 25 healthy men age from 45–60 years. All of men were treated with oral antidiabetic drugs or insulin. LOH was expected on the basis of low testosterone concentration ($\leq$5.9 nmol/l) and the index T/LH$\leq$1.
Results
The mean of duration of DT2 was 8.52±5.89 years. The TT level was lower in diabetic men compared with control group (6.05±2.65 vs 11.8±5.14 nmol/l). The mean T/LH ratio was 2.47±2.67. Of 62% of men had testosterone levels lower 5.9 nmol/l but only 30% had T/LH ratio below 1. There was inverted correlation between the T/LH ratio and BMI ($r=-0.24$, $P<0,05$), but not with duration of diabetes and age. The level of bT positive correlated with T/LH ratio ($r=0.35$, $P<0.05$).
Conclusions
In men with DT2 the prevalence of SLOH is about 62%, and it was more common then in population of men without diabetes. In all the patients with DT2 the possibility of SLOH should be investigated. In the other hand, men with SLOH also must be screening to exclude the symptoms of diabetes.

P622

Adiponectin increases insulin-like growth factor I-induced progesterone and estradiol secretion in human granulosa cells

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Adiponectin is a protein hormone mainly produced by adipocytes. It plays an essential role in the regulation of lipid and carbohydrate metabolisms. Adiponectin mediates its effects through mainly two receptors named AdipoR1 and AdipoR2. Some evidence in rodent and domestic animals suggests that adiponectin could also regulate female fertility and more particularly ovarian functions. However, its role in human ovary has never been investigated. The objectives of the present study were to identify adiponectin and adiponectin receptors 1 and 2 in human ovary and to determine the effects of human recombinant adiponectin on *in vitro* human granulosa cells (GC) steroidogenesis. We have also investigated which signaling pathways could be activated by adiponectin in human GC. We showed using Reverse Transcription-Polymerase Chain Reaction and Western blot that the mRNAs for AdipoR1 and AdipoR2 and the proteins are found in human GCs. In these latter cells, expression of adiponectin (mRNA and protein) was undetectable, whereas it was largely expressed in human theca cells. By ELISA assay, we detected higher levels of adiponectin in fluid follicular than in plasma. In the second part of our study, we observed that human recombinant adiponectin increased IGF-1-induced progesterone and estradiol (E2) production in human GCs without any variation

of StAR, p450scc and 3βHSD protein levels. However, we showed that adiponectin treatment increased IGF-1-induced E2 secretion and this was associated with an increase in the protein amount of p450 aromatase. Finally, we observed that adiponectin treatment rapidly increased the MAPK (ERK1/2 and p38) signaling pathway in human GCs. These findings significantly increase our understanding of the role of adiponectin on human GCs. However, further investigations are required to determine the role of adiponectin on other human ovarian cells including theca cells and also its potential implication in the polycystic ovary syndrome.

P623

Effects of thyroid hormones on ovary granulosa cells: regulation of proliferation, survival and function

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It is clinically evident that women suffering from thyroid disorders are associated with frequent occurrence of menstrual disturbances and impaired fertility, and these abnormalities are improved by restoring the euthyroid state. The exact mechanism for such reproductive aberrations is not well known; however, it is conceivable that thyroid hormones might have a direct role in ovarian physiology via receptors in granulosa cells. We evaluated the effect of thyroid hormones (T_3,T_4) on the proliferative activity, apoptosis and function of the human granulosa cells COV434. The cells have been treated with T_3 and T_4 (10^{-8M}) after dose response analysis. Cell viability, number and apoptosis of treated cells were evaluated by MTT assay, by cell counting, and by TUNEL assay. T_3 and T_4 were able to induce cell growth and viability. In particular T_3 showed the strongest effect, being able to induce a 40% increase in the cell number after 72 h of treatment. To better define the observed effects, cell cycle profile by FACS analyses has been performed. When the cells were cultured in serum-free condition to induce apoptosis, T_3 was able to induce a decrease in the cell apoptotic rate of 30% and the PI3K pathway seems to be involved in the survival effect of T_3. The ultrastructure of the cells exposed to THs has also been analysed by electron microscopy. In addition the treated cells showed a strong increase in the relative production of 17 beta estradiol, which was increased of 50% by the T_3, and of 30% by T_4 treatments. These results support our hypothesis that THs influence cell proliferation in human ovary granulosa cells.

P624

Successive degradation of StAR by soluble and membrane-bound mitochondrial proteases: plausible mechanism protecting steroidogenic mitochondria under 'protein stress'

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Steroidogenic acute regulatory protein (StAR) is a nuclear encoded vital mitochondrial protein that is essential for synthesis of steroid hormones in the adrenal and gonads. In these tissues, StAR mediates translocation of cholesterol into the inner mitochondrial membranes, where it is converted to the first steroid, pregnenolone. The roughly understood mechanism of StAR action is probably executed prior to StAR import, when the protein is paused at the surface of the outer mitochondrial membrane. Import, therefore, is presently perceived as a turning-off mechanism of StAR activity and leads to a rapid and excessive accumulation of non-functional StAR in the mitochondrial matrix. We postulated that the latter imposes a potential 'protein stress' scenario requiring a rapid degradation of the protein to avoid organelle damage. This study shows that, indeed, StAR is a rare example of a mitochondrial protein with a short half-life that is subjected to proteolysis by a cascade of ATP-dependent mitochondrial proteases. First, Lon protease degrades StAR upon its entry into the matrix. Then, StAR molecules that survived Lon, readily adhere onto the surface of the inner membranes where an inner-membrane protease complex is involved in the second phase degradation of the protein. By use of over-expression, siRNA, pulse-chase and promoter analysis approaches, our results suggest that it is the AFG3L2 protease/chaperone homo-oligomeric complex that is responsible for StAR degradation in the second phase of it elimination; AFG3L2 together with paraplegin (SPG7) function as proteases and chaperons involved in protein quality control and mitochondrial morphology; also, loss-of-function mutations in the latter cause terminal neurodegenerative disorders such as hereditary spastic paraplegia. Our results suggest that turnover of non-functional StAR in the mitochondria is reassured by a succession of protease actions that are critical to maintain the critical function of steroidogenic mitochondria posed under 'protein stress' circumstances.

P625

AdipoR1 and AdipoR2 Inactivation by RNA interference in the KGN human granulosa cell line: potential involvement of AdipoR1 in cell survival and AdipoR2 in steroidogenesis

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Adiponectin is one of the most abundant fat-derived hormones involved in a multitude of metabolism pathways. It acts as an anti-diabetic and anti-atherogenic adipokine. Adiponectin mediates its actions through mainly two receptors, AdipoR1 and AdipoR2. It has been postulated that although AdipoR1 and AdipoR2 consist of seven transmembrane helices, they are distinct from other G protein-coupled receptors. A role of adiponectin in ovarian physiology has been recently suggested. This hormone and its receptors have been identified in different ovarian compartments in various species. Thus, the aim of this study was to determine the effect of an inactivation of AdipoR1 and AdipoR2 mRNA by RNA interference (RNAi) on a human granulosa cell line (KGN cell line). We first observed in a few days the death of R1 cells that express AdipoR1 RNAi. These data were not shown with a control RNAi (scramble RNAi). On the opposite, R2 cells that express AdipoR2 RNAi are viable. Although AdipoR2 expression (mRNA and protein) was strongly reduced in R2 cells, no difference was seen in term of cell proliferation or viability ($\pm$ IGF-1 (5×10^{-8} M) or adiponectin (10 (g/ml)) if compared with KGN cells. Progesterone (P4) and Estradiol (E_2) secretions were increased in response to IGF-1 or FSH (5×10^{-8} M) compared to basal state in KGN and R2 cells. However, these levels of steroid hormones in R2 cells were lower in response to FSH and FSH-IGF-1 ($P<0.0001$) for P4 and E_2 and in response to IGF-1 for E_2 ($P=0.0059$). So, in KGN cells, AdipoR2 receptor could modulate steroïdogenesis stimulated by FSH or IGF-1. Lastly, we observed that human adiponectin induced quick and transient activation of the MAPK ERK1/2 pathway in KGN but not R2 cells. Taken together, these data suggest that, in human granulosa cells, AdipoR1 could act on cellular survival and AdipoR2 could regulate steroidogenesis.

P626

Progesterone suppression of the male hypothalamo-pituitary gonadal axis is partially reversed by the progesterone antagonist mifepristone

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Background
Daily injection of progesterone in men is reported to suppress the pituitary LH response to GnRH challenge. We hypothesized that the progesterone nuclear hormone receptor antagonist mifepristone (RU486) would reverse these effects.
Study design
Open–label randomised, three period crossover-over study in 12 healthy male subjects. Subjects were treated for 8 days with A/ progesterone 50 mg (IM) alone; B/ progesterone 50 mg + mifepristone 10 mg; C/ progesterone 50 mg + mifepristone100 mg with a 14 day washout between treatments. On days 1, 3 and 8 a GnRH challenge 1.4 μg/kg (IV), was carried out 2 h prior to study treatment administration. Blood samples were collected at -25, -15, -5, 15, 30, 60, 90 and 115 mins around the challenge and analysed for LH, FSH and testsoterone (T). Basal hormone levels and response to GnRH challenge (AUC0-2 h) were compared by ANOVA.

Results
Treatment with progesterone alone suppressed basal LH, FSH and T by 42, 43 and 64% on day 3 and 37, 46 and 62% on day 8 (relative to day 1). Concomitant treatment with RU486 dose dependently reversed the suppression of basal LH, FSH and T. In Group B (10 mg RU486), LH, FSH and T were increased by 16%, 35% and 126% on day 3, and by 18, 30 and 48% on day 8 compared to group A (no RU486). In group C (100 mg RU486), LH, FSH and T were increased by 34, 39 and 185% on day 3, and by 61, 36 and 108% on day 8 compared to group A (no RU486).
Progesterone treatment did not affect the LH, FSH or T response to GnRH challenge, and addition of RU486 had no affect on the response to GnRH.
Conclusions
Progesterone suppression of basal LH, FSH and T in men is mediated through the nuclear hormone receptor for progesterone.

P627

Isradipine inihibits oxytocin-induced contractions of isolated myometrium from late pregnant rat

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Preterm labour is a serious clinical problem in obstetrics, and affective treatment of this condition still far from satisfactory. The purpose of this *in vitro* study was to examine the effects of isradipine, a calcium channel antagonist, on oxytocin-induced contractions of myometrium. Myometral strips were removed from late pregnant (18th day) Wistar rats following decapitation and placed in a jacked tissue bath containing Krebs' solution and isometric contractions were evaluated. After recording the oxytocin-induced contractions (control, $n=7$), increasing concentrations of isradipine were applied to the tissue bath cumulatively. Single dose of isradipine was also tested on oxytocin-induced contractions in cacium-free conditions. The amplitude, frequencies (number of contractions for 10-minute) and area under curve (AUC) of contractions were evaluated at 10 min intervals before and after applications of isradipine. Wilcoxon Signed Ranks Test was used for statistical analysis. Of 1 ng/ml isradipine had no significant effect on the frequency, amplitude or AUC compared to control. Of 10 ng/ml of isradipine caused a significant decrease only in the amplitude and AUC values compared to control ($P<0.05$). Inhibitory actions of isradipine on oxytocin-induced contractions were more prominent at 0.1 μg/ml which was significant for the frequency, amplitude and AUC values ($P<0.05$). Of 1 μg/ml of isradipine completely abolished the contractions. Similarly, a single dose (1 μg/ml) of isradipine completely abolished the contractions when the extracellular Ca^{2+} was removed. Data from this study demonstrate that isradipine have inhibitory affect on oxytocin induced myometrial contractions in late pregnant rats. This result may warrant further investigations on the clinical potential of this agent in treatment of preterm labour.

P628

Obesity resulting from Gs-alpha mutations in the maternal (but not paternal) allele is a consequence of Gs-alpha imprinting in the central nervous system

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The female genital tract of the mouse was *in vivo* transfected using the reporter gene β-galactosidase and the liposome Lipofectamine as gene vector. All animals used were anaesthetised. DNA/Liposome complexes were injected through the infundibulum of the tubes in adult, immature and pseudopregnant females. Females that were at different stages of the ovarian cycle were also used. Transfection was analyzed using histochemical, immunological and molecular (Southern blotting, PCR and gene sequencing) procedures. Only epithelial cells appeared transfected in the female genital tract. In alla cases the most transfected areas were the lower region of the uterine glands and cells from the isthmus and juncture regions of the tubes. The hormonal stage of the female was crucial for transfection efficience. The highest number of transfections occurred during meta-oestrus and pseudopregnancy stages, when concentrations of progesterone are high and oestradiol is low. In these cases percentages of transfected epithelial cells were 12% in the uterus and 9% in the tubes. The duration of transgene expression reached a maximum of two weeks in the uterus and one week in the tubes. These data seems to be important regarding future applications of *in vivo* transfection technologies.

P629

Prolonged qt (corrected) dispersion in women with polycystic ovary syndrome

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Background
Polycystic ovary syndrome is the most common endocrinopathy among females in reproductive ages. PCOS is not accepted only a reproductive pathology but also accepted as a metabolic problem. Prolonged QT_c interval showing ventricular repolarization is a major risk factor for the arrhythmias, coronary heart diseases, and sudden cardiac death. Although hyperinsulinism and hyperandrogenism is known as common manifestations of PCOS, there is conflicting data about the QT interval and QT dispersion that are showing the ventricular repolarization. Considering these controversial data, we aimed to investigate the QTc interval and QT dispersion in Turkish women with PCOS.
Patients and methods
Thirty-one patients diagnosed as polycystic ovary syndrome were taken in to study. PCOS was diagnosed based on revised 2003 Rotterdam consensus criteria.
Results
There was no statistically significant difference between groups according to age, BMI, heart rate and systolic or diastolic blood pressure. Waist circumference was statistically higher in patients with PCOS compared to healthy controls. Patients had higher serum triglyceride (TG) and lower HDL-cholesterol levels compared to control group. QTc interval was significantly prolonged in patients compared to the control group. There was no correlation between serum total testosterone levels, DHEA-S levels or E2 levels. Additionally, QTc dispersion was significantly prolonged in patients compared to control group.
Conclusion
These findings suggest that besides the hyperandrogenic state affecting the lipid profile, arterial blood pressure and obesity, insulin resistance is the key factor determining the cardiovascular risk in patients with PCOS even in lean subjects.

P630

Free thiols in human spermatozoa: correlation with Na^+/K^+-ATPase, Ca^{2+}-ATPase activities and sperm motility

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The aim of the present study was to measure free thiols content, Na^+/K^+-ATPase, Ca^{2+}-ATPase activities in human spermatozoa of asthenozoospermic patients and normospermic donors, and evaluate any influence on kinetic sperm features, as well as correlation with peroxynitrite. In fact, membrane integrity and its composition are the basic characteristics of the sperm membrane, thus, it is evident that its composition is an important factor for membrane functions that can be modified upon oxidation. To reach our purposes, 70 infertile patients affected by idiopathic asthenozoospermia and 25 normal fertile donors were enrolled, according to WHO 1999 criteria.
Control spermatozoa exhibited Na,K-ATPase, and Ca^{2+}-ATPase activities, cytoplasmic Ca^{2+} concentration and free –SH content significantly higher than those of asthenozoospermic patients. Moreover, significant positive correlations were found between Na^+/K^+-ATPase and Ca^{2+}-ATPase activities and total sperm motility and sperm kinetic features, whereas significant negative correlations were found between $ONOO^-$ and Na^+/K^+-ATPase and Ca^{2+}-ATPase activities, and total SH content.
Peroxynitrite is able to reduce Na^+/K^+-ATPase and Ca^{2+}-ATPase activities, intracellular Ca^{2+} concentration, through possible depletion of free thiol content. Decrease in motility and loss of sperm function in idiopathic asthenozoospermia can be attributed to these sulfhydryl groups, important entities of the sperm membrane.

P631

Influence of polycystic ovary syndrome and obesity on vascular parameters (common carotid artery intima-media thickness – CCA-IMT, common carotid artery compliance – CP-CCA and endothelium function of the brachial artery – FMD) related to the process of atherosclerosis

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In order to determine the influence of PCOS and obesity on vascular parameters, we studied 25 patients with PCOS (10 with normal body mass index – BMI – and 15 obese) without classic cardiovascular risk factors (IGT or DM, arterial hypertension, dyslipidemia) and 23 control women (12 with normal BMI and 11 obese), pairwise matched for BMI, through a non-invasive method using high resolution ultrasound imaging. Global age range was 26.0 ± 4.7 years. The mean values of free testosterone in PCOS patients were significantly higher than the means in controls, independently of BMI. The means of HOMA-IR and the area under the curve for insulin in obese PCOS patients were significantly higher than the ones observed in PCOS patients with normal BMI and Controls. The groups were formed according to the presence or absence of PCOS and obesity – PCOS group ($n=25$) versus Control group ($n=23$), independently of BMI and normal BMI ($n=22$) versus obesity group ($n=26$), independently of PCOS presence. The means of CCA-IMT was higher in PCOS group than in Control group (49.1 ± 1.0 vs 47.2 ± 1.0 mm.10^{-2}, $P<0.05$) and similar between normal BMI and Obesity groups (49.1 ± 1.0 vs 47.3 ± 1.0 mm.10^{-2}, $P=$NS). It was not observe any influence in CP-CCA in PCOS group vs Control group (1.9 ± 0.1 vs 1.7 ± 0.1 $N^{-1}.m^4.10^{-10}$, $P=$NS) and in normal BMI vs Obesity group (1.7 ± 0.1 vs 1.9 ± 0.1 $N^{-1}.m^4.10^{-10}$, $P=$NS) as well as in FMD in PCOS group vs Control group (6.8 ± 1.1 vs $9.3 \pm 1.1\%$, $P=$NS) and in normal BMI vs Obesity group (88.3 ± 1.1 vs $7.8 \pm 1.1\%$, $P=$NS). In conclusion, in young women without cardiovascular risk factors, the presence of PCOS had influence on the increase of CCA-IMT. Thus, CCA-IMT might be the initial marker of the atherosclerosis process in these groups of patients.

P632

Impact of the type of diabetes in pregnancy upon pregnancy outcome

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Background and aims
It is well known that most of the pregnancy complications (maternal, fetal, & neonatal) increased noticeably in diabetic pregnancy compared with non diabetics, our aims of this study is the impact of the type of diabetes in pregnancy upon pregnancy outcome.
Methods
This is a crosse study analyzed 210 pregnant women who are undergoing antenatal follow up in diabetic pregnancy clinic, and then delivered in Aljalaa Maternity Hospital, Tripoli – Libya in the period from 1.1.2003 up to 31.12.2004. The diabetic women were categorized by the type of diabetes into 3 categories based on the WHO criteria (IDDM, NIDDM,GDM).
Results
GDM represent about half cases of diabetes associated with pregnancy (49%), then NIDDM which represents about 33% & IDDM represent about 18%. pregnancy- induced Hypertension (PIH), pre –Eclampsia (PE),% of cesarean section, polyhydramnios, preterm labour (PL) prelabour rapture of membrane (PLROM), Macrosomia, congenital malformation(CM), prenatal mortality (PNM), R D S, Neonatal metabolic abnormalities, are presented in the table.
Conclusions
GDM increases chance of C/S, induction, Polyhydramnios & PLROM, while IDDM increases rate of PIH & PE, while, incidence of prematurely, IUFD & IUGR increases with IDDM, however Macrosomia increases NIDDM. For neonatal complication IDDM associated with highest risk of CHD which comments CM, hypocalcaemia & PNM, while NIDDM increases chance of polycythemia & RDS and GDM increases rate of hypoglycemia and hyperbilirubinemia. This study revealed that despite the improvement of medical care still the diabetic pregnancy associated with highly unaccepted percentage of adverse outcome compared with non diabetic.

P633

Advanced glycosylated end products are associated with anovulatory markers in women with polycystic ovary syndrome

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Background
Chronic anovulation constitutes one of the cardinal features of Polycystic Ovary Syndrome (PCOS) and Anti-Müllerian hormone (AMH), a granulosa cell product, is a well documented marker of anovulation. Recent data have shown that oxidative stress is involved in the pathophysiology of anovulation. A marker of oxidative stress, Advanced Glycosylated End products (AGEs), is implicated to the pathogenesis of the syndrome and have been localized with high intensity in the granulosa cells of polycystic ovaries. The aim of this study was to investigate whether AMH is linked with oxidative stress markers in PCOS.
Methods
Biochemical, hormonal and ultrasonographic parameters from 37 anovulatory PCOS (PCOS-Anov) and 23 regularly ovulating PCOS (PCOS-Ov), were compared with the corresponding ones from 11 anovulatory non-PCOS women (Non-PCOS Anov) and 25 controls. All subjects were age and BMI matched.
Results
AMH values were statistically significantly higher in PCOS-Anov in comparison to PCOS-Ov and other groups (7.63 ± 3.12 in PCOS-Anov, $> 4.92 \pm 2.50$ in PCOS-Ov, $> 3.66 \pm 1.4$ in Non-PCOS Anov $>$ and 4.02 ± 1.27 ng/ml in controls, $P<0.001$). A similar pattern of AGEs distribution values was observed (8.70 ± 1.65 in PCOS-Anov, $> 7.43 \pm 1.79$ in PCOS-Ov, $> 5.21 \pm 0.09$ in Non-PCOS Anov $>$ and 5.85 ± 0.89 U/ml in controls, $P<0.001$). Follicle number was significantly higher in PCOS-Anov in comparison to all other groups. Additionally a significant positive correlation between AMH and AGEs was observed (r:0.326, $P<0.01$).
Conclusions
In conclusion, the significant positive correlation between, the AGEs, an oxidative stress markerwith the stronger marker of anovulation AMH, suggest a link of oxidative status with anovulation in PCOS.

P634

A systematic review and a meta-analysis on adiponectin levels in women with polycystic ovary syndrome

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Background
Conflicting results regarding adiponectin levels in women with polycystic ovary syndrome (PCOS) have been reported. To evaluate adiponectin levels in PCOS, a systematic review of all studies comparing adiponectin levels in women with PCOS to healthy controls and a meta-analysis of those involving women with similar Body Mass Index (BMI) were performed. Influence of possible effect modifiers, such as insulin resistance (IR) and testosterone, was investigated. Influence of obesity was investigated through a 'nested' meta-analysis after within-study BMI stratification and appropriate pooling.
Methods
Literature search was conducted independently through MEDLINE, EMBASE, Cochrane CENTRAL (through June 2008), references from relevant studies and personal contact with the authors. Thirty-one studies, reporting data on 3469 subjects, were reviewed and sixteen included in the meta-analysis.
Results
Women with PCOS demonstrated significantly lower adiponectin values (Weighted Mean Difference (95% CI) –1.71 (-2.82 to -0.6), $P<10^{-4}$), yet with significant between-study heterogeneity. In larger studies and in studies with modest difference in IR between PCOS and control groups, no significant difference in adiponectin was observed. IR was the only significant covariate in the univariate meta-regression model. Data on high molecular weight (HMW) adiponectin is limited (three studies).
Conclusions
After controlling for BMI-related effects, adiponectin levels seem to be lower in women with PCOS compared to non-PCOS controls. Hypoadiponectinaemia was present in both lean and obese women with PCOS when compared with

non-PCOS counterparts. Low levels of adiponectin in PCOS are probably related to IR but not to testosterone. Further investigation is needed for HMW adiponectin levels in PCOS.

P635

Gonadotropin releasing hormone-induced histone modifications and gonadotropin gene expression

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Gonadotropin releasing hormone actively de-represses expression of the gonadotropin subunit genes through several actions that target the chromatin. We have shown in the past that both LHβ and FSHβ genes are repressed by histone deacetylases (HDACs) in αT3-1 gonadotrope cells and that this repression is overcome by exposure to GnRH which facilitates HDAC removal via CaMKI-mediated phosphorylation. At the LHβ gene promoter, both histone acetylation and phosphorylation increase quite dramatically following GnRH treatment. The H3 lysine four is trimethylated (H3K4me3) at the LHβ gene promoter prior to treatment, while at the FSHβ promoter this modification is increased following GnRH exposure. However at both promoters there is a loss of histone H3 after GnRH treatment, indicating nucleosomal repositioning. H3K4me3 is commonly seen at the promoters of actively transcribed genes, and is recognized by chromo-tudor and PHD domain proteins. It is commonly associated with histone phosphorylation and acetylation and several studies indicate the requirement of all three modifications for transcriptional activation to proceed. The histone acetyl transferase (HAT) GCN5, which is found at the LHβ gene promoter, was shown in other systems to bind preferentially to the phosphorylated H3S10, indicating that the phosphorylation might signal recruitment of the HAT complex to certain genes. Our current working model is that the H3K14ac and pH3S10 are bound by GCN5 which recruits the larger co-activator complex to signal elongation, possibly with the help of additional proteins that recognize specifically the H3K4me3.

P636

Candidate gene analyses in Caucasian patients with primary ovarian insufficiency

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Primary ovarian insufficiency (POI) is a heterogeneous disorder characterized by primary (PA) or secondary (SA) amenorrhea associated with increased levels of gonadotropins. POI affects about 1% of women before the age of 40 years. A major genetic component has been suggested for idiopathic POI due to the frequent familiarity for this defect. Indeed, FMR1 premutations can be found in 10–15% and BMP15 mutations in 2–5% of POI patients. Numerous other candidate genes have been described but the frequency of their involvement is still uncharacterized in large POI series. Here, we report the mutational analysis of six candidate genes: GDF9 (PA = 36, SA = 206), INHA (PA = 24, SA = 172), BMPR1B (PA = 18, SA = 30), FSHR (PA = 14, SA = 7), NOBOX (PA = 10) and GPR3 (SA = 83). Our cohort included a total of 251 POI Caucasian women (12–40 years, FSH > 30 U/l) affected with PA (44) or SA (207), in familiar (PA = 13, SA = 66) or sporadic (PA = 31, SA = 141) form. Genetic screening was performed by dHPLC and direct automatic sequencing of genomic DNA and revealed several novel variations in TGFbeta family correlated genes: a) two variants involving the proregion sequence of GDF9 gene (c.117G > T → p.E39D; c.362C > T → p.T121I) in 3 SA out of 242 cases; b) two missense variants in the BMPR1B signal peptide sequence (c.11G > A → p.R4Q; c.16G > A → p.A6T) in 3 SA and a 3′UTR alteration (c.*9G > C) in 2 PA and 1 SA out of 48 cases; c) a missense substitution (c.832C > T → p.R278W) in INHA gene in 1/196 cases. All identified variants were in the heterozygous state and none was found in 100 control alleles. No variations were found in FSHR, NOBOX and GPR3 genes. In conclusion, we used a candidate gene approach leading to the identification of several new variants associated with POI. Alterations in several TGFbeta family correlated genes with a prevailing ovarian expression may frequently contribute to POI pathogenesis.

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Penile size and testicular volume in healthy Lithuanian newborns

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Aim
Of the study was to establish normal penile size and testicular volume in healthy Lithuanian newborns.
Methods
Of 1204 newborn boys were examined in Panevėžys Hospital due to genitometry. Those with cryptorchidism or hypospadias were excluded. Penis size was measured in 1042 and testicular volume in 712 healthy newborns. The stretched penile length was measured between the pubic ramus and the tip of the glans and the diameter – in the midshaft of the penis. Testicular size was determined with Prader orchidometer.
Results
The mean penile length was 35.7 ± 4.5 and lower and upper cut-offs (± 2.5 S.D.) were 24.5–47.0 mm. The mean penile midshaft diameter was 12.3 ± 1.0 mm (9.7–14.7 mm). Average testicular volume was 0.9 ± 0.3 ml. Penile size, diameter and testicular volume correlated positively between each other and with born body length, weight and gestational age respectively.
Conclusion
Stretched penile length of Lithuanian healthy newborns corresponded to the reported international data. Neonatal penile diameter and testicular volume also represent intrauterine androgenisation.

P638

The effects of stress on the histological structure of rat testis

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Objective
Unexplained infertility is 15% among all infertility cases. Many forms of stress, including psychological and physical can effect male fertility and reproductive activity. In this study, we aimed to investigate possible histopathological effects of physical and psychological stress on rat testis at light microscopic level.
Methods
Eight adult, male Sprague Dawley rats were used in this study. Rats were randomly divided as control ($n = 4$) and stress ($n = 4$) groups. Chronic mild stress (CMS) model of depression was performed to the stress group along for two weeks. During the test rats consumed foods and water freely. At the end of the test, rats were slept with ketamin HCI. Then weight of removed testes were measured with sensitive scales and fixed in Bouın's solution for histopathological evaluation. Following, haematoxylin–eosin dyed preparations were examined at light microscopical level.
Results
There was no statistical difference between two groups in terms of testicular weights ($P > 0.05$; independent samples t-test). In the light microscopic analysis of the CMS performed rats, germinal epithelium thickness of seminiferous tubules was the same with that of control group. In the interstitial connective tissue, intensive hyalinization regions were completely surrounding the seminiferous tubules. Vacuolisations in hyaline regions were remarkable. Vacuolar degeneration connecting with cytoplasmic bulge was seen in the Sertoli cells.
Conclusions
With the help of derived findings, it is mentioned that stress can be the reason for the clear structural changes which can cause deficiency of function in the testis. Finally we suggest that stress can cause male infertility.
Aknowledgement
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P639

Effect of lamotrigine and carbamazepine on selected reproductive hormones, lipid profiles and ovarian histology in female rats

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Objectives
This study was conducted to evaluate the effect of lamotrigine (LTG) & carbamazepine (CBZ) on reproductive hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH) & total testosterone), lipid profiles (total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) & very low density lipoprotein (VLDL)), ovarian weight & histology in non epileptic female rats.
Materials and methods
Thirty-two sexually mature female Sprague–Dawley rats were included in this study, divided randomly into 4 groups, each one included eight rats. Blood samples collected from one group (eight rats), then dissected before starting the treatment & experimental parameters were measured. The other 3 groups, group I received distilled water & considered as control group, group II received LTG & group III received CBZ for 56 days. After treatment, blood samples were collected from animals then killed & dissected for measuring of previously mentioned parameters.
Results
LTG & CBZ caused insignificant changes in serum FSH, LH & total testosterone. LTG & CBZ treatment insignificantly affect lipid profiles & weights of ovaries. Ovaries of LTG & CBZ treated rats did not show features of polycystic ovaries & their histology appeared similar to normal tissue. Numbers of corpus leutum & numbers of follicular cysts did not change significantly in these ovaries.
Conclusion
LTG & CBZ did not produce changes in reproductive hormones, lipid profiles & ovarian histology which were characteristic of PCOS in non epileptic female rats.

P640

Effects of electromagnetic fields of cellular phone on testosterone and progesterone hormones rate in Syrian Hamsters (*Mesocricetus auratus*)

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In this study, the effects of exposure to a 900 MHz electromagnetic field (EMF) emitted from cellular phones on serum testosterone and progesterone hormones rate of adult male Syrian Hamster were evaluated. 72 male Hamster divided in 3 groups include: 1) control group, without any radiation, 2) exposed for 10 days and under 900 MHz EMF (emitted from cellular phone) for 1 h daily, 3) exposed for 50 days and under 900 MHz EMF for 1 h daily. In end of experiment, blood samples collected for determine of the testosterone and progesterone concentration in the serum. Results showed that in long term exposure to EMF (group 3) testosterone levels were increased (6.96 ng/ml, $P<0.01$), but between groups 1 and 2, did not significant changes (3.21 & 3.65 ng/ml, respectively). progesterone level were significantly declined in groups 2 and 3 in compared with control group (progesterone level in control, short and long term exposure groups was 14.90, 11.63 and 4.75 ng/ml, respectively). In this study, progesterone level in hamster serum has been decreased by short-term and long-term exposure to 900 MHz. but testosterone level didn't significant change in short-term exposure to 900 MHz EMF of cellular phone. In conclusion, long-term exposure to cellular phones EMF may affect the reproductive hormonal balance and impair endocrine homeostasis and it may cause peripheral effects.
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P641

Is there a need for withdrawal test with oral micronized natural progesterone in the evaluation of women with polycystic ovary syndrome?

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Objective
To investigate the effects of oral natural micronized progesterone on hormonal and metabolic parameters in patients with polycystic ovary syndrome (PCOS) and to answer the clinical question if induction of withdrawal bleeding is a necessity for the comparison of hormonal and metabolic data in PCOS subjects.
Design
Prospective clinical study.
Setting
Academic medical centre.
Population
Twenty eight reproductive-aged women with PCOS.
Main outcome measures
Blood sampling was collected at baseline, following 7 days of oral natural micronized progesterone (200 mg) administration and post withdrawal bleeding. At these three stages hormonal parameters and HOMA-IR index were assessed in all patients.
Results
Oral natural micronized progesterone administration did not alter significantly insulin sensitivity index and androgen levels; however LH was decreased when post bleeding values were compared to baseline. Nevertheless, following oral natural micronized progesterone administration,progesterone and 17OH-progesterone concentrations were raised, and HOMA-IR was lowered, whereas androgens levels were not altered, in comparison to baseline.
Conclusions
The induction of withdrawal bleeding, with this regimen, does not appear to be a necessity for the assessment of hormonal and metabolic profile in anovulatory women with PCOS.

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Gonadal function in male mountain bikers

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Objectives
Mountain biking has become increasingly popular worldwide. Mountain bikes, are a common source of significant injuries including chronic overuse injuries affecting the genitourinary tract. Erectile dysfunction and infertility are some of the reported urogenital problems in male riders. Our aim was to evaluate the testicular function in male mountain bikers.
Methods
Thirty male professional mountain bikers with a mean age of 23.4 ± 2.5 years (range, 19–27) and mean body mass index (BMI) of 23.2 ± 1.6 kg/m^2 (range, 19.5–25.7) were recruited to the study. Twenty-two non-biker healthy male controls with a mean age of 25.5 ± 6.1 years (range, 17–45) and a mean BMI of 22.3 ± 2.0 kg/m^2 (range, 19–29) were also included in the study. In the study group, the minimum duration cycled was at least one year for each cyclist. None of the cyclists had a history of biking related head or urogenital trauma that required treatment. Fasting blood samples were obtained from all study participants for the measurement of glucose, insulin, leptin, total testosterone (TT), free testosterone (FT), sex-hormone binding globulin (SHBG), leutinizing hormone (LH) and follicle-stimulating hormone (FSH). FT (calculated FT-cFT) and bioavailable testosterone (bioT) were calculated from SHBG and TT using the method of Vermeulen. The presence of insulin resistance was investigated by using the homeostasis model assessment (HOMA) score in both groups.
Results
The study and control groups were comparable in terms of age and BMI. Basal hormonal levels including insulin, leptin, LH, FSH, SHBG, TT, glucose and HOMA scores were similar between the groups. However, bioT, cFT and FT levels were significantly lower ($P<0.05$) in the mountain bikers than those in controls. Despite the lower mean testosterone levels in the study group, the levels of LH and FSH were within normal range in all cyclists.
Conclusion
The study indicates that professional male mountain bikers have lower testosterone concentrations with no accompanying significant increase or decrease in the concentrations of FSH and LH. Low testosterone levels may be both testicular and extratesticular in origin.

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The effects of metformin therapy in women with idiopathic hirsutism

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Hirsutism, which is characterized by excessive growth of terminal hair in a male pattern, is a common clinical condition in women. Among its etiologies, idiopathic hirsutism (IH) is considered to be one of the most common forms of hirsutism. We have previously shown the presence of insulin resistance in women with IH and our aim was to investigate the effects of metformin therapy in women with IH. The study was approved by the local Ethics Committee. Sixteen women with IH (mean age 21.6±1.1 years, BMI: 24.9±0.8 kg/m^2) and 13 healthy women (mean age 27.1±0.5 years, BMI: 22.4±0.9 kg/m^2) were included in the study. The presence of insulin resistance was investigated by using basal insulin levels, insulin responses to oral glucose tolerance test (OGTT) and HOMA score in both groups. Patients with IH had significantly ($P<0.05$) higher basal insulin levels (20.6±4.9 vs 8.1±1.2 mU/l), area under the curve (AUC) of insulin during OGTT (10659.0±2496.4 vs 3509.6±708.3 mU/dlx 2 h), insulin levels at 2 h of OGTT (125.9±36.2 vs 13.9±2.1 mU/l) and HOMA-IR (3.6±0.7 vs 1.5±0.2) in comparison to control subjects. Patients were treated with metformin (1700 mg/day) for 6 months and insulin resistance parameters were re-evaluated. Although it did not reach a significant level, basal insulin level decreased to 13.8±1.6 mU/l, AUC of insulin during OGTT decreased to 7725.8±1992.1 mU/dlx 2 h, insulin levels at 2 h of OGTT decreased to 52.0±12.3 mU/l and HOMA-IR decreased to 2.5±0.3. Our results confirmed the presence of insulin resistance in women with IH. Although limited, 6 months of metformin therapy had beneficial effects on insulin resistance in women with IH. Long term effects of metformin therapy in women with IH should be investigated.

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Impact of gestational impaired glucose tolerance test on pregnancy outcome

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Background and aims
The high prevalence of diabetes and IGTT in women of childbearing age in our community provides an opportunity to assess prospectively the risks of adverse outcome in pregnancy of these conditions.
Method
This retrospective analysis data of a 114 pregnant women diagnosed to have gestational impaired glucose tolerance test, in the period from beginning of January 2001 to the end of December 2004 from maternal & neonatal documents, and controls were obtained by selecting and comparing with the next woman to deliver without IGTT.
Results
There was statistical difference between the mean ages among both groups ($P<0.001$).Inviewofparity,revealed that 63.2% of IGTT cases were multipara, and this confirm the relationship between multiparity and increased risk of IGT and GDM. The mean age for IGTT cases was 36.3. In view of the maternal age, the highest incidence ofIGTT was in the 36 year old group, while the lowest in the 32. The mean age for IGTT cases was 36.3±5.32 S.D., while in normal women 32.9.
Conclusions
Increase incidence of hypertensive disorders of pregnancy 3 fold (12.3%) compared with normal cases (4.4%), PLROM and BOH 6 fold, preterm labor 3 fold (11.4 vs 4.4%) and delivery by caesarean section 4 fold (62.3 vs 14%). Of 6.3 S.D., there was statistical difference between the mean ages among both groups ($P< 0.001$).

P645

A monocentric study of 360 consecutive patients presenting with premature ovarian failure

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Premature ovarian failure (POF) encompasses a heterogeneous spectrum of conditions, with phenotypic variability among patients. The etiology of POF remains unknown in most cases. This led us to set up a French network on POF for the purpose of better characterizing POF patients and understanding the mechanisms involved in this pathology. Over the last 10 years, we have evaluated 360 women who were referred to our center with a diagnosis of POF, and performed a study of clinical, biological, histological, morphological and genetic data relating to these patients. Seventy-seven percent of the patients presented with normal puberty and secondary amenorrhea. Family history was present in 14% of the patients, clinical and/or biological autoimmunity in 29%. The presence of follicles was suggested at ultrasonography in 45% of the patients, and observed in 28% at histology; the negative predictive value of the presence of follicles at ultrasonography was 80%. A genetic cause of POF was identified in 22 patients, 8 of whom had chromosomal abnormalities other than Turner's syndrome, 5 evidenced FMR1 pre-mutation and 9 showed molecular alterations in candidate genes possibly or certainly associated with POF (FSHR, GDF-9, BMP-15 or meiosis gene and Congenital Disorders of Glycosylation). Two patients had autoimmune polyendocrine syndrome (APS) type 2 and 1 with multiple autoimmune diseases. POF remained idiopathic in all the other cases. Over 57% of POF patients experienced BMD alteration, highlighting the importance of estrogen therapy. Our data indicate that global phenotyping of POF patients is of importance to improve clinical management and to orient the search for the identification of genetic mutations, particularly the screen for FMR1 premutation and FSHR mutations. This is expected to be relevant to the detection, in the near future, of women who are at risk for POF and to the development of new therapeutic approaches.

P646

Effect of an oral contraceptive on emotional distress of women with polycystic ovary syndrome: a prospective study

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Background
The symptoms typically associated with polycystic ovary syndrome (PCOS) such as acne, hirsutism, irregular menses, amenorrhoea, obesity and infertility are a major source of psychological morbidity and can negatively affect quality of life (QOL). Limited data are available regarding effects of different treatment modalities on emotional distress of women with PCOS.
Objective
To determine potential impact of treatment on health-related quality-of-life (HRQL), emotional well-being and depressive symptoms in PCOS patients.
Methods
We assessed changes in quality of life and psychological well-being in 26 PCOS patients prospectively by using disease-specific PCOS questionnaire (PCOQ18), hospital anxiety and depression scale (HADS) and general health questionnaire-28 (GHQ-28) before and after 6 months of treatment of drospirenone and ethinyl estradiol alone or combined with metformin. Clinical and endocrine parameters were also evaluated before and after treatment.
Results
Main complaints of the patients were hirsutism and irregular menses. In association with this, the body hair and menstrual problems domains were the areas most negatively affected followed by the emotions domain at baseline. After 6 months of treatment, satisfactory menstrual cycles were attained and hirsutism was significantly improved in all patients. The treatment had a positive impact on the emotions domain of PCOQ18 and mean HADS scores ($P<0,05$). There was a trend for improvement in hirsutism domain of the PCOQ18 ($P=0.06$). Other domains of PCOQ18 or GHQ-28 mean scores did not show a significant change.
Conclusion
Oral contraceptive therapy alone or combined with metformin for 6 months improves emotional well-being of PCOS patients, along with improvement of hirsutism and menstrual disturbance.

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Spermatogenesis in men with late-onset hypogonadism, receiving testosterone-gel

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The prevalence of LOH among middle-aged men, who want to have kids, is increasing. According to HIM study (Mulligan T, Frick MF 2006), the prevalence of LOH is 34% in men, aged 45–54. There are 3 wide-spread forms of testosterone treatment of LOH: oral, i/m forms, and gels. Oral forms of treatment are known to have a rather weak effect. I/m forms of testosterone treatment have a significant negative effect on spermatogenesis down to azoospermia, causing decrease in LH and FSH levels. The effect transdermal forms of testosterone treatment on spermatogenesis is not studied.
Objective
To evaluate the influence of testosterone-gel (Androgel) therapy in men with LOH on the parameters of spermatogenesis.
Material and methods
Of 16 men with clinically and laboratory approved LOH, aged 45–58 years old were included in the study. All the patients were receiving Androgel (testogel) 50 mg daily. Spermatogenesis was assessed after 6 months of treatment. Total and calculated free testosterone levels were evaluated after 1 and 6 moths of treatment. Spermatogenesis was assessed according to WHO recommendations: sperm count (millions/ml), motility, percent normal forms and viability of spermatozoa were used. Categorical variables were presented as median and quartile range.
Results
Total and calculated free testosterone levels were normal in all the patients after 1 and 6 months. After 6 moths of therapy sperm count was 37.5 (11.0;55.0) millions/ml, motility (a+b) – 14.0 (6.0;28.0) %, percent of normal forms – 7.0 (1.0;12.0), viability – 86.0 (61.0;96.0) %.
Conclusion
AndroGel treatment has some negative effect on motility and morphology of spermatozoa, though it might have only a slight effect on sperm count. Further investigation with a bigger amount of patients, spermatogenesis assessment before and during the therapy is needed. Androgel treatment might be preferable to using i/m forms in such patients.

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Prostatic secreted proteins in mice and rats: Identification using mass spectrometric analysis and the hormone dependent expression

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It is not surprising that mouse prostate gland is anatomically very similar to that in the rat, which consists of the ventral prostate (VP), dorsolateral prostate (DLP) and anterior prostate (AP). However, the basic biological function of the prostate, prostatic secretion, has been suggested to be diverse between the two species and yet poorly understood. We have identified major secreted proteins from separate prostate lobes of the mouse as well as the rat by mass spectrometric analysis. Hormone dependency of these protein mRNAs was also examined in both species. In mice, the VP secretes spermine binding protein, serine protease inhibitor KT3 and a 91 kDa hypothetical scavenger receptor, while the DLP/AP secrete a protein similar to immunoglublin binding protein (IgBPLP) and experimental autoimmune prostatitis antigen protein 2 (EAPA2). Prostate secretion in mice was very different from that in rats being only IgBPLP and PSP94 in common. Castration of animals led to a decrease in the mRNAs of these secreted proteins. In rats, a quick androgen response was apparent in the VP as compared with other lobes. In the mouse, however, large decreases in mRNAs were evident in all of the lobes. Combined administration of androgen and estrogen showed synergistic effects on prostate secretion in rats but not in the mouse case. The present study has provided an understanding of the major secretory function of the mouse prostate, and has identified common aspects of secretory functionality between mouse, rat and human. The identified secretory proteins should be available as models of androgen-dependent gene regulation and are candidates as markers for prostatic differentiation. Some of the identified proteins may be useful as pathological markers associated with prostate disorders.

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Erectile dysfunction among HIV-infected men is associated with protease inhibitor containing HAART

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Background
Erectile dysfunction (ED) is an increasingly recognized condition among HIV-infected, however its prevalence and etiology remain unclear. Our aim was to asses prevalence and risk factors associated to ED in this population.
Methods
A cross-sectional study was performed, 88 male HIV-positive without hepatitis C or diabetes were studied. Patients were classified according antiretroviral treatment (ART): naïve, protease inhibitor (PI)-containing HAART and non-nucleoside (NN) containing HAART never exposed to PI. All patients completed standardized questionnaires regarding sexual function (IIEF-15, International Index of Erectile Function), and hypogonadal symptoms (Androgen Deficiency in Aging Men, ADAM; Aging Male's Symptoms Scale, AMS). total testosterone (TT), sex hormone binding globulin (SHBG) and albumin tests were performed to calculate FT. ED was defined as IIEF-15$\leq$25 and hypogonadism as FT$<$6.5 ng/dl. Univariate and multivariate logistic regression analyses were performed to asses risk factors associated to ED.
Results
Mean age 42 years (25–68), 100% caucasian, mean time with HIV infection: 7.8$\pm$5.6 years, median CD4+ cells: 465 cells/mm^3 (IQR, 365–676), 84% received HAART. ED was diagnosed in 47/88 (53.4%) patients, according to ART, naive 10.6%, NN 42.6% and IP 46.8%. In univariate analyses, ED was associated with increasing age (odds ratio (OR) 1.07 for a 1-year increment, CI 1.01–1.14, $P<0.02$) and longer duration of PI therapy (OR 1.03 for a 1-month increment, CI 1.005–1.05, $P<0.02$). In multivariate analysis, ED was associated with increasing age (OR 1.1, $P=0.04$) and longer duration of PI therapy (OR 1.04, $P=0.01$) too. Only 23% of patients with ED were treated with a phosphodiesterase-5-inhibitor, and it was effective in 81%. Hypogonadism was a risk factor for a moderate-severe ED (OR 4.2, $P<0.05$).
Conclusions
ED was associated with age and cumulative use of PI-containing HAART. Hypogonadism is a risk factor of ED in HIV infected men.

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Abstract withdrawn.

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Screening of maternal thyreopathies in Slovakia: is it worth it?

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Background
Newborn screening is the only systematic work-up of thyroid disorders in Slovakia with unambigously proven positivities. There is wide discussion regarding the screening of thyreopathies in specific risk groups such as pregnant women.
Aims
To study the gynaecologist and endocrinologist co-operation in screening pregnant patients from region of Liptov and Orava for autoimmune thyroiditis.
Subjects and methods
The participants were 183 pregnant patients (mean age 29 years) in the first trimester without previous endocrinological investigation. They underwent one day out-patient examination and were screened for TSH, fT4, fT3 levels, thyroid antibodies against thyroglobuline and thyroid peroxidase. At the same time, thyroid ultrasonography was performed blindly to determine the patients' functional thyroid status.
Results
Based on positive thyroid antibodies, autoimmune thyroiditis was diagnosed in 36 (19.7%) pregnant women. From these, 29 (15.8%) were euthyroid, 5 (2.7%) were hypothyroid, and 2 (1.7%) had hyperthyroidism (Graves disease). Moreover, 23 (12.5%) patients were shown to have non-autoimmune nodular and/or cystic changes in thyroid gland. The papillary thyroid cancer was diagnosed in one patient.
Conclusions
The practice management guidelines for thyroid disorders in pregnant females are missing in Slovakia. High prevalence of autoimmune thyroiditis in our pilot study argue for their early intervention. At least, it is reasonable to screen the patients with positive family history and/or personal history of thyroid diseases at the beginning of the pregnancy or even better preconceptually.

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Temporal dexamethasone treatment: responses in uterine proliferative mechanisms induced by neurogenic stimulation

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Mechanical vagino-cervical stimulation (vcs) or mating stimulates hypothalamic neurons that regulate pulsatile secretion of gonadotropin-releasing hormone and the gonadotropins which affect decidualization, an essential for successful mammalian pregnancy, via induced ovarian steroidogenesis. Progesterone-regulated deciduomal growth is promoted by paracrine factors plus the uterine matrix metalloproteinases (MMPs) that remodel the decidual tissue, and nitric oxide (NO), a regulator of uterine vascularity. Dexamethasone (Dexa), a synthetic glucocorticoid, is an established inhibitor of uterine growth. The purpose of the study was to evaluate the time-related inhibitory effects of Dexa on (1) the enzymatic activities of MMPs, and of inducible nitric oxide synthase (iNOS), an isoform involved in NO biosynthesis; and (2) on progesterone secretion during decidual proliferation that was triggered by the neurogenic signals of copulomimetic vcs followed by deciduogenic stimulation via surgical uterine trauma during artificially-induced pseudopregnancy (PG). Female rats (210–240 g; under 12L: 12D) were subjected to vcs (proestrus and estrus) and uterine trauma (day 4 PG) for PG/decidualization induction. Rats ($n=6$/group) were subcutaneously injected with Dexa (1.5 mg/day) for 3 days (PG day 1–3, 4–6, 7–9, 10–12 and 13–15). Animals were killed on the last injection day for analysis of serum progesterone by RIA, MMP activity by substrate zymography, and iNOS activity by western blot. Comparable temporal inhibition by Dexa was noted for decidual weights and iNOS activity which peaked after PG days 4–6 and 7–9. Decidual MMP (72 and 92 kDa) activities were maximally reduced following PG days 4–6 Dexa treatment. However, serum progesterone levels were equally ($P<0.0001$), but asynchronously inhibited by Dexa on PG days 9 and 12. The data indicate that deciduomal iNOS/NO system and MMP activity appeared to be linked to the overall decidual metabolic mechanism that responds to Dexa inhibition. The time-related reductions in decidual growth, iNOS and MMP activities were apparently not mediated by serum progesterone.

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Hyperprolactinemia in polycystIc ovary syndrome

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Background
Hyperprolactinemia is frequently reported with polycystic ovary syndrome (PCOS). However, there is a controversy whether they share a common mechanism or have cause–result relationship or just are coincidental. The objective of the present study was to identify the cause of hyperprolactinemia in patients with PCOS.
Methods
We retrospectively evaluated our outpatient admission records for PCOS, hirsutism, oligomenorrhea, or secondary amenorrhea. PCOS diagnosis was made after excluding other causes of hirsutismus and in accordance with Rotterdam criteria. Patients to be included in the study required not to receive any medication for PCOS within the last 6 months. Fasting serum glucose, insulin, FSH, LH, total and free testosterone, DHEA-S and lipid profile were determined. If hyperprolactinemia was present, in the second step prolactin levels were determined by polyethyleneglycol (PEG) precipitation method, in order to exclude macroprolactinemia.
Results
During the study period, 117 women (median 24.5 years, range 16–40) was diagnosed with PCOS. The median prolactin level was 15.4 (normal value 6–30) ng/ml. Nineteen (16.2%) had elevated levels of prolactin (median 41, range 30.5–118.2 ng/ml). Two patients were receiving antipsychotics, and 3 were using antidepressants. Nine of the remaining 14 patients had mild hyperprolactinemia, and PEG precipitation method revealed normal prolactin levels. Pituitary MRI showed microadenoma in two, and pituitary gland heterogenity in other two patients. One patient was diagnosed with hypothyroidism and Hashimoto thyroiditis. In univariate analysis, the serum prolactin levels did not correlate with any of the the following parameters: HOMA-IR score, total testosteron, free testosteron, DHEA-S, LDL-C, HDL-C, triglyceride, LH or FSH.
Conclusions
Since hyperprolactinemia is not a clinical manifestation of PCOS, patients with increased PRL levels should be investigated for other causes of hyperprolactinemia.

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Serum androgen levels correlate well with metabolic parameters in polycystic ovary syndrome

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Background
In polycystic ovary syndrome (PCOS), in response to stimulation by luteinizing hormone (LH), the ovarian theca cell synthesizes androgens. However, associations of serum androgen levels with metabolic parameters yet to be determined. We aimed to investigate these associations.
Methods
We retrospectively evaluated our outpatient admission records for PCOS, hirsutism, oligomenorrhea, or secondary amenorrhea. PCOS diagnosis was made after excluding other causes of hirsutism and in accordance with Rotterdam criteria. Patients to be included in the study required not to receive any medication for PCOS, or for other conditions associated with insulin resistance, within the last 6 months. Fasting serum glucose, insulin, FSH, LH, total and free testosterone, DHEA-S levels and lipid profile were studied. The correlations between these parameters were determined.
Results
Median fasting serum glucose levels of 167 women (median age 25, IQR 25 to 75, 21 to 30) participated in the study was 4.67 mmol/l (4.38 to 4.94), HOMA-IR index was 2.04 (1.44 to 3.78), HDL-C was 52.00 (41.00 to 62.75), LDL-C was 95.50 (80.25 to 119.00), triglyceride was 106.00 (76.00 to 150.50) mg/dl, total testosterone was 60.40 (40.17 to 93.58) ng/dl (normal, 6–82), free testosterone was 2.0 (1.45 to 2.80) pg/ml (normal, 0.29–3.18), DHEA-S was 263.90 (180.83 to 379.20) mcg/dl (normal, 35–430). Serum total testosterone levels correlated well with LH (r: 235, P: 0.021), whereas negatively correlated with LDL-C (r: −0.290, P: 0.021). Free testosterone levels correlated with insulin (r: 0.372, P: 0.003) and HOMA-IR index (r: 0.372, P: 0.005), while negatively correlated with HDL-C (r: −0.289, P: 0.018). Serum DHEA-S levels negatively correlated with LDL-C (r: −272, P: 0.023).
Conclusions
Androgen levels in women with PCOS may be manipulated with manipulation of metabolic parameters such as LDL-C and HDL-C and serum insulin levels. Specifically, manipulations to increase serum HDL-C may decrease the levels of free testosterone, the most bioavailable androgen.

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Abstract

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Objective
In order to determine the plasmatic levels of leptin in adult Wistar rats (90 days + 7 days) which had undergone specific types of stress, a prospective study was done in which the stimulus was presented in the proestrus stage and the samples, recovered by decapitation 24 h after the initial stimulus, were centrifuged and stored for analysis of leptin by radioimmunoassay (RIA).
Methods
The rats were divided into four groups: Group I ($n=14$) the control group, Group II ($n=16$) underwent laparotomy, being anesthetized with 2.2 tribromoethanol 2.5% 1 mg/100 ml weight, Group III ($n=15$) suffered stress caused by lack of light for 24 h and Group IV ($n=15$) were given 24-hour lighting stimulus (cold light, 20 W).
Results
There was no variation in weight for any of the groups. In Group I, leptin levels varied from 0.476 to 6.714 ng/ml, with a median of 2.667 ng/ml; in Group II, from 0.384 to 1.448 ng/ml, with a median of 0.895 ng/ml; in Group III, from 0.484 to 2.346 ng/ml, with a median of 1.856 ng/ml; in Group IV, from 1.003 to 3.181 ng/ml, with a median of 2.024 ng/ml.
Conclusion
It was concluded that the plasmatic levels of leptin suffered significant reductions in adult Wistar rats suffering from stress produced by surgery, when compared with other groups.

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Use of estradiol benzoate in presynch method in lactating dairy cows

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The aim of this study was to determine of pregnancy rate when Estradiol Benzoate (EB) used in presynch method than to routine presynchronization protocol in lactating dairy cows (*N*: 210). In this study, cows divided in two treatment groups: control group (*N*: 100) and main group (*N*: 110). Control group cows received two injections of Cloprostenol (750 μg/Case, IM) with 14 d interval. Then, they received an injection of Gonadorelin (100 μg/Case, IM), 7 d after cloprostenol (750 μg/Case, IM) injected, followed by an injection of Gonadorelin (100 μg/Case, IM) 48 h later, finally cows artificial inseminated (AI) 16–18 h later. In main group, cows received an injection of EB (1 mg/Case, IM) instead of last injected Gonadorelin 24 h after cloprostenol injection. In main group, AI performed 48 h after EB injection. Detection of cow pregnancy performed at 42 ± 3 d via rectal palpation. Estrus rate in control and main groups were 63 and 71%, and pregnancy rate were 44 and 41.1%, respectively. Also, pregnancy rate in estrus and non estrus cows compared in two groups that in control group were 44.4 and 43.2%, and in main group were 43.4 and 35.5%, respectively. Overall, no significant differences were detected between two synchronization methods in present study. In conclusion, Estradiol Benzoate can be use as a suitable instead to GnRH in presynch protocol.

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Prolonged treatment with *N*-acetylcysteine and L-arginine restores gonadal function in patients with PCO syndrome

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Introduction
Nitric oxide (NO) plays a wide spectrum of biological actions including a positive role in oocyte maturation and ovulation. Free radicals have been shown elevated in PCOS and therefore would be responsible for quenching NO that, in turn, would play a role in determining oligo or amenorrhea connoting PCOS. We recently demonstrated that the combined administration of *N*-acetylcysteine (NAC) and arginine (ARG) is able to exert an antioxidant action leading to an increase in NO availability in patients with type 2 diabetes mellitus, another condition characterized by insulin-resistance and endothelial dysfunction.
Aim
Aim of the study was to evaluate the effects of a prolonged treatment with NAC and ARG in patients with PCOS, focusing on their ovarian function as well as on some metabolic parameters.
Materials and methods
Eight patients with PCOS displaying oligo-amenorrhea from at least 1 year underwent a combined treatment with NAC (1200 mg/die) plus ARG (1600 mg/die) for 6 months. Menstrual function, glucose and insulin levels, and in turn HOMA index, were monitored.
Results
Menstrual function was restored as indicated by the number of uterine bleedings under treatment (3.00, 0.18–5.83 vs 0.00, 0.00–0.83; $P<0.02$). Also, a well-defined biphasic pattern in the basal body temperature in 21 out of a total of 24 cycles under treatment suggested ovulatory cycles. The HOMA index decreased under treatment (2.12, 1.46 4.42 vs 3.48, 1.62 5.95; $P<0.05$).
Conclusions
This preliminary, open study shows that prolonged, oral treatment with combined administration of NAC and ARG determines a clear increase in the number of menstrual cycles, likely ovulatory, in patients with PCOS. These data therefore support the hypothesis that the treatment acts by increasing NO availability counteracting exaggerated free radicals that connote the PCOS. These finding have to be verified in a more prolonged, double blind, placebo controlled study in PCOS patients.

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Does the point of time of menstrual irregularities appearance influence clinical, metabolic, hormonal profile and ultrasound findings in PCOS women?

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Background
PCOS is characterized from chronic anovulation, clinically expressed as menstrual irregularities, hyperandrogenism and polycystic ovaries on ultrasound. A growing body of data indicates that PCOS is a life existing disease starting before puberty and lasting post menopause, as hormonal and metabolic changes exist through the life span of the patient. Since the presence of menstrual irregularities constitutes a prerequisite for the diagnosis of PCOS, we wonder if the timing that menstrual disorders appear has any effect on several parameters of PCOS.
Aim of the study
To compare anthropometric, hormonal, metabolic profile and ultrasound findings in PCOS women who presented menstrual disorders from menarche with the corresponding data obtained from patients who developed PCOS later in life.
Patients and methods
Eighty-nine PCOS women were evaluated. In 49 subjects menstrual irregularities were present from menarche (Group A), whereas in 40 women these irregularities emerge at least three years post menarche (Group B). In each subject clinical, hormonal and metabolic profile were assessed and in each subject ovarian ultrasound and OGTT were carried out.
Results
Anthropometric and clinical parameters were comparable among the two groups as well as hormonal-metabolic profile and ultrasound findings.
Conclusions
These data indicate that despite the timing that menstrual disorders are installed, clinical, hormonal, metabolic profile and ultrasound findings are not affected. These findings imply that regardless the timing of PCOS diagnosis, the lifetime course of the disease is in progress. Accordingly, early recognition of subjects prone to develop PCOS and lifestyle modification is mandatory in order to avoid long-term consequences of the disease.

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Adiponectin levels and its relation to indices of insulin resistance in women with PCOS

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Objectives
Insulin resistance and hyperinsulinaemia are implicated in the pathogenesis of the polycystic ovary syndrome (PCOS) in the majority of the cases. Adiponectin is adipose tissue-specific protein and its correlation with insulin resistance is well established. The aim of the study was to access the correlation between adiponectin and anthropometric and metabolic parameters in a group of women with PCOS.
Methods
Thirty-one women with PCOS (age: 25.7 ± 4.0 years, BMI: 25.8 ± 6.6 kg/m^2; mean ± S.D.) and twenty-three age and BMI respective controls were examined. Anthropometric measurements were conducted by bioelectric impedance (Tanita). There was no significant difference in waist circumference between PCOS and controls ($P=0.119$). In all subjects serum concentrations of glucose, insulin (with HOMA calculation), C-peptide, cholesterol, HDL, LDL triglycerides, adiponectin, testosterone, SHBG, DHEAS, estradiol, basal cortisol were determined. PCOS was diagnosed using ESHRE/ASRM criteria.
Results
There was neither significant difference in adiponectin plasma concentration between PCOS and control group (7.9 ± 3.7 vs 9.1 ± 3.1 μg/ml; $P=0.194$), nor there was significant difference in insulin levels (19.04 ± 7.5 vs 14.1 ± 5.4 mIU/l) and HOMA index (3.9 ± 2.8 vs 2.6 ± 1.1) in both groups. Only in PCOS group was found significant negative correlation between levels of adiponectin and waist circumference ($P<0.05$) and total body fat mass ($P<0.05$). There was significant positive correlation of adiponectin with HDL ($P<0.01$) and SHBG ($P<0.01$) and negative correlation with insulin ($P<0.01$), C-peptide ($P<0.05$), HOMA index ($P<0.05$) and estradiol ($P<0.05$). None of these correlations was found in control group.
Conclusion
There is no difference in plasma adiponectin concentration between our group of women with PCOS, and age and BMI matched healthy controls. Significant statistical correlation of adiponectin with anthropometric and metabolic parameters only in PCOS group, could indicate on intrinsic influence of specific form of insulin resistance in this group of women.

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Preterm menopause and some of its related factors among over 40 years old women

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Goal
To study Prevalence of Preterm menopause and its relation to obesity; to study relation of DM and the age of the first menorrhoea among women over 40 years old.
Materials and methods
The study was performed on 640 menopause women (with no menorrhoea during the past 12 months) who participated in 'sugar and lipid' study of Tehran during 2006 to 2008. With no history of hysterectomy. Preterm menopause was defined as menopause before 48 years old. For studying relation of factors like obesity and DM to incidence of Preterm menopause, just the subjects who had been menopaused maximally in past 36 months were selected ($n=212$). Preterm menopause was the dependent factor, and BMI and DM were the indepent factors of this study.
Findings
The average age of menopause was 48.3 years (47.9–48.8; CI=0.95), and was significantly lower among subject who had their first menorrhoea before 12 years old (47.5; CI=0.95). Prevalence of Preterm menopause Was 35.8%; among the study subjects. It was significantly higher among the subject with had the first menorrhoea before 12 years old (41.5% comparing to 33.8%; $P<0.05$). Logistic Regression Analysis test showed that probability of Preterm menopause is higher among DM patients than other subjects (OR=1.4–3.1; $P<0.05$). There wasn't any significant relation between obesity and Preterm menopause.
Conclusion
The study showed that there is a direct relation between DM and the age of the first menorrhoea with Preterm menopause; but there wasn't any significant relation between obesity and Preterm menopause.

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Does idiopathic hyperandrogenemia exist?

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Some of the patients with hyperandrogenemia are characterized by regular cycles and normal ovaries on USG and named as idiopathic hyperandrogenemia (IHA). To clarify the pathophysiology of IHA we have investigated both the adrenal and ovarian functions, and insulin resistance in 20 patients with IHA and 10 healthy women. The study was approved by the local Ethics Committee. The patients have at least one increased serum androgen. ACTH stimulation test, buserelin test and an OGTT were performed. Patients with IHA and healthy women did not differ in age and BMI. Basal DHEAS, 17-OHP, 11-deoxycortisol (11-S), androstenedione and free testosterone levels were significantly ($P<0.05$) higher in the patients than in the controls. Both peak and area under the curve (AUC) 11-S and AUC DHEAS responses to ACTH test were significantly higher in women with IHA than in the control group ($P<0.005$). Peak androstenedione, AUC androstenedione and AUC 17-OHP responses after buserelin stimulation were significantly ($P<0.05$) higher in the patients than in the control women. Fasting blood glucose levels were similar between the groups. Three (15%) of the patients had impaired glucose tolerance (IGT). Basal insulin, peak insulin, AUC insulin responses to OGTT and HOMA-IR were significantly ($P<0.005$) higher in the patients group than in the controls. The present study clearly shows that high androgen levels in the circulation in IHA patients are in both, adrenal and ovary in origin. A subset of IHA patients are characterized by insulin resistance. Whether IHA is the earliest stage of fullblown PCOS should be clarified by prospective studies.

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Ovulation induction and multicentric follow-up of 21 pregnancies in 14 patients

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Objective
To determine the outcome of pregnancy in hypopituitary women.
Background
Rare situation, not studied enough: only two previous studies concerning 9 and 19 patients (Overton et Hall).
Design
Multicentric, descriptive, prospective and retrospective study.
Material and methods
Twenty-one pregnancies were registered in 14 hypopituitary women (defined as gonadotropic deficiency associated with at least another pituitary deficiency) with median age 32.5 years (27–41), from 3 French endocrinology centers in the universitary hospitals of Paris (Kremlin-Bicêtre), Toulouse and Grenoble.
Results
The primary outcome was the pregnancy: 93% of patients completed at least one pregnancy. Twenty-one pregnancies were obtained with 17 live births (1 set of twins and 5 miscarriages).
The secondary outcomes were the results of ovulation induction: 95% of ovulation and 56% of pregnancies by stimulation with gonadotrophins; the results of luteal phase support which were better with hCG (associated or not with progesterone), than with progesterone alone; the adaptations of levothyroxine and the other substitutive therapies.
Pregnancy or deliveries' complications, newborns' health and measurements were also studied. Two complications were noted: oligo-hydramnios and pre-eclampsia, but no acute hypophyseal deficiency. About 62.5% of patients had a physiological delivery. The 17 newborns were healthy, with median length 50 cm (45–52) and median weight 3234 g (2080–4250), two of them were under the 3th percentile.
Conclusion
Pregnancy can be considered and managed in hypopituitary women, it requires the collaboration of endocrinologists, reproductive physicians and obstetricians for a successful outcome.

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The effects of subclinical hypothyroidism treatment in PCOS women with metabolic syndrome

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Aim
The aim of study was to explore the effects of subclinical hypothyroidism (SH) treatment in PCOS women with metabolic syndrome (MS) and PCOS women without MS.
Methods
The 34 PCOS women were divided in two groups: 1) PCOS women with SH and the MS ($n=23$, age 32.9 ± 9 years, BMI=31.2 ± 3.1 kg/m^2; waist size=87 cm) and 2) PCOS women with SH without MS ($n=11$, age 30.5 ± 7.5 years, BMI=23.4 ± 1.5 kg/m^2; waist size=75 cm). The diagnosis of PCOS was established according to the clinical, hormonal (elevated LH and serum androgens) and ultrasonographic findings. The diagnosis of SH was established according to the TSH>4.2 mIU/ml with normal level of FT3 and FT4. The diagnosis of MS was established according to the high waist size and high insulin and lipids level. All patients were treated with low dose of L-thyroxin (25–50 μg).
Results
PCOS and SH women with MS had significantly higher levels of serum testosterone than PCOS and SH women without the MS (3.42 ± 0.91 vs 2.14 ± 0.77 nmol/l), significantly higher TSH (9.52 vs 5.78 mIU/l), and levels of total cholesterol, LDL cholesterol, CRP. Menstrual cycle irregularity was frequently in group PCOS and SH women with MS. After the six months treatment, women had normal or limited TSH, level of PRL significantly decreased (from 639 to 435 and from 393 to 310 μIU/ml) and level of CRP (from 5.7 to 3.8 and from 3.7 to 2.7 mg/l) in both group. In PCOS and SH group with MS significantly decreased fasting insulin (from 211 to 143), BMI (from 32.9 to 27.1), testosterone (from 3.42 to 2.7) and waist size (from 87 to 81) as well. The changes were and in the level of total cholesterol, triglycerides, HDL and LDL cholesterol. The correlation between TSH and amenorrhoea was significant ($r=0.41$).
Conclusion
These data support an important role of SH treatment in support metabolic control and insulin sensitivity in PCOS women.

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Total ghrelin levels in obese and non-obese patients with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is associated with adiposity and metabolic changes predisposing to insulin resistance. Because the recently discovered GH secretagogue, ghrelin is intimately involved in the control of appetite and weight regulation, we investigate ghrelin levels in non-obese and obese PCOS patients. Ten obese (BMI = 32.50 ± 1.57 kg/m^2) and ten non-obese (BMI = 20.45 ± 0.51 kg/m^2) patients with PCOS and their respective controls were evaluated. Fasting ghrelin was significantly lower in non-obese PCOS (51.82 ± 26.83; $P < 0.05$) as well as in obese PCOS (42.65 ± 26.91; $P < 0.05$) in comparison with controls (non-obese controls 120.11 ± 58.42; obese-controls 96.33 ± 37.34; $P < 0.05$) matched for age and body mass index. In conclusion, women with PCOS had lower fasting ghrelin independently of their BMI, compared to the controls and there were no differences between fasting ghrelin levels among non-obese and obese women with PCOS. The ghrelin level in women with PCOS reflects the metabolic and hormonal changes which are characteristics of the syndrome.

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Our experience in treatment of infertility in patients with Klinefelter syndrome (mosaic karyotype 46,XY/47,XXY)

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Introduction
Until 1996, men with Klinefelter syndrome (KS) were considered to be infertile, but with the development of testicular sperm extraction (TESE) and intracytoplasmatic sperm injection (ICSI) it is now possible to extract viable spermatozoa from the testes using surgical biopsy for the future injection into an ovum. A minority of men with KS have viable sperm in their ejaculate and might, therefore, be able to provide native sperm for ICSI.
Materials and methods
We hold 4 successful programmed *in vitro* fertilization (IVF)/ICSI cycles to couples with man's factor of infertility because of KS (karyotype 46,XY/ 47,XXY) in our clinic.
The results of initial spermograms presented in Table 1.

Table 1

	Volume, Ml	Concnetration million/ml	Motility, % AB (A + B)	Normal Morphology, %
No 1	1.2	0.4	2 (0+2)	4
No 2	0.6	0.005	0	5
No 3	0.8	1.1	12 (2+10)	9
No 4	0.6	0	0	0

Results
We used native sperm in 3 patients, and spermatozoa in one case were received by multifocal TESE.
IVF/ICSI cycles were routine. ICSI was performed for all cases. The embryo on the stage of 6 or more blastomeres underwent preimplantation genetic diagnostics (PGD). The aim of this diagnostics was to select only female embrios for the further implantation. Pregnancy and labor of KS patients' wives were ordinary and didn't differ from normal. Children were examined by pediatrician, no abnormalities were found. All children had a normal karyotype.
Conclusion
Pregnancy can be achieved in KS patients' families, using IVF/ICSI method.

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Periodontal disease in polycystic ovary syndrome

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Background
Periodontal disease (diseases of the tissues around teeth) and polycystic ovary syndrome (PCOS) are common disorders in women with a significant public health impact. Both disorders appear to be associated with diabetes and cardiometabolic risk. There are no published data regarding periodontal disease in PCOS.
Objective
To determine periodontal status in women with PCOS compared to healthy women.
Methods
We studied 25 non-obese PCOS patients with normal glucose tolerance (age: 22.5 ± 3.6 y, BMI: 23.2 ± 3.1 kg/m^2) and 12 age- and BMI-matched healthy controls. All of the participants were non-smokers. Periodontal clinical parameters including probing depths (PD), clinical attachment levels (CAL), gingival index (GI), bleeding on probing (BOP) and plaque index (PI) were recorded during early follicular phase of the menstrual cycle. As a potential contributor to periodontal status and PCOS, selected oxidative stress biomarkers were also assessed. Nitric oxide in terms of nitrite and nitrate were measured in both gingival cervicular fluid (GCF) and blood samples while myeloperoxidase levels measured in GCF only.
Results
PD, CAL, GI, BOP and PI were significantly higher in PCOS patients compared to controls ($P < 0.01$ for all). GCF myeloperoxidase levels were also significantly higher in the PCOS group ($P < 0.05$). There was a non-significant trend of an increase of GCF nitrite and nitrate levels in PCOS group; whereas serum nitrite and nitrate levels were similar between the PCOS and control groups.
Conclusion
Our results suggest that the susceptibility for periodontal disease is significantly increased in patients with PCOS compared to age- and BMI-matched healthy young women, and that local/periodontal oxidant status appears to be affected in PCOS.

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Selected cytokines are associated with markers of insulin resistance in polycystic ovary syndrome

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The polycystic ovary syndrome (PCOS) is associated with features of the insulin resistance syndrome and altered glucose homeostasis. Factors that play an important role in these processes are still emerging. Pro-inflammatory cytokines may be involved in development of insulin resistance in PCOS. The purpose of this study was to determine if a relationship exists between interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), hepatocyte growth factor (HGF), nerve growth factor (NGF), tumor necrosis factor alpha (TNF alpha), fibroblast growth factor-21 (FGF-21) and insulin resistance indices in PCOS.
Methods
Fasting insulin, glucose, C-peptide, lipid profile, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, sex hormone binding globulin (SHBG), 17-hydroxyprogesterone, IL-6, IL-8, MCP-1, HGF, NGF, TNF alpha, FGF-21 serum concentrations were analyzed in 19 women with PCOS and 15 age- and weight- matched healthy controls. Homeostasis model assessment insulin resistance (HOMA-IR) was calculated. Statistics: Mann Whitney test and partial correlations adjusted for BMI.
Results
Fasting insulin and C-peptide were significantly higher in women with PCOS than in control group ($P < 0.05$ for both), HOMA-IR tended to be higher in PCOS ($P < 0.06$). IL-6, MCP-1, HGF, NGF, TNF alpha, FGF-21 levels did not differ between groups. In women with PCOS, after BMI adjustment: (1) MCP-1 and HGF serum concentrations significantly positively correlated with fasting insulin ($P < 0.01$ for both) and HOMA-IR ($P < 0.05$ and $P = 0.001$, resp.), (2) IL-6 and IL-8 serum concentrations significantly negatively correlated with HDL cholesterol ($P < 0.01$ and $P < 0.05$, resp.), (3) IL-6 positively correlated with triglyceride concentrations ($P < 0.01$), (4) FGF-21 correlated significantly negatively with fasting glucose ($P < 0.05$).
Conclusions
In women with PCOS, serum levels of MCP-1, HGF and IL-6 are associated with markers of insulin resistance and FGF-21 was connected with fasting blood glucose. Supported with the grants NR 8759-3 and MSM 0021620814.

Steroid Receptors

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Increased fat mass in androgen receptor knockout mice is caused by decreased physical activity with no change in food consumption

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We have used an androgen receptor knockout (ARKO) mouse model with an in-frame deletion of the 2nd zinc finger of the DNA binding domain, which abolishes the genomic actions of the AR to investigate androgen regulation of fat mass. At 12 weeks of age, ARKO males have increased adiposity compared to wildtype (WT) males, with subcutaneous fat mass increased by 75% ($P<0.001$, $n\geq17$/group) and infrarenal fat mass increased by 36% ($P<0.05$, $n\geq17$/group). However, total body mass of ARKO males is decreased by 13% versus WT males ($P<0.001$, $n\geq17$/group) at 12 and 30 weeks of age. Mean voluntary physical activity at 12 weeks, measured by wheel running, is 86% lower in ARKO mice ($P<0.05$, $n=3$–4/group). At 24 weeks of age, following 12 weeks of a high fat diet (containing 60% fat), total body mass is not different between WT and ARKO mice ($n=11$–12/group). Subcutaneous fat mass remains increased by 66% compared to WT males ($P<0.001$, $n=11$–12/group), however there is no difference in infrarenal fat mass. Average weekly food intake is not different, but mean voluntary physical activity is decreased by 49% in ARKO males compared to WT males ($P<0.001$, $n=11$–12/group). There is no difference in resting energy expenditure, fat oxidation or glucose oxidation rates between the two groups ($n=11$/group). This study suggests that increased adiposity in ARKO mice is in part due to decreased voluntary physical activity but not increased food consumption or decreased resting energy expenditure.

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Androgen receptor gene CAG(n) repeat polymorphism and coronary artery disease (CAD) in women

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Objective
Androgen may be detrimental for vascular health in women. Sensitivity to androgen is influenced by the CAG repeat length of the androgen receptor (AR) gene. We investigated possible associations between the CAG repeat polymorphism with the severity of CAD in women undergoing coronary angiography.
Methods
We examined 131 postmenopausal women (45–88 years). CAD severity was assessed by the number of coronary vessels with $>50\%$ stenosis. History of angina, number of myocardial infarctions (MI), hormonal and biochemical parameters were recorded. The number of CAG repeats ranged between 13 and 28. The mean lowest quartile corresponded to 19 and the highest to 22 repeats.
Results
Angina was more frequent in those carrying ≤19 repeats compared to ≥22 repeats ($P=0.037$, Fisher's exact). A higher percentage of women carrying ≤19 ARgene CAG repeats had 1 and 2 MIs (28.6 and 10.7%) compared to women with >19 repeats (18.2 and 1.45% respectively, $P=0.019$). Carriers of ≤19 repeats in their shorter allele had severe disease (≥2 vessels affected) more frequently compared to those carrying ≥22 repeats (39.2% vs 9.5%, $P=0.009$ Fisher exact). Cholesterol and LDL levels were negatively correlated with the number of repeats of the shorter allele ($r=-0.203$, $P=0.029$ and $r=-0.196$, $P=0.039$ respectively). Antilipid drug therapy was less frequent among carriers of longer repeats in both alleles ($P=0.025$). Mean SHBG levels were lower in women carrying the shorter CAG repeat number (<22 vs ≥22 repeats: 41 ± 17 vs 53.8 ± 30 nmol/l, $P=0.03$).
Conclusions
Shorter polyglutamine stretch in the androgen receptor gene, indicative of increased androgen action, is associated with more severe CAD in postmenopausal women undergoing coronary angiography, both concerning clinical manifestations and angiographic findings. This effect may be mediated by adverse lipid profile or SHBG levels. This association may support the adverse cardiovascular effect of life-long androgenic exposure in this highly selected group of women.

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Association study of the estrogen receptor alfa gene polymorphism and age-dependent endocrine changes in a Romanian population

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Objective
The aim of this study is the characterizing of genetic variation in PvuII and XbaI polymorphisms of the ESR1 gene associated with age-dependent endocrine, metabolic and cognitive changes in a representative sample of Romanian population stratified by age and sex.
Subjects and methods
Subjects, both genders aged between 20 and 80 yr were assigned to three lots 1) 177 subjects aged 55–80 years with moderately cognitive impairment (MCI) (MMSE<28); 2) 133 age-matched subjects without cognitive impairment (MMSE≥28); 3) 143 healthy subjects under 55 years. The haematological, biochemical profiles and cognitive performance were evaluated. Gene polymorphisms were assayed by using PCR-RFLP technique.
Results
The hematological, biochemical and hormonal profiles, correlated with the cognition and associated with both PvuII (IVS1-397 T/C) and XbaI (IVS1-351 A/G) polymorphisms proved that the aging process produces profound changes. The frequencies of genotypes and alleles of polymorphisms did not deviate from the Hardy-Weinberg equilibrium in lot 3 (under 55yr) and lot 1 with MCI. Of note, in men compared with women as whole group and in lot 2 (over 55 yr) neither the PvuII nor the XbaI genotypes were in Hardy-Weinberg equilibrium ($P<0.004$)). In older peoples (lot 2) there were significant differences between distributions of PvuII and XbaI genotypes compared to younger peoples. The percentages of both CC and AA genotypes significantly decreased whereas TC and AG genotypes increased with advanced age. The changes in endocrine function that involved a decrease of testosterone, DHEA, estrogens, TSH, growth hormone, insulin-like growth factor-1 concomitant with an increase of LH and FSH were associated with both PvuII and XbaI polymorphisms.
Concusions
Our results showed that the C and G alleles in ESR1 polymorphisms are associated with the endocrine, metabolic and cognitive alterations with advanced age. Further research is needed to determine the mechanism whereby ESR1 polymorphisms influence aging.
Acknowledgements
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Effect of the androgen receptor gene GGN repeat polymorphism on serum testosterone levels in healthy men

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Objective
The human androgen recteptor (AR) contains a polyglutamine and a polyglycine stretch that are highly polymorphic and are coded by a CAG and GGN repeat, respectively, in exon 1 of the AR gene. Although *in vitro* studies indicated a possible effect on AR gene tanscription and clinical observations suggest a modulation of androgen action, the functional significance of the GGN repeat remains unclear.
We wanted to assess whether the GGN repeat affects serum T levels in healthy men, which is the expected outcome through feedback regulation if the GGN repeat influences androgen action as shown to be the case for the CAG repeat.
Design and patients
A population based cohort study including 1485 healthy young, middle-aged, and elderly men. The study protocol was approved by the ethical committee of the Ghent University Hospital and written informed consent was obtained from all participants.
Measurement
Testosterone (T) and luteinizing hormone levels were determined by immunoassay; free T levels (FT) were calculated. Genotyping of the GGN repeat was performed using the sequencing technique.

Results
The GGN repeat number was significantly associated with circulating T and FT levels ($\beta=0.063$, $P=0.011$ and $\beta=0.058$, $P=0.006$ respectively). However, taking into account that age, BMI and CAG are already in the regression model, the GGN repeat could explain only 0.4 percent of the variation of both T and FT.
Conclusion
To our knowledge, this study is the first to find a significant positive association between the GGN repeat and androgen levels in a large cohort of healthy men. Although the present study adds credence to the view that the polyglycine tract in the AR modulates AR action, this effect appears to be only small so that its clinical relevancy remains questionable.

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ACTH-independent Cushing's syndrome in pregnancy with normal adrenal glands: ectopic receptors to chorionic gonadotrophin hormone (HCG)?

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Cushing's syndrome during pregnancy is a rare disorder. A 32-year-old pregnant woman presented with Cushing's syndrome. She had had a medical abortion complicated with bleeding for anencephaly by 15 weeks of her first pregnancy. At the beginning of her second pregnancy, typical clinical signs of Cushing syndrome developed: extensive red stretch marks on the abdomen and thighs, excessive weight gain (24 kg over 17 weeks) and high blood pressure (150/90 mmHg). By week 22 of gestation, plasma cortisol diurnal rhythm was absent (Midnight cortisol level: 40 μg/dl;N:3–7 μg/dl) and there was a paradoxical increase in plasma cortisol after a 1-mg dexamethasone overnight suppression test (30 μg/dl; Normal <1.8). Basal urinary free cortisol was 3 fold above the upper limit and ACTH levels were suppressed. Blood HCG levels were 14 785 UI/l. The diagnosis of ACTH-independent Cushing's syndrome was established. MRI scans revealed normal adrenal and pituitary glands. Spuriously, by 26 weeks of gestation, clinical signs slightly dwindled with a 2 kg weight loss whereas blood HCG levels decreased to 10 840 UI/l. As hypertension was controled with labetalol and nicardipine, and gestational diabetes with diet, no further treatment with metyrapone was proposed. By week 28, signs of preeclempsia (high blood pressure, moderate oedema, platelets fall, increase of liver enzyme levels) led to perform a caesarean section (CS). The newborn was a normal virilized male who weighed 1490 g. The mechanisms by which pregnancy-induced Cushing's syndrome occurred in this patient are unclear. Aberrant responsiveness or hyperresponsiveness of adrenocortical cells to a non-ACTH and non-CRH substance produced in excess in pregnancy should be considered. Ectopic adrenal receptor for HCG are suspected. Nevertheless the last assessment 1 week after CS showed the persistence of ACTH-independent Cushing's syndrome leading to suspect a Carney complex. A genetic study is in progress.

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Non-classical rapid effects of glucocorticoids on the beta cell function in response to glucose in healthy men

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Glucocorticoids suppress insulin secretion, inhibit glucose uptake in peripheral tissues, and promote gluconeogenesis in the liver. These effects are known to be mediated via genomic mechanisms of slow onset. Despite recent evidence on rapid non-genomic glucocorticoid signaling in several organs, there is no information on rapid glucocorticoid effects on carbohydrate metabolism in humans.
Here we present data on the rapid effects of hydrocortisone on the metabolic response in a frequently sampled intravenous glucose tolerance test (FSIGT). Ten healthy men were recruited in a randomized placebo-controlled cross-over study. They received intravenously a bolus of placebo/0.6 mg/kg hydrocortisone and 4 min afterwards 330 mg/kg glucose. During the following 180 min, blood samples were taken for the measurement of glucose, insulin and C-peptide. Minimal model analysis was performed for the calculation of beta cell function, hepatic insulin extraction and insulin sensitivity.
Hydrocortisone attenuated the rise in plasma glucose following glucose administration during the first 2 h of the study ($P=0.017$), but led to higher plasma glucose concentrations during the last hour ($P=0.004$). Hydrocortisone increased the first phase insulin secretion ($P=0.003$) and decreased the late phase insulin secretion ($P=0.03$). Minimal model analysis revealed that hydrocortisone reduced the total hepatic extraction of insulin ($P=0.009$) without significantly changing insulin sensitivity.
In summary, we present here evidence that the administration of glucocorticoids a few minutes before an intravenous glucose load induces non-classical changes in beta cell function and hepatic insulin clearance, but does not modulate insulin sensitivity during the FSIGT.

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Calculated free testosterone as 'gold standard' for diagnosis of hypogonadism among HIV-infected men

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Background
HIV-associated hypogonadism is a prevalent endocrine disorder, total testosterone (TT) test is not useful for its diagnosis, Endocrine Society clinical practice guidelines have recently proposed calculated free testosterone (FT) as screening test of hypogonadism in HIV infected men, but no clinical study has been published using this approach in HIV population to date. Our aim was to study prevalence and risk factors for hypogonadism in HIV-infected men using FT.
Methods
About 90 caucasic HIV-infected men -without HCV infection or diabetes- were studied. Patients were classified by antiretroviral treatment: naïve, protease inhibitors (PI)-containing HAART and non-nucleoside (NN)-containing HAART (never exposed to PI). All patients completed standardized questionnaires regarding hypogonadal symptoms (AMS, ADAM). Early morning TT, SHBG and albumin tests were performed to calculate FT. Hypogonadism was defined as FT <6.5 ng/dl. Logistic regression analyses were performed to asses risk factors associated to hypogonadism.
Results
Mean age was 42 years (25–68). Participants had been HIV-positive for a mean of 7.8 ± 5.6 years, median CD4+ count: 465 cells/mm^3 (IQR, 365–676). About 84% were receiving HAART and 31.5% reported lipodystrophy. Hypogonadism was observed in 13.3%; belonging 50% to PI group and 50% to NN group. In univariate analyses, hypogonadism was associated with increasing age (odds ratio (OR) 1.13 for a 1-year increment, CI 1.04–1.2) and longer duration of HIV-infection (OR 1.12 for a 1-year increment, CI 1.007–1.3). In multivariate analysis, the association persists. AMS and ADAM questionnaires had a specificity of 37.7 and 37.7% respectively to diagnose hypogonadism in HIV patients.
Conclusions
Prevalence of hypogonadism is remarkable in HIV patients and is associated to older age and duration of HIV infection. Diagnosis of hypogonadism must include FT in any HIV-infected men, because of clinical implication and absence of specific predictive disease factors or useful screening scales in this population.

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Rat female derived adipose tissues respond to estrogens and vitamin D metabolites of in vivo

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We have previously reported that treatment *in vivo* with estrogenic compounds or vitamin D metabolites increased the specific activity of creatine kinase specific activity (CK) in different organs both in intact and in ovariectomized female rats. In the present study we assessed the hormonal responsiveness of para-uterine fat from female and epididymal fat from male rats. Injection of immature female rats with 5 μg estradiol-17β (E_2), 50 μg of estrone (E1), 50 μg estriol (E3), 500 μg diethylstilbestrol (DES), 500 μg cumestrol (Cum) or 50 μg genistein (G) resulted

in increased CK by all compounds tested. When female rats were injected with different anti-estrogens only tamoxifen stimulated CK in fat, whereas raloxifene, tamoxifen methiodide or ICI 164 384 were ineffective, but inhibited E_2-stimulated CK. Injection of female rats with E_2 at different days of the menstrual cycle resulted in highest increased CK in proestrous, lower response in diestrous, even lower at metestrous and no response at oestrous. E_2 induced CK in the para-uterine fat of immature rats was age dependent and was active after ovariectomy with increased response at different time after surgery. CK induction by E_2 was inhibited by both inhibitors of protein and RNA synthesis. Induction of diabetes lead to increased CK in para-uterine fat, but to abolishmed response to E_2. Fat cells from either intact female or male as well as gonadectomised rats responded to injection of either 0.5 ng of 1, $25(OH)_2D_3$ or 5 ng 24, $25(OH)_2D_3$ with increased CK. In conclusion, fat tissues from female rats both intact and after ovariectomy, responded to different estrogens and vitamin D metabolites by increased CK similar to the uterus itself. Since fat cells express ERs mRNA, it might be considered as a new target for estrogens and has to be considered when treated with HRT, both for its beneficial and hazardous effects.

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Androgen insensitivity in the genesis of the mild penile size abnormalities in young belarussian men

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Androgen insensitivity leads to a defect of virilization in karyotypic males despite supraphysiologic androgen levels. Because of an impaired regulatory mechanism of the gonadotropin-androgen axis, both LH and T in serum may be elevated, leading to a distinctively high value of the LH×T multiplication product, which has been termed as the androgen sensitivity index (ASI). The aim of our present study was to investigate a cohort of young males with light decrease in penile size for impaired ASI. About 177 nearly healthy post-pubertal boys at the age of 16 up to 26 years old, permanently living on the territory of the Republic of Belarus, were examined to evaluate their physical, sexual and hormonal state (TSH, FSH, T, Free T, PRL, SHBH, E2). ASI has been calculated according to the standard formula, the normal range reported by Hiort *et al.*, ranging from 1930–9970 ng*IU/l^2 (mean, 15 590 ng*IU/l^2), was used for comparison. Penile size of $\geq$9.5 cm had been determined in 22.6% (40 from 177), 8.5–9.5 cm in 36.2% (60 from 177) and $\leq$8.5 cm in 41.2% (73 from 177) of all examined. The 3-rd group was considered to have subnormal penile size as it was less, then reported by Wessells *et al.* (8.85–10.7 cm) for European population, while the 1-st group was taken as control. The average ASI index in 3-rd group was 31 767 ng*IU/l^2 (10 000–83 950) while in control group 24 845 ng*IU/l^2 (4393–39 480), $P<0.0001$. In the 3-rd group also were determined evident elevated levels of LH, T and Free T in comparison with the control group. That means, that androgen insensitivity could be named as one of the leading reasons for subnormal penile size forming in young belarussian men and high frequency of that phenomenon could not just be considered as a national particularity.

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Effects of seocalcitol, TTNPB, phytol or their combinations on MNU-induced carcinogenesis of mammary gland and nuclear receptors expression in mammary tumours of female Sprague-Dawley rats

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1-methyl-1-nitrosourea (MNU) is a well characterized carcinogen that induces adenocarcinomas in rat mammary gland with high specifity. This model has proven to be of resemblance to human breast cancer. Synthetic analogs of vitamin D_3 and retinoids have shown some promise as chemopreventive agents against chemically induced mammary gland carcinogenesis in rodents.
Female Sprague-Dawley rats were given 50 mg/kg MNU i.p. on 46th and 52nd day of age and were treated with Seocalcitol (the derivate of vitamin D_3, 7 μg/kg per week), TTNPB (the RAR-selective retinoid, 0.7 μg/kg thrice per week), Phytol (500 mg/kg thrice per week) or their combinations after the first tumour was observed in animal (approx. 100th day of age) until the end of experiment. The results have shown that combination of TTNPB and Phytol markedly reduced the number, volume and burden of tumours when compared to animals treated with TTNPB alone. Also treatment of rats with the combination of Seocalcitol and Phytol decreased number of tumours in comparison with the groups of rats (TTNPB alone or Phytol alone). Furthermore, treatment with combination of TTNPB and Phytol inhibited tumour progression. MicroPET data showed the changes of size and number of tumours during the experiment. RT-PCR method has shown that application of Seocalcitol, TTNPB, Phytol or their combinations changed expression of VDR, RAR and RXR subtypes and other nuclear receptors in mammary gland tumours. This observation was also supported by using EMSA analyses. The VEGA grant 2/0022/08.

Signal Transduction

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The MAPKinases ERK1/2 take centre stage in somatotroph physiopathology

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Somatotroph pituitary adenomas are characterized by unrestrained hormone secretion and cell proliferation alterations. In those tumors, the only mutation so far unequivocally identified is the *gsp* oncogene (Gsα protein gain of function mutation). Nethertheless, there is no clear difference in the clinical phenotypes of patients bearing tumor with the gsp oncogene (gsp+) or not (gsp−). In addition, an overexpression of the wild-type Gsα protein has been observed in a subset of gsp− adenomas. To apprehend the role of Gsα alterations in the initiation and progression of GH-secreting adenomas, we have recently developed doxycline-dependent Gsα expressing cell lines derived from the rat pituitary GH4C1 cells. Using these conditional cell lines, we show that induction of the expression of the gsp oncogene as well as overexpression of the wt Gsα protein, which both disrupt the cAMP pathway, induces a chronic activation of the MAPKinases ERK1/2 cascade. This ERK1/2 pathway upregulation is involved in the sustained activation of the human PRL and GH promoters observed in both cell lines. Our results are in close correlation with clinical observations and demonstrate how a slight overexpression of Gsα can cause physiological disorders similar to the *gsp* oncogene ones. We are currently investigating the molecular mechanims, downstream of Gsα, responsible for unrestrained ERK1/2 activity. In both cell lines, we show that the Src kinases are involved in sustained ERK1/2 activation. Moreover co-immunoprecipitation experiments suggest a direct recruitment of Src by Gsα. Src could in turn activate Ras and Rap1 which are both activated and contribute to ERK1/2 hyperactivation. Together, these results put forward a novel Gsα-Src-Ras/Rap1-ERK1/2 signaling pathway as a critical component in somatotroph physiopathology and could be relevant to develop new therapeutical molecules.

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Retinol-binding protein 4 activates TGB-β1 expression and apoptosis via phosphorylation of JNK and MAPK in HEK cells

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Serum retinol-binding protein 4 (RBP4), a new adipocytokine, was reported to increase insulin resistance. RBP4 was significantly elevated in type 2 diabetic patients with microalbuminuria and macroalbuminuria as compared with normoalbuminuric patients. Some adipocytokines have been found to induce cell injury. Therefore, we investigated whether RBP4 could modulate JNK, p38MAPK, ERK and TGF-β1 expression in HEK293 cells by using transfection of p38MAPK and JNK small interfering RNA (siRNA). HEK cells were grown without transfection or with transfection with p38MAPK and JNK small interfering RNA (siRNA) plasmid. Cells were also transfected with control siRNA. HEK cells were stimulated with RBP4 (0, 2.5, 12.5, 25 and 59 μg/dl) to modulate the expression of TGF-β1 and phosphorylation of JNK, ERK and p38MAPK. Our results showed that the addition of RBP4 significantly increased phosphorylation of JNK, p38MAPK and ERK, and TGF-β1 expression in a dose-dependent pattern in untransfected cells. The transfection of p38MAPK and JNK siRNA significantly decreased the expression of p38MAPK and JNK, but also markedly attenuated RBP4-activated expression of TGF-β1. Additionally, the transfection of p38MAPK and JNK siRNA attenuated RBP4-activated caspase expression and DNA fragmentation in HEK cells. In conclusion, our results demonstrated that RBP4 can increase TGF-β1 expression and induce apoptosis through the activation of JNK, p38MAPK and ERK phosphorylation in HEK293 cells.

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Identification of septin 3 as new protein-protein interaction partner of TrkB

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The melanocortin 4 receptor (MC4R) plays a prominent role in hypothalamic weight regulation. Activation of this receptor results by so far not understood mechanisms in a decrease of food-intake and an increase of energy expenditure. Recently a functional role downstream of MC4R signalling for brain derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase B receptor (TrkB) was reported. TrkB signalling influences several neuronal processes like synapse formation and long-term potentiation Mutations in human TrkB gene *NTRK2* cause severe hyperphagia, obesity, developmental delay and deficits in memory and learning.To understand the physiological role of TrkB in hypothalamic weight regulation and to identify further downstream signalling pathways of TrkB we used different fragments of TrkB as baits in a yeast-two-hybrid screen against prays derived from a human brain cDNA library. We found an interaction of the Shc binding site containing juxtamembrane domain of TrkB with the neuron specific Septin Sept3b. This interaction was confirmed by *in vitro* Gluthathion S-Transferase (GST)-pulldown experiments using GST-tagged Sept3 and the HA-tagged intracellular TrkB domain. Furthermore we could show *in vitro* that the interaction is independent of the phosphorylation of the Shc.-binding site of TrkB and splice variants of Sept3, but dependent on a short sequence motif that has previously been described to be important for the intracellular trafficking of TrkB. In differentiated PC12 cells we could show intracellular colocalization of both full-length proteins. Our results provide a first hint for a connection between BDNF signalling and Sept3 that has to be further elucidated in a neuronal cell system.

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A newly identified loss-of-function mutation in helix 5 reveals new insights into signalling mechanisms of the thyrotropin receptor

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In two siblings suffering from congenital hypothyroidism we identified a homozygous missence mutation Ala579Val in transmembrane helix 5 of the thyrotropin receptor (TSHR) gene which motivated us to investigate molecular details of this mutation.
We were interested, firstly, in the functional effects regarding signal transduction and, secondly, in the particular structural properties of the wild type receptor and the Ala579Val mutant. The aim was to gain deeper mechanistic insights into the inactivation resulting from mutations at this position and to understand the biochemical properties of other potentially participating amino acids. We analysed structural-functional relationships of the TSHR by molecular modelling using the latest crystal structures of GPCRs as templates for TSHR homology models and we designed and functionally tested side chain mutations at this position to Ser, Gln, Phe, Met and Leu by determination of b-TSH induced intracellular cAMP formation.
The functional characterization of the patients' mutation revealed a complete loss of function. Moreover side chain mutations to Leu, Met, and Gln at this position resulted in a complete or partial loss of signalling capability. By contrast, substitutions to Ser and Phe were behaved like the wild type protein. Investigations of TSHR homology models suggest, that substitution of the small Ala to a more bulky side chain causes a signalling incompetent receptor conformation. Especially side chains that are branched at the beta-carbon are structurally not tolerated and lead to completely impaired signalling.
In summary, investigation of this newly identified mutation, Ala579Val, of the TSHR helps to explain functional properties as well as structural changes caused by the mutation. Our findings provide significant structural and functional implications for mechanisms of signal transduction of the TSHR and homologous glycoprotein-hormone receptors.

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Nuclear orphan receptor Nur77 is a mediator of p53-dependent apoptotic response

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Nur77 is a nuclear orphan receptor belonging to the steroid receptor superfamily. A role for Nur77 has been described in proliferation, differentiation and apoptosis, as a result of its induction in response to multiple signal transduction pathways. Previous studies have suggested that physical interaction between Nur77 and the tumor suppressor p53 can prevent p53 ubiquination and subsequent degradation by hMdm2. This suggests a possible role for Nur77 as a regulator of p53-dependent signals.
To further investigate the interaction between these two proteins, we first studied if p53-dependent apoptotic responses are modified by the absence of Nur77. Wildtype and Nur77 mouse embryo fibroblast were sensitized by infection with the oncoviral protein E1A and subsequent treated with Doxorubicin or Cisplatin. We observed that apoptosis was partially reduced in absence of Nur77, even though p53 was equally estabilized in these cells when compared to the Wildtype. Thus, these results indicate that Nur77 is a mediator of p53-dependent apoptotic responses.
A possible regulation of Nur77 by p53 was also explored by analyzing Nur77 mRNA levels after treatment with genotoxic agents. HCT116 colorectal carcinoma cells cultured in presence of Doxorubicin or Cisplatin showed a higher increase on Nur77 mRNA when compared with their counterpart p53−/− . Sequence analysis showed the presence of a canonical p53-binging site whitin Nur77 gene. By cloning this site on a luciferase reporter system we were able to detect an increase on the reporter activity in the presence of both endogenous or transfected p53. Site-directed mutagenesis on this sequence led to a marked reduction on the reporter signal confirming the specificity of the regulation.
Taken together our results indicate that Nur77 is a p53-responsive gene that is mediating, at least in part, p53-dependent apoptotic responses.
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On the importance of the selenium status for the inflammatory response

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Many endocrine disorders bear an immunological component and involve local cytokines as paracrine signals. Moreover, certain auto-antibodies appear as causative pathological agents in some of the most common endocrine diseases e.g. in type I diabetes mellitus or different forms of autoimmune thyroid disease (AITD). Recently, selenium (Se) status and selenoproteins have emerged as important modifiers of the inflammatory response, AITD or sepsis. Notably, mortality risk of patients with severe sepsis appeared negatively correlated to serum Se concentrations and prognosis improve upon Se supplementation in a recent multicentric trial. In an attempt to study the importance of the Se status for inflammation-dependent pathologies, we subjected mice on different Se supply to an LPS-induced acute phase response. First, we tested whether the acute phase

induced alterations affect preferentially the essential trace element Se or rather represent a more general impairment of the hepatic trace element metabolism. Among all these trace elements tested (including Fe, Cu, and Zn), only Se showed a very pronounced decline during the acute phase response. Next, we analyzed the importance of the baseline Se status for the response. Se-concentrations in serum and liver declined to approx. 50% of control values 24 h after LPS injection in a Se-supplemented group, whereas Se-poor animals maintained a constant value at a low baseline. More importantly, cytokine production was strongly affected by the Se-status. The Se poor animals displayed significantly augmented concentrations of the circulating cytokines IL6 ($P \leq 0.05$) and MCP1 ($P \leq 0.01$) compared to the Se-supplemented mice. This effect was especially pronounced in the male mice and pointed to certain sex-specific differences. We conclude that Se-based adjuvant supplementation efforts might improve selenoprotein expression and immune function during inflammation avoiding an exaggerated induction of pro-inflammatory cytokines. This mechanism might underlie the positive Se supplementation results which have been observed in recent AITD trials. Further studies are needed to test which patients are in need of Se supplementation and how their inflammatory response or autoimmune antibody load respond to an improved Se supply.

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Orexins activates protein kinase C-mediated Ca^{2+} signaling in cultured rat dorsal root ganglion neurons

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Orexins, the novel pluripotent hypothalamic peptides, have been shown to exert important roles in the regulation of multiple physiological functions and behaviors including appetite, sleep and wakefulness and energy homeostasis through neural and endocrine mechanisms. Evidence emerging from recent research indicates that orexins may be involved in many other physiological functions. Our previous results have suggested that orexin-A and B activate Ca^{2+} signaling in cultured rat dorsal root ganglion (DRG) neurons implicating a role in nociception, and the aim of the present study was to investigate whether this orexin receptors mediated signaling involves PKC pathways in this sensory neurones. Following enzymatic digestion and mechanical agitation the DRG neurons were cultured *on* coated coverslips and loaded with 1 μmol Fura-2 AM. $[Ca^{2+}]_i$ responses were quantified by the changes in 340/380 ratio for individual DRG neurons using the imaging system consisting of CCD camera coupled to an inverted microscope with a 40× (1.30 NA) objective. All data were analyzed by using unpaired *t* test, $P<0.05$ defining statistical significance. The non-peptide OX_1 selective receptor antagonist SB-334867-A (1 μM) inhibited the orexin-A (200 nM) and orexin-B (200 nM)-induced calcium responses (57.2±4.2% versus orexin-A, $n=5$, and 65.9±3.6% versus orexin-B, $n=9$). The PKC inhibitor chelerythrine chloride also decreased the orexin-A (200 nM)-induced calcium responses (59±5.2% and 5±1.7% versus orexin-A, $n=7$ for both 10 and 100 μM). In conclusion, the results suggest that ORX A and -B cause an increase in free intracellular calcium through PKC pathway activation, which could be associated with nociceptive modulation and pain.

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High susceptibility haplotypes of the TSHR gene in Graves-Basedow disease

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The mechanisms triggering autoimmunity in the Graves-Basedow disease (GBD) are unknown, although the evidence for a genetic predisposition is well established. The thyrotropin receptor (TSHR) is a good candidate gene.

Objectives
To identify the main alleles/haplotypes of the TSHR gene, and to analyze the existence of TSHR susceptibility alleles to the GBD.
Material and methods
To establish the main alleles of TSHR gene, 54 polymorphisms (53 SNPs and 1 DIP) were selected including: 1) SNPs identified by sequencing both the promoter and 3′UTR regions, and 2) TagSNPs capturing most of the genetic variability of the gene. TagSNPs have been selected using the Haploview software and available data from HapMap project. These 54 polymorphisms were genotyped, using SNPlex technology, in 329 gDNA samples from: 192 control subjects and 137 GBD patients.
Results
In the case–control study of the 54 polymorphisms genotyped, after the multiple tests correction, a set of 10 SNPs showed a significantly different distribution between cases and controls ($P<0.05$), some of them with a high statistical signification ($P=10^{-5}$). The odds ratio obtained ranged from 2.08 to 5.15. All these significant SNPs were located in a region that covers from the position −6200 in the promoter, to the intron 1, remaining the rest of the gene free of significant associations. Beyond this analysis of individual markers, the main haplotypes of the TSHR gene were established, using the different blocks of linkage disequilibrium (LD). Two highly significant haplotypes, one protective and one predisposing to the disease, were identified.
Conclusions
A set of SNPs located in the 5′ region of TSHR gene (from the promoter up to intron 1) significantly associated to GBD was identified. The existence of LD among the associated SNPs allow to define the main haplotypes, two of them conferring susceptibility or protection to the disease.

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The effect of circulating estradiol concentrations on gonadotropin secretion in young and old castrated men

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Context
In men, a decline of mean circulating testosterone level is seen with increasing age. This decline is associated with an increase in the mean levels of LH and FSH, albeit insufficient to maintain testosterone at its original level. It has been speculated that a higher sensitivity of the hypothalamus and/or pituitary for the feedback effect of circulating testosterone in older men is responsible.
Objective
To compare the effect of experimentally varied plasma levels of estradiol on the LH and FSH secretion in young and old men, in almost absence of testosterone.
Methods
In 10 healthy, castrated young men and 11 healthy, castrated old men (both male-to-female transsexuals after gonadectomy) plasma estradiol levels were experimentally varied with estradiol patches (the first week 100?g/day, the second week 50?g/day, the third week 25?g/day and the fourth week no patch was applied). We monitored plasma levels of LH and FSH after every week.
Results
The mean plasma bioavailable E2 levels in the two groups ranged between 13.6 and 104.0 pmol/l. LH and FSH were inversely related to peripheral estradiol levels. Mean LH and FSH levels were lower in the old group at all time points however, the difference only reached statistical significance in the last week of the study when no patch was applied and estradiol levels were extremely low.
Conclusions
Circulating E2 levels markedly inhibit gonadotropin release in both young and older men. Observations that the difference between gonadotropin levels of young and old men is highest when E2, T and inhibin levels are very low, does not support the hypothesis of an age related increasing sensitivity of the hypothalamus for the negative feedback of E2, but rather supports the hypothesis of a deficient hypothalamic feed-forward drive.

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